Freie Universität Berlin

Multivalent Dendritic Polyglycerol Anions for Diagnostic and Therapeutic Applications

Dissertation

to obtain the academic degree

Doctor rerum naturalium (Dr. rer. nat.)

submitted by

Sabine Reimann

from Wesendorf

Department of Biology, Chemistry, Pharmacy

Institute of Chemistry and Biochemistry

Freie Universität Berlin

March 2017

Declaration of honesty

Hereby I declare and confirm that this PhD thesis is entirely the result of my own work and that no other sources than those cited have been used. All annotations which have been used from published or unpublished sources are identified as such. The shown illustrations have been created by myself or have been marked with the corresponding references.

_____

Sabine Reimann March 2017

The following project was carried out within the research group of Prof. Dr. Rainer Haag from **November 2012** until **December 2016** at the Institute of Chemistry and Biochemistry of the Freie Universität Berlin.

1. Reviewer: Prof. Dr. Rainer Haag, Freie Universität Berlin

2. Reviewer: PD Dr. Kai Licha

Day of defense: May 23rd, 2017

Acknowledgements

First of all, I would like to thank **Prof. Dr. Rainer Haag** for giving me the opportunity to work in his international research group and on this interesting topic.

Furthermore, I thank **PD Dr. Kai Licha** for being co-referee of this thesis.

Many thanks go to **Prof. Dr. Peter Fratzl** and **Dr. Wolfgang Wagermaier** for their collaboration and the opportunity to perform various measurements at the Max Planck Institute.

All former and present group members, especially my many lab colleges **Dr. Dominic Gröger**, **Dr. Aileen Justies**, **Dr. Olaf Wagner**, **Abbas Faghani**, **Christoph Schlaich**, and **Dr. Benjamin Ziem** I want to thank for the pleasant working atmosphere and collaboration.

Many thanks are addressed to my cooperation partners, **Dr. Jens Dernedde**, **Dr. Sebastian Riese**, and **Dr. Christian Kühne** from the Charité Berlin, **Dr. Pia Welker**, **Ingo Steinke**, as well as **all other members of the epiios GmbH**, **Prof. Dr. Gundula Schulze-Tanzil** and **Dr. Tobias Schneider** from the Paracelsus Medical University, as well as **Falko Neumann** for making this highly interdisciplinary work possible.

The service of the **core facility BioSupraMol** I would like to thank for performing numerous sample measurements.

Dr. Pamela Winchester, **Dr. Katharina Achazi**, and **Prof. Dr. Michael Schirner** are acknowledged for proofreading and **Jutta Hass** for her help regarding administrative issues.

My biggest thank is addressed to **my family and friends** for their patience, open ears, and support during the last years.

Table of Contents

1 Introduction

In the last decades, the field of nanomedicine has gained much attention in the natural and life sciences. As the interface between nanotechnology, chemistry, and biology, this highly interdisciplinary topic is expected to give rise to new forms of treatment in critical areas such as cancer,[1] bacterial diseases,[2] and viral infections,[3] especially in the context of tissue regeneration, diagnostic applications, and theranostics.[4] In the past, a variety of different nanomaterials (1 – 100 nm) have been explored including micelles, polymers, liposomes, hydrogels, and inorganic particles.[1, 4b] These materials can be functionalized with a multitude of different moieties and tuned in their physiochemical properties to achieve improved pharmacokinetic and -dynamic characteristics, enhanced therapeutic efficacy, and reduced toxicity in comparison to small molecules.[5] As a result, various nanoscaled therapeutic and diagnostic agents have been approved by the US Food and Drug Administration (FDA) or have entered clinical trials (**Figure 1**). An excellent overview about nanoparticle-based medicines was recently published by Bobo et al.[5]

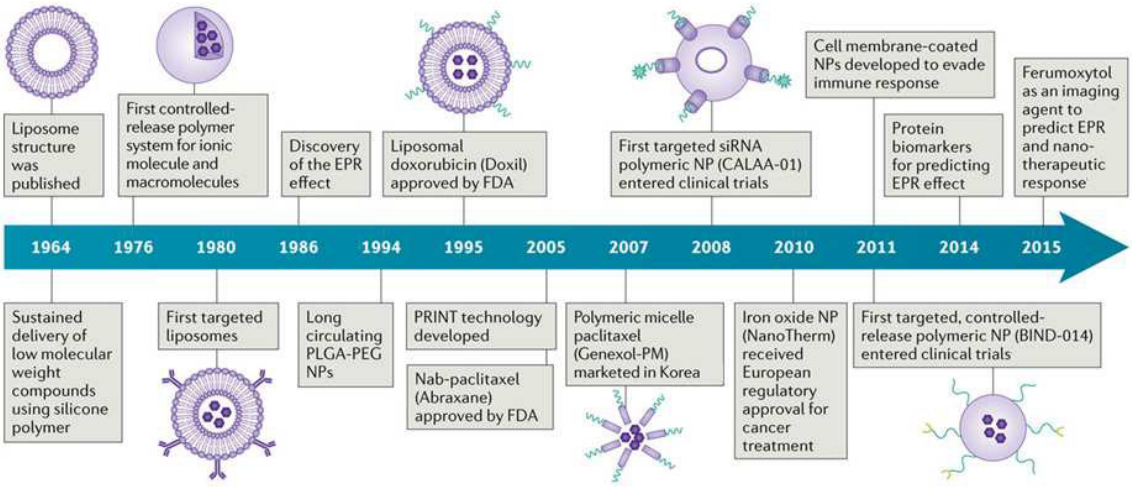

Figure 1. Timeline of major developments in the nanomedicine field and their entrance into clinical trials. EPR = enhanced permeability and retention; FDA = US Food and Drug Administration; nab = nanoparticle albumin-bound; NP = nanoparticle; PLGA = poly(lactic-co-glycolic acid); PEG = poly(ethylene glycol); PRINT = particle replication in non-wetting template; siRNA = small interfering RNA. Modified with permission from Macmillan Publishers Ltd.: Shi et al., *Nat. Rev. Cancer*,[1] Copyright 2016.

Before clinical approval, safety risks of the respective nanomaterial have to be evaluated, which requires an extensive research on its interaction with biological surroundings on the molecular, cellular, and organ level.[5-6] In the past, numerous empirical *in vitro* and *in vivo* studies have been carried out to unravel the complex interplay of particle properties and biodistribution, cellular uptake, protein adsorption, and clearance mechanisms.[7] The size of the nanoparticle, for instance, influences the

circulation half-life and elimination pathway from the body. While small particles (< 5 nm) are excreted via the kidney, larger particles (> 200 nm) are cleared through the liver and spleen.[7] These mechanisms can be biased by the choice of scaffold flexibility, molecular weight, degree of branching, particle shape, and implementation of cleavable linkages.[8] However, the size of nanomaterials in biological systems often differs dramatically from the size determined by characterization techniques like dynamic light scattering (DLS) or nanoparticle tracking analysis (NTA). Immediately after entering a biological milieu, the surface of the particle is covered with non-specific proteins, which results in the formation of a protein corona.[9] The subsequent protein denaturation initiates a signaling cascade that either results in phagocytosis by activated macrophages and/ or aggregation of the nanomaterial, which alters its size.[5] This in turn leads to undesired biodistribution patterns and unpredictable pharmacokinetics. Moreover, hydrophobicity and surface charge were found to have an impact on the *in vivo* application of the nanoparticle due to cytotoxic effects. Cationic compounds, for example, are usually associated with a higher cytotoxicity and lower biocompatibility in contrast to anionic structures.[7, 10]

The combination of nanoscaled architectures and anionic species exploiting multivalent effects therefore represents a promising approach for the development of therapeutic and diagnostic agents with maximized biocompatibility and efficacy. In this PhD thesis, stable and degradable dendritic polyglycerol anions were synthesized, evaluated for their targeting properties toward inflammation, bone, as well as cartilage, and investigated concerning their binding affinity to different proteins in order to gain more information about their interactions with biological systems.

1.1 Multivalency

Multivalency is a key principle in biological systems, which plays a pivotal role in cell-cell recognition and the binding of proteins to cell membranes and small molecules.[11] Multivalent processes describe strong yet reversible interactions, in which multiple ligands on one entity, such as cell surfaces or molecules, simultaneously bind to multiple receptors on another entity (**Figure 2**).[12] It is often associated with a much higher kinetic and thermodynamic stability, stronger binding, and higher specificity compared to monovalent binding events.[13] Although the term multivalency is widely used in different scientific fields, its definition is ambiguous and closely related concepts such as chelation and avidity are used synonymously.[14] The chelate effect, for instance, describes a similar principle, in particular cooperative interactions between multiple linked binding partners, but mainly refers to the complexation of a central molecule or metal ion and has its origin in inorganic and organometallic chemistry.[12, 14d, 15] Avidity, in contrast, is related to antibody-antigen interactions and comprises the overall strength of multiple affinities of individual non-covalent interactions, which are dependent on the number of valences of both the immunoglobulin and antigen.[16] Since avidity and chelation are usually associated with processes where the number of individual ligand-receptor interactions (N) is < 10,[12, 14d] the term multivalency in the following will refer to higher valent processes (N > 10).

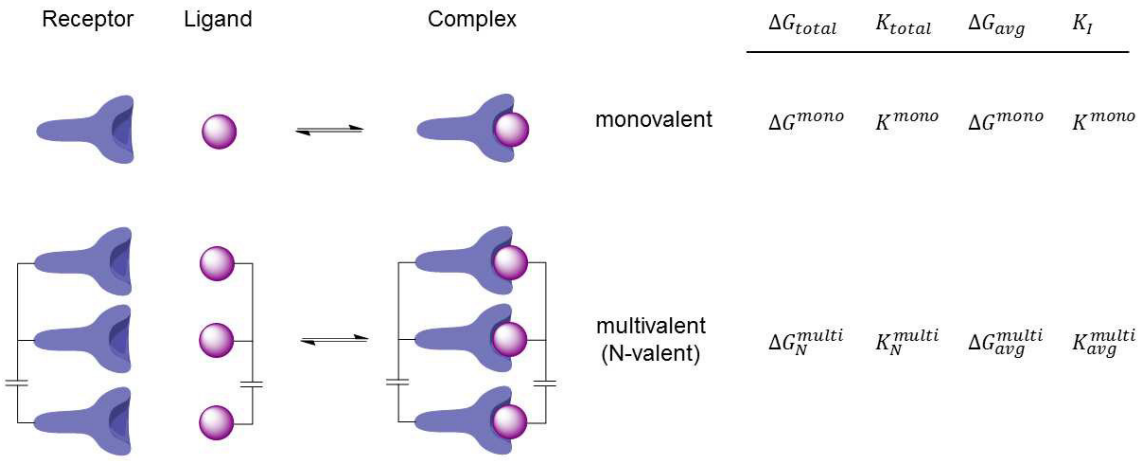

Figure 2. Comparison of mono- and multivalent interactions and their thermodynamic parameters such as the total free energy (ΔG), total binding constant (K_{total}), and average free energy (ΔG_{avg}).[12]

Multivalent interactions differ fundamentally from its monovalent counterpart in terms of thermodynamics and kinetics.[17] However, an understanding of both is crucial for the design and development of multivalent inhibitors with high efficacy. In multivalent processes a series of association and dissociation steps is involved and rebinding

easily occurs, which eventually leads to lower dissociation rates of multivalent complexes and therefore higher kinetic stability.[13b] Thermodynamic stability, on the other hand, is mainly determined by the change in the free energy (ΔG) that occurs during binding. In a monovalent system, only a bound and an unbound state contributes to ΔG^{mono}, while in multivalent binding events the total free energy difference (ΔG_N^{multi}) is related to N monovalent associations and the average free energy (ΔG_{avg}^{multi}) between a single ligand and single receptor is given by $\Delta G_N^{multi}/N$.[12, 14a] The enhancement of the free binding energy in multivalent interactions can be quantified by the following equations:

$$\Delta G_N^{multi} = \alpha \Delta G^{mono} \quad \text{(Equation 1)}$$

$$\Delta G_N^{multi} = N \Delta G_{avg}^{multi} = \alpha N \Delta G^{mono} \quad \text{(Equation 2)}$$

$$K_N^{multi} = (K_{avg}^{multi})^N = (K^{mono})^{\alpha N} \quad \text{(Equation 3)}$$

As obvious from these equations, ΔG_{avg}^{multi} can be greater, equal, or less than the average free energy of the analogous monovalent interaction, depending on the degree of cooperativity (α).[12] The cooperative effect describes how binding of one ligand to a receptor affects the binding affinity of the others.[18] Multivalent interactions can either lead to positive (synergistic, $\alpha > 1$), negative (interfering, $\alpha < 1$), or no (additive, $\alpha = 1$) cooperativity, depending on whether one interaction favors or disfavors the other or has no influence on further ligand binding.[15] Cooperativity of a multivalent system can be influenced by a large number of factors, for instance, by changes in the binding enthalpy, translational and rotational entropies of each individual ligand involved in the binding event, or by changes in solvation and structure of the receptor.[14a] So far, cooperativity has mostly been determined and quantified for low valent systems such as for the binding of oxygen to hemoglobin, protein folding, and formation of pseudorotaxanes.[15, 19] However, in these systems, the number of effective binding interactions is known, while this is not the case for most multivalent systems.[12] Moreover, the majority of multivalent interactions exhibit negative cooperativity, although binding affinities were found to be much higher than those of the monovalent counterparts.[12] This was impressively demonstrated by a trivalent system based on a vancomycin receptor and a trivalent D-Ala-D-Ala ligand, which showed a $4 \cdot 10^{10}$ fold increased binding strength compared to the respective monovalent interaction although a negative cooperativity was found.[13a] Therefore, Whitesides and co-workers introduced an enhancement factor β, which reflects the strength of a multivalent

association relative to the corresponding monovalent binding, even if the multiplicity of interactions is unknown.[12] This factor is defined as:

$$\beta = \frac{K_N^{multi}}{K^{mono}} \quad \text{(Equation 4)}$$

From a thermodynamic view, the enhancement of enthalpy or entropy leads to a lower free binding energy (ΔG) and a higher thermodynamic stability.[12] The enthalpy change can either be increased by multivalent interactions due to a conformational change of the binding site of a receptor, which leads to a more favorable second binding event,[20] or diminished if, e.g., steric repulsion interferes with further interactions.[12] However, this highly depends on the structure of the ligand and the binding site of the receptor. With increasing rigidity of a multivalent entity, spatial mismatches become more likely, which results in enthalpically less favored binding, unless the geometric fit between the ligand and receptor is adjusted up to a few picometers.[12]

Entropy plays a central role in multivalent interactions and insufficient knowledge about this thermodynamic quantity has resulted in many synthetic multivalent scaffolds with a lower or comparably high efficacy as their monovalent analogs in the past.[21] A simplified estimation of the thermodynamics of a multivalent system can be achieved by comparing the free binding energies of two ideal systems.[12] In the first system N independent monovalent complexes are formed, which are composed of N monovalent ligands and N monovalent receptors. The second, multivalent system is characterized by a N-valent receptor that bind to a N-valent ligand and create a N-valent complex. The free energy difference between both systems ($\Delta\Delta G$) then can be expressed as:[14a]

$$\Delta\Delta G = \Delta G_N^{multi} - N\Delta G^{mono} = (N-1)\,T\Delta S^{mono} - T\Delta S^{conf} \quad \text{(Equation 5)}$$

This equation suggests that multivalent interactions are more favorable due to a relatively smaller entropy loss compared to monovalent binding events, which results in more negative free energy and higher affinity.[14a] However, this assumption is only true under specific conditions. In monovalent associations, the total entropy only slightly decreases if a complex is formed and thus the translation, rotation, and solvation entropy is changed since entropy is maintained by remaining free ligands.[14a] In contrast, in multivalent systems the translation, rotation, and additionally conformational entropy (ΔS^{conf}) are already lost if the first binding event occurs, while further intramolecular binding is entropically enhanced if there are no additional enthalpic costs.[12, 14a]

1.2 Design of Multivalent Binders

Multivalent interactions are involved in various biological processes such as the uptake of viruses or bacteria by their host cells and the recruitment of leukocytes to the site of inflammation.[12] The latter is achieved by the interaction of leukocytes with the activated endothelium and mediated by the recognition of glycan ligands by cell adhesion molecules (CAMs) like selectins (see Section 1.3).[22] In chronic inflammation, a prolonged extravasation of leukocytes eventually leads to severe tissue damage,[23] which can be suppressed by inhibiting the interaction between selectins and their corresponding carbohydrate ligands. Monovalent binding events between selectins and the glycan moiety are relatively weak, while affinity dramatically increases if ligands are presented multivalently.[14a] The development of multivalent inhibitors is therefore promising for the treatment of inflammatory diseases. However, the concept of multivalent compounds can also be adopted for numerous other applications.

When it comes to the design of multivalent binders, the nature of the receptor and ligand has to be taken into account. In order to minimize the loss of conformational entropy and achieve tighter binding, the implementation of rigid and preorganized spacers into the scaffold backbone instead of flexible linkers is recommended.[17, 24] However, the length of the linker in rigid systems has to be precisely adjusted to avoid spatial mismatches.[12] The evaluation of the right geometric fit certainly needs some insights into the spacing of the respective binding sites and requires extensive and highly time-consuming experimental and computational studies.[17, 25] Unlike rigid linkers, flexible spacers facilitate the maximum number of binding events without steric strain and allow sampling of the optimal conformational space.[17] Moreover, a higher valency is more preferable since a low number of binding valences is considered to be less efficient due to entropic reasons.[14a] This was, for instance, demonstrated for agents blocking virus-[26] and selectin-binding[27] and inhibitors of several bacterial toxins.[28] As a result, multivalent drugs can be administered in smaller doses compared to the respective monovalent agents with similar efficacy. In addition, enhanced affinity of multivalent ligands results from steric stabilization, which is primarily related to large particles bound to a biological surface.[26c, 29] In this motif, the attachment of a large multivalent species to the surface sterically shields the receptor from reaching further ligands without actually binding to all receptor sites.

Since polymeric architectures can combine the above-mentioned features in one scaffold, they are interesting platforms for the development of synthetic multivalent binders. However, the choice of the polymer backbone is highly critical in this context, as it contributes to the overall properties of the compound such as the viscosity and solubility, which is, for instance, influenced by the size and molecular weight,[30]

shape,[31] and polydispersity index (PDI)[32] of the polymer. The application of linear, random-coil architectures can lead to highly viscous solutions, whereas the impact of spherical agents (e.g. hyperbranched or dendritic polymers) on the viscosity is negligible. Moreover, rigid scaffolds are often poorly water soluble compared to more flexible architectures, which makes them less worthwhile for *in vivo* applications.[17]

One of the most advanced multivalent systems so far is a poly-L-Lysine (PLL) (**Figure 3**) dendrimer of the 4th generation covered with naphthalene disulfonate, which is known as VivaGel® (SPL7013). This polymer is currently in clinical development as a topical vaginal gel for the prevention of the sexual transmission of HIV-1.[33] However, clinical trials of structurally related compounds were stopped at phase III due to their limited efficacy and observed higher infection rates in some cases.[34] Hence, the clinical outcome of VivaGel® must be awaited.

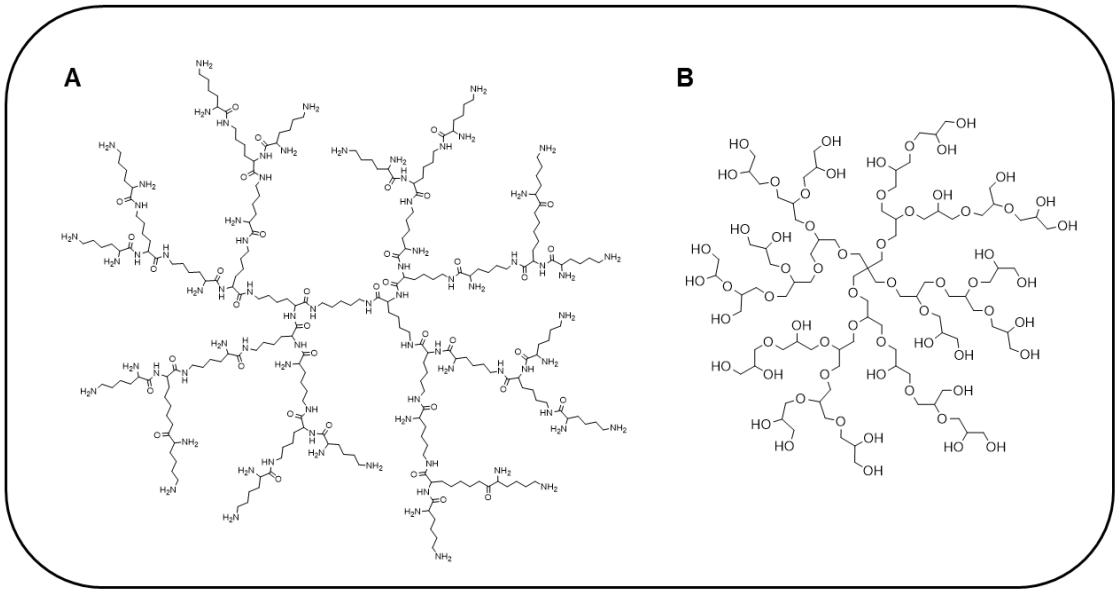

Figure 3. Chemical structures of (a) poly-L-lysine (PLL) and (b) dendritic polyglycerol (dPG).

Other highly potent antiviral agents have been derived from dendritic polyglycerol (dPG). Initially introduced in 1999 by Sunder et al., dPG can be prepared in one step by an anionic, ring-opening multibranching polymerization (ROMBP) of glycidol on the multigram scale.[35] By slow monomer addition on a partially deprotonated polyvalent starter, dPGs can be synthesized with defined molecular weights and degrees of branching on a large scale.[36] Moreover, morphologies can be modulated for the respective application by preparing different architectures like self-assembled supramolecular structures[37] or nanogels,[38] while surface charges can be controlled simultaneously by conjugating with anionic[39] or cationic[40] groups in order to obtain multivalent binding sites. Glycoarchitectures and anionic derivatives are particularly

promising as anti-viral or anti-inflammatory agents. In recent studies, Papp et al. designed sialic acid-modified nanogels derived from dendritic polyglycerol (dPG) with sizes between 3 – 50 nm. Larger particles with comparable degrees of functionalization of sialic acid showed a drastically increased inhibitory activity against influenza virus, although the ligand spacing was not adjusted to the structure of the receptor.[26f] By conjugating galactose moieties onto the dPG scaffold in order to mimic naturally occurring selectin ligands, potent selectin binding inhibitors have been developed. The multivalent display of the carbohydrate moieties resulted in significantly higher binding affinities toward L-, P-, and E-selectin in a competitive *in vitro* assay, compared to an analogous galactose tetramer of pentaerythritol.[27e] Additional sulfation of the sugar moieties gave even lower IC_{50} values up to the nanomolar range. IC_{50} values in this study were determined in a concentration dependent, competitive surface plasmon resonance (SPR)-based binding assay (**Figure 4**), which was established by Enders and co-workers.[27c] In this assay, selectin-IgG chimeras were immobilized on gold nanoparticles in order to mimic leukocytes, whereas the minimal motifs of selectin ligands were coupled to the surface of a BIAcore sensor chip that represented the endothelium. L-selectin-coated Au nanoparticles were then passed over the sensor chip, which resulted in a binding signal that was set to 100 % and served as reference. Pre-incubation of the Au nanoparticles with a potential inhibitor perhaps led to a reduced binding signal that was dependent on the inhibitor concentration. IC_{50} values related to a concentration that caused 50 % reduction of binding.

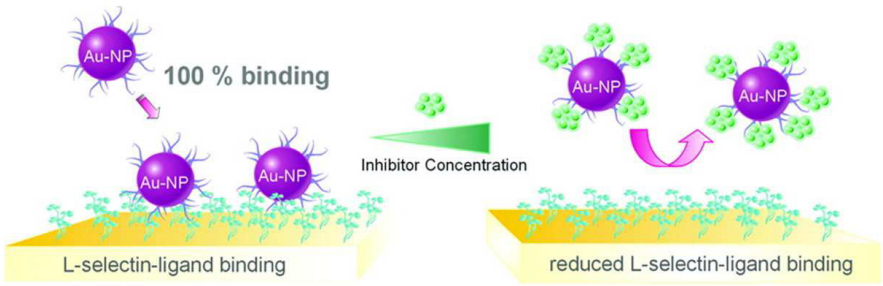

Figure 4. Schematic illustration of the surface plasmon resonance (SPR)-based concentration dependent competitive L-selectin binding assay. Adapted with permission from American Chemical Society: Weinhart et al., *Biomacromolecules*[39] Copyright 2011.

Along with its high biocompatibility[41] and the manifold options of modification, the dPG scaffold is a powerful platform for developing multivalent nanosystems for therapeutic and diagnostic applications with a high target specificity. The following chapters will describe strategies for achieving a higher selectivity and specificity toward bone, cartilage, and inflammation.

1.3 Inflammation

Inflammation is an essential part of the innate immune response, triggered by microbial infections or physical injuries, in order to remove harmful stimuli such as toxins or pathogens and initiate wound healing.[42] Acute inflammation is a time-limited process characterized by redness (*rubor*) due to an increased blood flow, swelling (*tumor*) caused by an increased vascular permeability and leaking of plasma fluids, heat (*calor*) as a result of vasodilation and the metabolic activity of inflammatory mediators, pain (*dolor*) due to perivascular changes and the release of pain transmitters, and loss of function (*functio laesa*), which has multiple causes.[23, 43] A notable progress has been made to understand the molecular and cellular mechanisms involved in the acute inflammatory response and has been characterized best for bacterial infections.[42] The first step within this complex process is the coordinated recruitment of leukocytes out of the blood vessel to the area of injury, which proceeds in a cascade like fashion (**Figure 5**).[22]

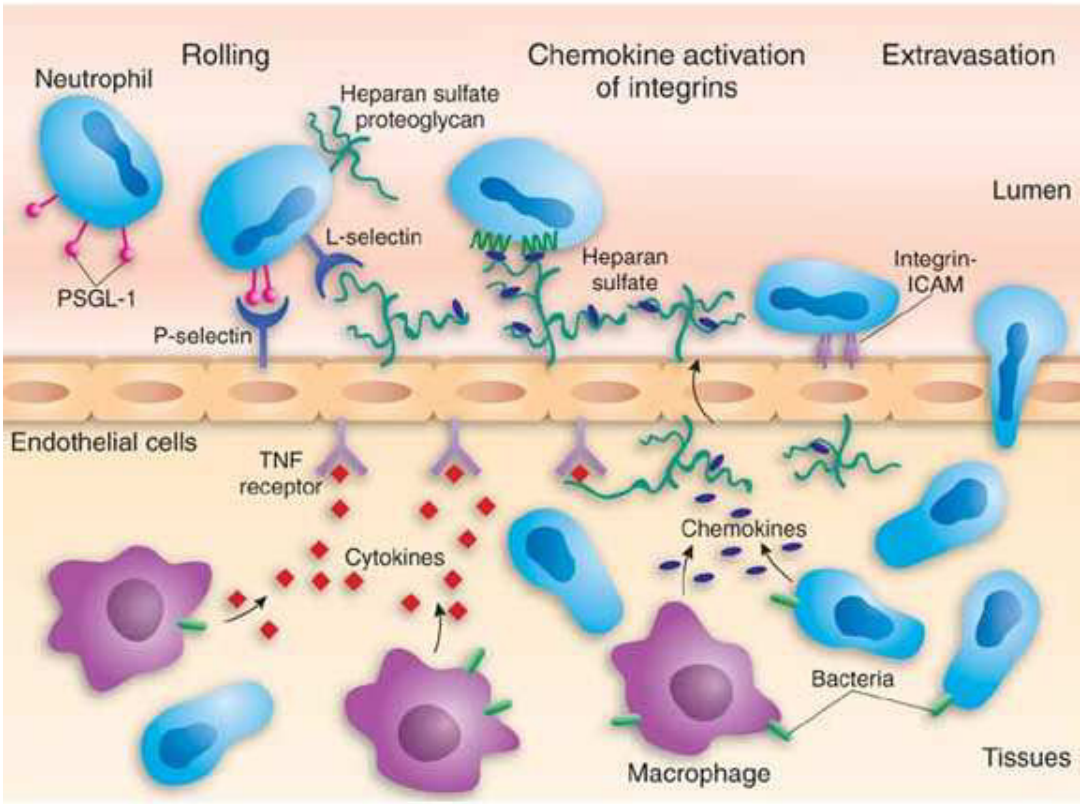

Figure 5. Schematic illustration of the mechanism of the leukocyte-adhesion cascade in inflammatory processes. Reversible interactions of selectins with P-selectin glycoprotein ligand-1 (PSGL-1) mediate tethering of circulating leukocytes and rolling on the activated endothelium. Endothelium-bound chemokines initiate the activation of integrins on the leukocyte surface to trigger decelerated rolling and firm adhesion. Signaling factors direct the extravasation to the site of inflammation through a chemoattractant gradient. Pathogens are eliminated upon recognition by antibodies or other complement components. Adapted with permission from Nature Publishing Group: Parish, *Nat. Immunol.*[44] Copyright 2005.

The initial recognition of tissue damage by infections or mechanical injuries is mediated by activated tissue-resident immune cells like macrophages, dendritic cells, and mast cells, which induce the production of pro-inflammatory cytokines, chemokines, and other chemoattractants.[42, 45] By binding to their respective receptors, cytokines like Interleukin-1 (IL-1) and tumor necrosis factor α (TNFα) initiate the upregulation of CAMs such as selectins through signal transduction.[22, 44] Selectins are transmembrane glycoproteins expressed on leukocytes (L-selectin), activated platelets (P-selectin), and inflamed endothelial cells (E-selectin).[22] The transient binding to their glycosylated ligands triggers the tethering of circulating leukocytes and subsequent rolling. In addition, endothelium-bound chemokines activate integrins on the leukocyte surface to induce decelerated rolling and firm adhesion by binding to CAMs of the immunoglobulin superfamily such as intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1).[22, 46] Finally, adherent leukocytes transmigrate via a para- or transcellular pathway through the endothelium of the blood vessel and are directed to the source of inflammation through sensing of a chemotactic gradient, which is generated by activated host cells within the inflamed tissue.[22, 47] Upon recognition by opsonization by antibodies or complement components, pathogens are eliminated by phagocytosis in order to repair the damaged tissue.[42, 47] Involved in the increased permeability of blood capillaries, chemotaxis of leukocytes, and opsonization of microorganisms, for instance, the complement system is one of the key mediators of inflammation.[48] The complement can be activated by the classical, alternative, and lectin pathway and encompasses > 30 plasma proteins, which serve as cellular receptors or regulation proteins to release bioactive pro-inflammatory anaphylatoxins like C3a and C5a or form the membrane attack complex (MAC) to induce the lysis of pathogens.[48-49]

However, if the time-limited acute inflammatory response fails to accomplish the removal of the invading agent, inflammatory processes can persist, which leads to a prolonged extravasation of leukocytes and tissue damage due to the excessive release of reactive oxygen (ROS) and nitrogen species (RNS).[23, 42] In addition to pathogens, autoimmune reactions can also result in chronic disorders like osteoporosis, diabetes type II, and rheumatoid arthritis, although the underlying causes are still poorly understood.[42-43]

1.4 Targeting Inflammation

Due to their involvement in the molecular mechanisms of inflammation, the targeting of CAMs, pro-inflammatory cytokines, and complement factors has become a promising approach to prevent the ongoing tissue destruction in chronic inflammatory diseases. However, a permanent suppression of the immune response should not be the goal for long-term treatment but is indeed useful for short-term therapy. Especially the development of selectin inhibitors has become a promising approach for anti-inflammatory agents, which is related to their essential role in leukocyte recruitment and development of metastases in cancer.[50]

The most important and best characterized, naturally occurring selectin ligand is the P-selectin glycoprotein ligand-1 (PSGL-1), which shows a high affinity towards all three selectins.[51] The interaction of PSGL-1 with L-selectin, for instance, has been reported to account for 60 % to 70 % of all recruited leukocytes in vitro by a process known as secondary tethering, in which free flowing leukocytes are captured by activated white blood cells rolling on the endothelium.[51b, 52] PSGL-1 is a homodimeric transmembrane protein present on leukocytes and activated endothelial cells, that contains sulfated tyrosine residues at the N-terminus and several N- or O-linked carbohydrates like sialylated and nonsialylated Lewis^X (sLe^x, Le^x) and Lewis^a (sLe^a, Le^a) saccharides.[50a, 53]

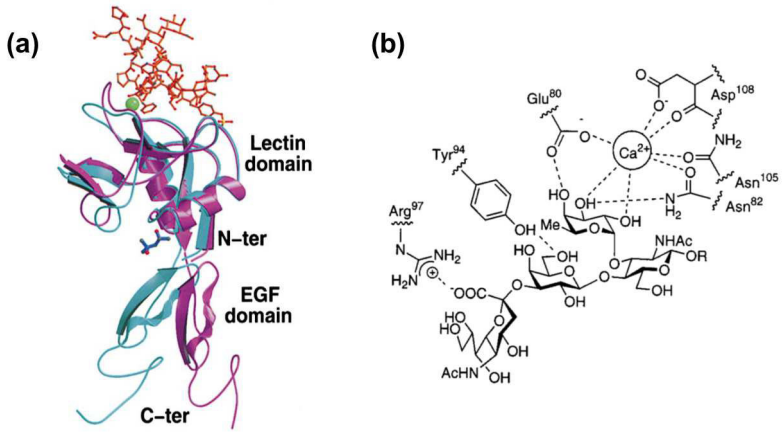

Figure 6. (a) A ribbon-stick representation of the postulated interaction of P-selectin with a glycosulfopeptide (SGP-3), structurally similar to P-selectin glycoprotein ligand-1 (PSGL-1). SGP-3 is depicted in orange, the structure of complexed P-selectin is shown in purple, that of unbound P-selectin in blue, and the calcium anion in green. Adapted with permission from Cell Press: Somers et al., *Cell*,[54] Copyright 2000. (b) Hypothesized interaction of E-selectin with sLe^x including Ca^{2+} coordination, hydrogen bonding to tyrosine, acid and amino acid chains, and ion pairing with arginine. Adapted with permission from American Chemical Society: Simanek et al., *Chem. Rev.*,[53] Copyright 1998.

The recognition of sLe^x by terminal calcium-dependent (C-type) lectin domains of selectins (**Figure 6**) is considered to be responsible for leukocyte adhesion, as the

carbohydrate was found to be the minimal structural motif for all known selectin specific ligands.[50a, 51a, 53] However, an additional binding to sulfated tyrosine residues on the sugar moiety was found to be critical for L- and P-selectin, whereas the affinity of E-selectin was not affected by sulfation of the ligand.[51a, 55] Crystal structure analysis with P-selectin revealed its binding to sulfated tyrosine by contacting arginine (Arg85) and histidine (His114) residues of the lectin domain.[54]

With elucidation of the molecular mechanisms involved in these binding events, various synthetic oligosaccharides based on sLe^x have been investigated for their application as selectin inhibitors.[55] However, the use of the carbohydrate itself as anti-inflammatory drug failed in clinical trials, probably due to its low enzymatic stability, poor bioavailability, and low biological affinity toward selectins.[56] K_d values for the interaction of sLe^x with E- and P-selectin were reported to be 0.5 mM and 8 mM[57] respectively, compared to PSGL-1, which shows a K_d of 320 nM[58] for binding to P-selectin. The substitution of sugar moieties and modification of peptide chains did not substantially improve selectin affinities of low molecular weight compounds, whereas the multivalent presentation of sLe^x and its mimetics on liposomes, for instance, led to a 100-fold increased binding compared to the monomeric ligand.[49-50] In a competitive selectin inhibition assay with polymerized liposomes containing sLe^x-like sugar moieties, Bruehl et al. revealed the strong influence of anionic groups on selectin binding.[59] Surface functionalization with multiple sulfate esters, in order to mimic the physiological ligands, resulted in a four orders of magnitude better L-, E-, and P-selectin inhibition compared to the neutral and cationic architectures. Interestingly, for sulfated liposomes containing no carbohydrates, a significantly reduced binding affinity was verified in case of L- and P-selectin, which demonstrates the crucial role of sulfates in the recruitment of leukocytes and hence inflammatory response.

In recent years various sulfated polysaccharides such as heparan sulfate, fucoidan, and dextrane sulfate (**Figure 7**) have been identified as potential selectin inhibitors.[60] With an average molecular weight of 30 kDa, heparan sulfate (HS) is a linear glycosaminoglycan (GAG) composed of disaccharide repeating units of uronic acid (glucuronic or iduronic) and glucosamine (**Figure 7**).[61] It is expressed on cell surfaces as well as extracellular matrices, and is able to transport pro-inflammatory cytokines trough the endothelium and present it to leukocytes.[44] In addition, by binding to a variety of different proteins, HS participates in various physiological processes like lipid metabolism, growth factor regulation, and blood coagulation.[62] The latter results from its interaction with antithrombin III leading to a conformational change, which mediates the inhibition of thrombin and proteases factor Xa.[61] Today, heparin, a structurally related GAG (**Figure 7**), is the standard anti-coagulant for prevention of thrombosis.[63]

However, due to its non-human origin and possible severe side effects such as bleeding and heparin-induced thrombocytopenia (HIT) after intravenous (i.v.) administration, there is still an ongoing need for synthetic mimetics with comparable properties and less side effects.[61, 64]

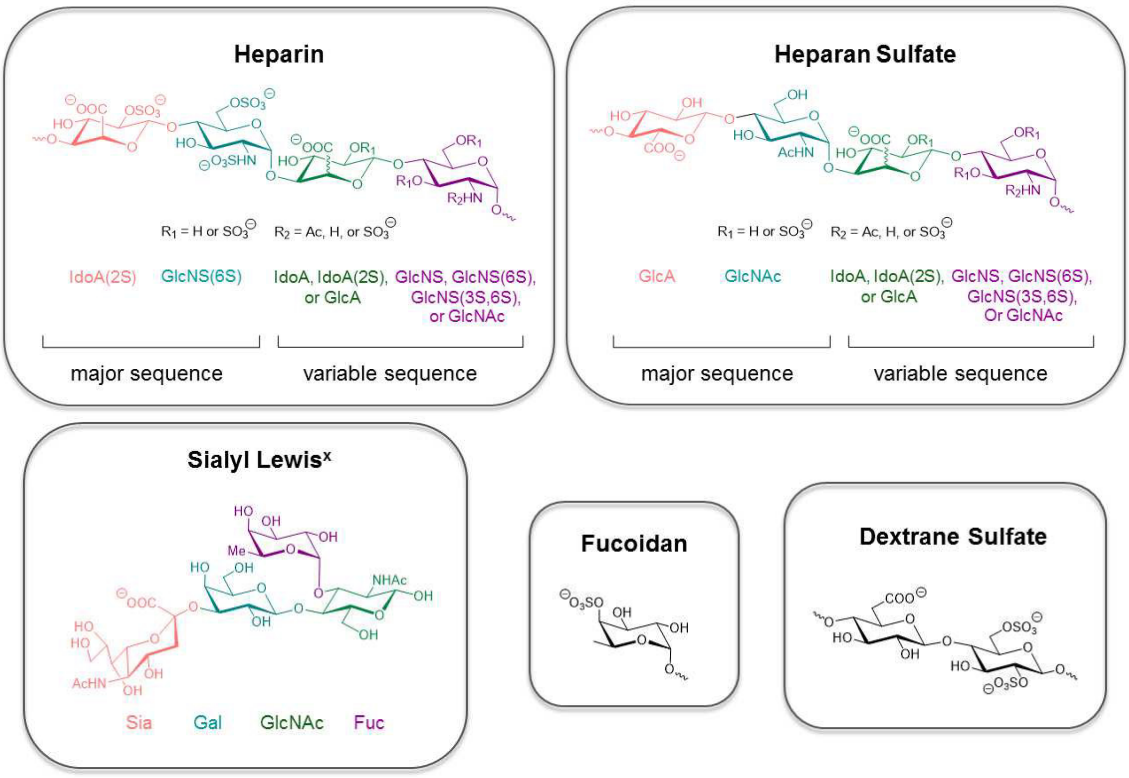

Figure 7. Chemical structure of different carbohydrate-based L- and P-selectin inhibitors.

One approach to a fully synthetic heparin analog was developed by Türk et al., which combined the anti-inflammatory properties of polysulfates with the high biocompatibility of dPG.[41, 64-65] This sulfated compound efficiently inhibited the complement activation and exhibited a much lower anticoagulant effect in vitro compared to heparin.[64] Recent studies revealed multiple targets of dendritic polyglycerol sulfate (dPGS) within the inflammatory response (**Figure 8**). In a competitive, concentration-dependent surface plasmon resonance (SPR)-based binding assay, a strong affinity to L- and P-selectin was demonstrated, which increased with size and surface charge.[66] Moreover, by binding the complement factors C3 and C5, dPGS suppresses release of the anaphylatoxin C5a and inhibits the activation of the complement system.[66a] In addition to further in vitro experiments,[39, 67] dPGS and its derivatives have also been applied in vivo either as therapeutic agents, e.g. in a contact dermatitis model,[66a] or as a diagnostic tool for allergic asthma,[68] rheumatoid arthritis,[69] and mammary carcinoma,[70] which clearly demonstrates its potential as a drug candidate for inflammation-related diseases. However, recent biodistribution

studies with radiolabeled dPGS amine revealed its accumulation in liver and spleen in contrast to neutral dPG, which was observed even 21 days post i.v. injection and hampers its use for treatment of patients.[71] As the anionic polymer was found to bind to a variety of blood proteins,[72] the formation of aggregates and a protein corona, which increases the hydrodynamic diameter of the compound and results in its recognition by the reticuloendothelial system (RES), is suggested. Although, the exact coherences are still not fully understood. In order to unravel the underlying mechanisms, which lead to organ accumulation, more information about the structure-activity relationship are needed. Investigating the influence of size and charge on the biodistribution, metabolism, and excretion pathway might be useful to fine tune physiochemical properties of the respective polymer for in vivo applications.

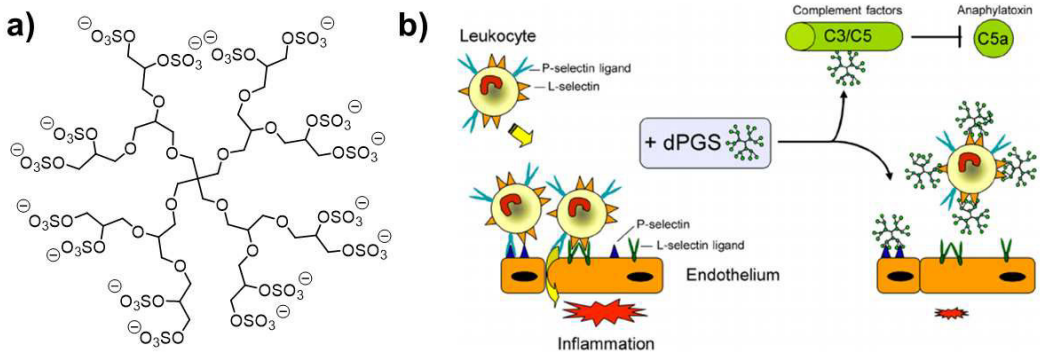

Figure 8. (a) Idealized chemical structure of a small dendritic polyglycerol sulfate (dPGS) with a pentaerythritol starter. Sodium cations are not shown here for clarity reasons. (b) Schematic illustration of the anti-inflammatory effect of dPGS by simultaneous binding of L- and P-selectin and complement factors C3 and C5. The former prevents leukocyte extravasation to the site of inflammation, the latter suppresses the release of the pro-inflammatory anaphylatoxin C5a and inhibits the activation of the complement system. Adapted with permission from National Academy of Sciences: Dernedde et al., *Proc. Natl. Acad. Sci.*,[66a] Copyright 2010.

In order to avoid the observed enrichment in organs of the RES, two approaches are feasible: the application of dPGS with a lower degree of functionalization or cleavable sulfated architectures. However, within the first, a reduced selectin binding, due to a lower amount of sulfate groups, is expected.[66b] In contrast, by implementation of degradable linkers into a neutral dPG scaffold and subsequent sulfation, anti-inflammatory properties can be obtained along with an enhanced renal clearance. Besides, also core cleavable architectures based on other polymers, such as Boltorn type polyesters, are conceivable. Hence, one goal of this thesis was to develop shell and core cleavable dPGS analogs with distinct degradation profiles and to evaluate their anti-coagulant and anti-inflammatory properties including L-selectin binding and inhibition of the complement activation.

1.5 Bone

Bone is a unique connective tissue which, is the main component of the skeletal system in all higher vertebrates and one of the best characterized biological materials.[73] Bone has several mechanical and metabolic functions: it provides structural support to the body, protects various organs from injury, enables movement by muscle attachment, produces red and white blood cells in the bone marrow, stores a high amount of inorganic minerals, and is actively involved in calcium and phosphate homeostasis.[73-74] It shows a complex hierarchal structure with remarkable mechanical properties due to the composition of the extracellular matrix (**Figure 9**). The combination of stiff and brittle hydroxyapatite (HA) nanoparticles and elastic collagen I fibrils simultaneously leads to rigidity and high resistance against fracture.[75] The adult human skeleton comprises 206 different bones that vary in size, shape, and mechanical features, depending on their function and position within the body.[76] Ear bones, for instance, are porous, highly mineralized, and stiff for acoustical reasons, whereas the skull is more tough and dense in order to prevent the brain from external harm.[77] Long bones such as the femur are characterized by a dense outer layer and a spongy interior.[75] The hard outer shell is formed by cortical (compact) bone, which accounts for 80 % of the bone mass in the human skeleton.[78] The porosity of cortical bone of < 5 % is related to the presence of marrow cavities and blood vessels but can increase up to 30 % depending on the proceeding remodeling processes.[79]

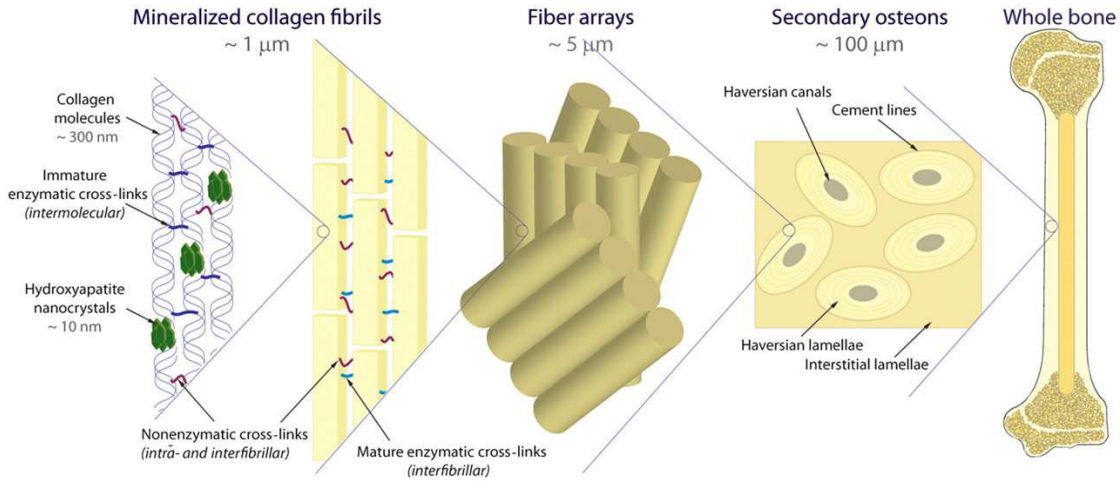

Figure 9. Schematic representation of the hierarchical structure of cortical bone in the human skeleton. Stabilized by cross-links and hydroxyapatite nanoparticles, collagen fibrils self-assemble to lamellae fiber arrays to form osteons. Concentrically surrounded by lamellae, Haversian canals are present at the center of the osteon, which contain blood vessels and nerves. At the outer boundary, the highly mineralized cement line separates the osteon from the surrounding tissue. Adapted with permission from National Academy of Sciences: Zimmermann et al., *Proc. Natl. Acad. Sci.*,[80] Copyright 2011.

In cortical bone, mineralized collagen fibrils are often self-assembled in a twisted plywood-like manner to form cylindrical structures with a diameter of around 200 µm, called osteons.[75, 81] At the center of each osteon, a channel that contains blood vessels and nerves, known as Haversian canal, is concentrically surrounded by lamellae.[81] Although present in all larger mammals, Harversian systems are missing in smaller animals like mice due to the lower thickness of the cortical bone.[82] At the outer limits, a 5 µm thick highly mineralized boundary termed cement line, separates the osteon from the surrounding tissue.[81] Except at joints where articular cartilage is present, the outer surface of cortical bone is covered by the periosteum, a fibrous connective tissue that contains blood vessels as well as nerve fibers and plays a crucial role in bone growth and fracture repair.[78] Perpendicular to the central canal, perforating Volkmann's canals enable the communication of Haversian canals with each other and the periosteum and thereby ensure the nutrition of the osteon.[83] The inner surface of cortical bone is covered by the endosteum, a membranous structure which is in contact with the bone marrow space and represents the boundary to trabecular bone.[78] Trabecular or cancellous bone is a highly porous network of lamellar bone, showing a rod- and plate-like structure with a pore size of up to 1 mm.[84] In cancellous bone only around 20 % of the volume is made up of bone, while the rest is filled with bone marrow and hematopoietic stem cells, which can differentiate to red and white blood cells.[74-75]

Bone is a highly dynamic living tissue as it undergoes permanent remodeling processes, longitudinal growth at the growth plate, and adapts to external stimuli in order to improve its functionality.[77-78, 85] Remodeling is a beneficial feature to remove dead bone, change the grain to alter mechanical properties, heal micro fractures, and improve blood supply.[77] These processes are regulated by the interplay of bone-resorbing osteoclasts and bone-forming osteoblasts (**Figure 10**).[85] During resorption multinucleated osteoclasts form a sealing zone also known as Howship's lacunae and release hydrogen ions via H-ATPase proton pumps and chloride channels through the ruffled cell membrane.[78, 86] This process leads to a reduced pH of 4 – 4.5 within the pit, by which HA nanoparticles are dissolved. Moreover, various enzymes such as cathepsin K, matrix metallo-proteinase 9, gelatinase from cytoplasmic lysosomes, and tartrate-resistant acid phosphatase (TRAP) are secreted in order to degrade the organic matrix.[78] Afterwards, osteoclasts move away from the resorption site and either undergo fission into mononuclear cells or apoptosis.[78, 85] Osteoblasts then fill the cavity with a new lamellar bone matrix, which is primarily composed of collagen I and termed osteoid.[85] By the secretion of membrane-bound matrix vesicles, containing phosphate and calcium ions, mineralization of the osteoid is induced.[78] In cortical bone

this process leads to the formation of new Haversian systems called secondary osteons.[87] During deposition, osteoblasts are surrounded by the bone matrix and become osteocytes, which reside inside lacunas and communicate with each other and cells on the bone surface through a canalicular network and gap junctions.[88] Osteocytes are known to be involved in bone remodeling processes by sensing strain and stress signals and the release of proteins such as sclerostin, fibroblast growth factor 23 (FGF-23), and receptor activator of nuclear factor kappa-B ligand (RANKL), which stimulate bone resorption and formation as well as homeostasis of minerals.[89] Maintenance of the bone mass density and microstructure highly depends upon the balance of anabolic and catabolic processes.[90] With aging and in diseases like rheumatoid arthritis, osteoporosis, bone metastasis, or Paget's disease this balance is altered, favoring either bone formation or resorption. Osteoblasts and osteoclasts have therefore become the main targets for the treatment of malfunctioning bone to improve its biomechanical properties.

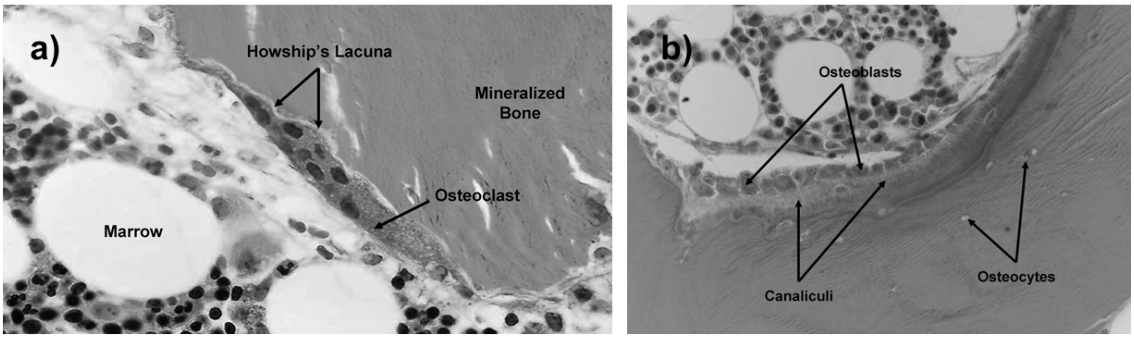

Figure 10. The different cell types within bone. (a) Osteoclasts resorb bone and create a cavity at resorption site known as Howship's lacunae. (b) Osteoblasts secrete a new bone matrix at the resorption pit called osteoid, which is then mineralized. During deposition cells get buried in the matrix and become osteocytes, which reside inside the bone and communicate through a canalicular network. Adapted with permission from American Society of Nephrology: Clarke, *Clin. J. Am. Soc. Nephrol.*,[78] Copyright 2008.

1.6 Targeting Bone

With growing knowledge about bone biology, numerous bone-specific therapeutic agents have been developed in recent years, targeting either the metabolic activity of osteoblasts and osteoclasts, or the inorganic part of the bone by chelation of calcium ions (**Figure 11**).[90] Initially introduced as an antibiotic, tetracycline exhibits a strong affinity to growing bone and dentine as evident from its properties to stain the teeth of children yellow and inhibit the skeletal growth.[86] Due to its strong binding of HA, tetracycline derivatives were conjugated as targeting ligand to various hormones such as β-Estradiol[91] and 3-Estron[92] for instance. However, the complex chemical structure as well as the low stability during modifications limits its application as a targeting moiety.[73] Based on the amino acid structure of osteopontin, sialoprotein, and other calcium binding proteins, acidic oligopeptides derived from aspartic and glutamic acid were developed due to their strong affinity to HA.[93]

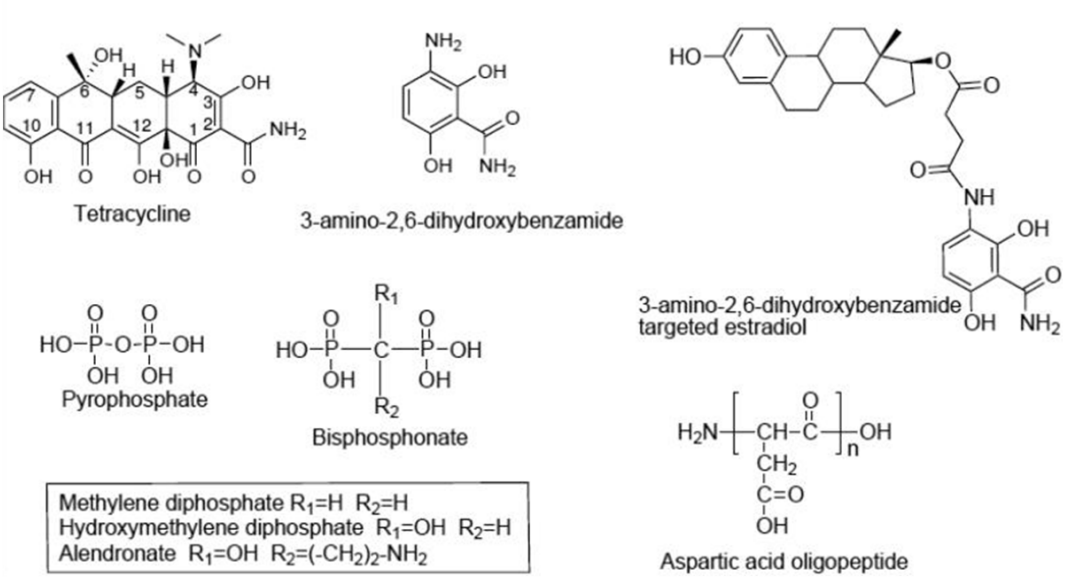

Figure 11. Overview of common bone targeting moieties and their chemical structures. Adapted with permission from Elsevier: Low et al., *Adv. Drug Delivery Rev.*,[86] Copyright 2012.

Sekido et al. demonstrated an optimal binding for hexapeptides of glutamic and aspartic acid, whereas an increased chain length did not result in an enhanced affinity.[93d] Since molecules of low molecular weight often exhibit short circulation half-lives and the incorporation of multiple targeting ligands could increase the therapeutic effect if loaded with drugs, high molecular weight compounds of oligopeptides have been developed.[86] Copolymers of aspartic acid octapeptides and HPMA or polyethylene glycol (PEG), for example, showed a prolonged blood circulation time and strong bone-targeting properties.[94]

However, bisphosphonates (BPs) still represent the predominant group of bone targeting moieties, which can be classified into highly potent nitrogen-containing agents and less effective nitrogen-free compounds.[90] Former BPs like risendronate, ibandronate, and alendronate disrupt the mevalonic acid pathway by inhibiting the farnesyl pyrophosphate synthesis, which causes either a functional loss or apoptosis of osteoclasts.[95] In contrast, nitrogen-free BPs like clodronate and etidronate act as non-hydrolyzable adenosine triphosphate (ATP) analogs, which are incorporated by osteoclasts and lead to apoptosis and reduced bone turnover.[96] In addition to their interaction with bone-resorbing cells, BPs have also been reported to be uptaken by osteoblasts, influencing the proliferation of the cells.[95c, 97] However, their strong affinity to bone is mainly related to their complexation of calcium cations.[98] In recent years, particularly bisphosphonates of low molecular weights have been clinically approved for the treatment of osteoporosis, myeloma, osteogenesis imperfecta, or Paget's disease (**Figure 12**).[99] Other therapeutic approaches combine BPs with protein-based drugs, selective estrogen receptor modulators (SERMs), or cytostatic agents.[73, 90, 100]

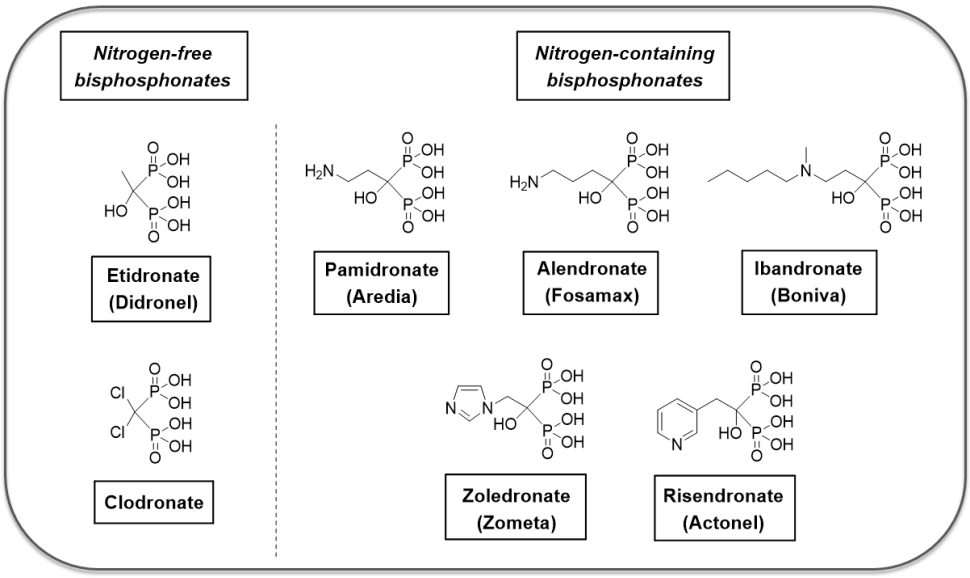

Figure 12. Chemical structures of clinically approved low-molecular weight bisphosphonates, classified into nitrogen-containing and nitrogen-free compounds. Modified with permission from American Academy of Pediatrics: Graham et al., *Pediatrics*,[98] Copyright 2007.

Moreover, bisphosphonate groups have also been introduced as targeting ligands for diagnostic applications in order to visualize metabolically active osteoclasts or image bone abnormalities such as osteogenic sarcoma and bone metastases.[101] Nevertheless, when applied in vivo, low molecular weight BP compounds are known to cause severe problems like osteonecrosis of the jaw, esophageal cancer, or atrial fibrillation.[102] In this context, polymeric architectures have gained an increasing

amount of interest as bone-specific drug delivery systems, as they offer several advantages: They potentially maximize the local therapeutic effect by carrying the drug to the specific destination if functionalized with targeting ligands, enhance the solubility of hydrophobic agents, increase the circulation half-life of the drug, and protect it from destruction by body components or fluids along with variable drug concentrations, which can be adjusted for the respective application.[103] In recent years, a variety of synthetic polymers has been functionalized with alendronate or other BPs and utilized for the targeted delivery of bone morphogenetic proteins, hormones, and further therapeutics. These scaffolds include PEG,[104] HPMA,[105] Boltorn-type polyester,[106] PLGA,[107] and liposomes[108] as well as copolymers of PLGA and PEG[109] (**Figure 13**).

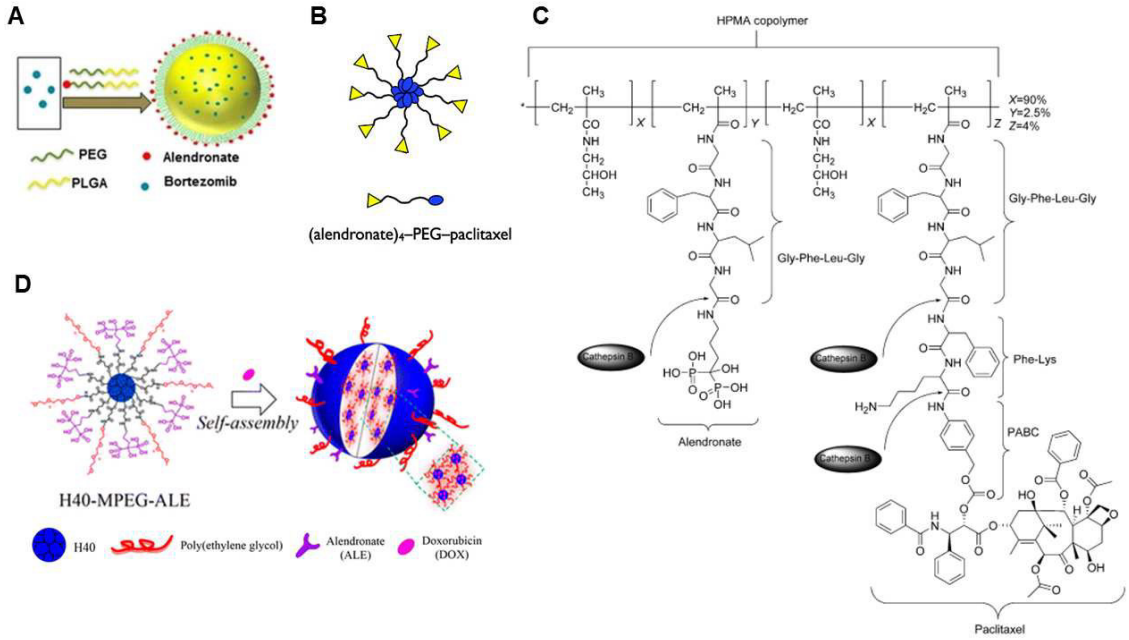

Figure 13. Overview of alendronate-functionalized bone-specific drug delivery systems derived from polymeric scaffolds. (a) Schematic illustration of alendronate containing poly(lactide-co-glycolide) (PLGA) nanoparticles functionalized with polyethylene glycol (PEG) and loaded with the therapeutic agent Bortezomib. Modified with permission from National Academy of Sciences: Swami et al., *Proc. Natl. Acad. Sci.*,[109] Copyright 2014. (b) Schematic illustration of alendronate and Paclitaxel conjugated PEG, which self-assembles to bone-specific micelles. Modified with permission from American Chemical Society: Clementi et al., *Mol. Pharmaceuticals*,[104] Copyright 2011. (c) Chemical structure of a cathepsin B cleavable poly(2-hydroxypropyl methacrylate) (HPMA)/ peptide copolymer functionalized with paclitaxel and alendronate. Adapted with permission from Wiley: Miller et al., *Angew. Chem. Int. Ed.*,[105b] Copyright 2009. (d) Micelles composed of Boltorn™-type polyester (H40) and PEG functionalized with alendronate and loaded with doxorubicin. Modified with permission from American Chemical Society: Chen et al., *Bioconjugate Chem.*,[106] Copyright 2012.

However, despite their high potential as therapeutic agents for the treatment of bone related diseases, a bone-specific synthetic polymer is still not available on the market

due to high production costs, insufficient in vivo studies, poor target specificity, or the associated toxicity of BPs.[86, 110] One promising approach to overcome these limitations would be to introduce anionic groups other than bisphosphonates into a highly biocompatible polymeric scaffold such as dendritic polyglycerol, which may cause less toxic effects. Moreover, the presentation of multiple anionic groups on the surface of a polymer could increase its target specificity by multivalent binding. In order to demonstrate the target specificity of the respective compound, the incorporation of fluorescent dyes is vital. Besides, a combination of different imaging techniques such as confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM) is a conceivable method to verify the binding to mineralized compartments. However, bone-targeting moieties that are alternative to BP first have to be identified and the respective polyanion should be examined concerning its biocompatibility and toxicity in vitro. When it comes to in vivo applications, not only the targeting moiety itself but also the amount of ligands on the polymer plays a crucial role for biodistribution and off-target side effects, which would have to be adjusted for the respective purpose. Since bone specificity can also vary on the tissue's condition, compounds should also be investigated in bone-related diseases like rheumatoid arthritis.

1.7 Articular Cartilage

Cartilage is a highly specialized connective tissue that is found in various areas of the body and exhibits different functions related to its mechanical and biomechanical properties. In the nose and ear, for example, cartilage provides structural support of the surrounding tissue in order to maintain the shape of the organ, facilitate breathing, or collect sounds, whereas tracheal cartilage prevents organs from collapsing during inhalation, and articular cartilage (AC) withstands compressive, tensile, and shear stress during joint motion.[111] In contrast to bone, cartilage exhibits only a low potential of regeneration due to its avascular, alymphatic, and non-innervated extracellular matrix, which is sequestered by chondrocytes.[112] The cells are usually supplied with nutrients by diffusion via the perichondrium or in case of AC via the synovial fluid in the joint cavity, produced by the synovial membrane.[113] Although chondrocytes usually do not divide in mature cartilage, they play a crucial role in regulating the formation and resorption of the ECM.[114] Related to an anoxic environment, they undergo metabolic processes through anaerobic glycolysis and create a dense network of mainly collagen II fibers, in which glycoproteins, noncollagenous proteins, and anionic proteoglycans like aggrecan are embedded.[114-115] Depending on the composition of these components as well as the amount of resident cells, cartilage can be classified into three major types: elastic cartilage, which is found in the auricle and epiglottis, fibrous cartilage, which is present in the meniscus and annulus fibrosus, and hyaline cartilage, which is the most abundant type and appears in the nose, trachea, and nucleus pulposus.[111] The latter is also found in AC, which covers the end of long bones and forms the frictionless and smooth surface of diarthrodial joints.[116] The extracellular matrix of AC can be divided into several layers, based on the type and arrangement of collagen fibers, shape and activity of cells, and content of water and proteoglycans (**Figure 14**).[115]

In contact with the synovial fluid, the acellular superficial zone represents the uppermost surface, which contains a low amount of proteoglycans and a dense mesh of fine collagen fibrils parallel to the joint surface including type I, II, and III.[115] The subjacent deepest zone additionally comprises chondrocytes of a round morphology, which produce the superficial zone protein (SZP), a large proteoglycan that functions as lubricant after secretion into the synovial cavity.[115, 117] In the underlying transitional area, ellipsoidal chondrocytes form a poorly organized network of covalently bound type II, VI, IX and XI collagen fibers, which is less hydrated and less dense than the articular surface.[115, 118] The role of collagen type VI is not yet fully understood but probably ensures matrix attachment of surrounded chondrocytes by binding to type II collagen.[118-119] The deep radial zone consists of collagen type II, IX, and XI fibers,

which are organized perpendicular to the joint surface with chondrocytes stacked and arranged in radial columns along those fibers.[115] The deep zone is separated from the calcified layer by an undulating basophilic line called tidemark.[120] With a thickness of around 5 µm, it represents the boundary between calcified and uncalcified cartilage and is visible in histochemical stainings using hematoxylin and eosin.[121] The tidemark is assumed to be a highly dynamic interface, which can undergo molecular changes and remodeling processes as it contains enzymes like alkaline phosphatase and ATPase, as well as phospholipids based on calcium complexes.[122] The underlying calcified area of 20 µm to 250 µm thickness shows the highest proteoglycan concentration within cartilage and a vascularized structure.[115, 121] Hypertrophic chondrocytes produce entangled collagen type X fibers, which can be mineralized if anchored to the subchondral bone.[115] This semipermeable layer is also known as cartilage-bone interface and permits the diffusion of small molecules (< 500 Da) through the tissue.[123] Moreover, it keeps the structural integrity intact during movement, when tensile, compressive, and shear stress is transferred from the AC to the much stiffer bone.[121] However, little is known about the structure and composition of this area and its molecular modifications in malfunctioning joints.

	Collagens types	Proteoglycans content	Water content	Cell density and shape	Collagen fibers orientation
Surface area (superficial and deepest)	I, II, III			+ Circle	Parallel to the joint surface
Transitional area	II, IX, XI			++ Circle	Randomly organized
Deep area	II, IX, XI			+ Stack	Perpendicular to the joint surface
Calcified area	X			+ Hypertrophic	Entanglement
Subchondral bone	I and III				

Figure 14. Structure of articular cartilage, which is organized in four zones. The classification is based on the content of water and proteoglycans, type and orientation of collagen fibers, and activity, shape, and density of chondrocytes. The latter is denoted with + (moderate) and ++ (high). A histological section of articular cartilage of the knee stained with Alcian Blue and photographed under a light microscope is shown on the left. Adapted with permission from Elsevier Ltd.: Clouet et al. *Drug Discovery Today*,[115] Copyright 2009.

1.8 Targeting Cartilage

The selective targeting of cartilage is a promising approach for the treatment of arthritic diseases, which are characterized by the degradation of AC including surface roughening, appearance of cracks, and cell apoptosis (see Section 1.9).[115, 124] Current therapies mainly focus on pain relief by the administration of non-steroidal anti-inflammatory drugs (NSAIDs) or intra-articular injection of hyaluronic acid and corticosteroids while cartilage destruction continues.[115, 125] Conventional therapeutics are limited in their efficacy by a too fast clearance out of the joint or an inefficient drug delivery to the target tissue.[126] Systemically administered agents, for instance, have to cross blood vessels and pass the synovial membrane, which has a low permeability, in order to reach the joint cavity.[127] Once there, drugs are rapidly cleared into extra-synovial spaces, which results in poor cartilage penetration.[127b] Moreover, due to the avascular structure of cartilage, compounds can only enter the tissue by diffusion, which is aggravated by the dense ECM that acts like a filter for molecules bigger than ~ 6 nm.[118, 128] Recent studies with multiple drugs and formulations for intra-articular injections demonstrated a longer residence time in the synovial fluid of high molecular weight compounds compared to small particles (< 10 kDa).[129] The implementation of therapeutic drugs into polymeric scaffolds with enhanced cartilage specificity is a promising strategy to overcome short-term availability and rapid clearance and could enhance the efficacy of drug delivery systems. Since arthritic disorders already affect more than 200 million people all over the world[130] and our ageing population will worsen the problem, there is an obvious need for novel approaches to efficiently and selectively target cartilage in order to treat these diseases.

Due to the high amount of keratan sulfate, hyaluronic acid, and chondroitin sulfate within the ECM,[114] cationic compounds are the most widely used ligands for cartilage targeting (**Figure 15**). The binding affinity of quaternary amines to collagen type II and cartilage was initially reported in 1975 and was further investigated in the following decades for pyridinium derivatives.[131] Based on these results, radiolabeled amines were applied in vivo as radio diagnostics, which showed an improved enrichment in cartilage tissue.[132] Amine-functionalized tantalum oxide particles were used for the imaging of AC by x-ray computed tomography[133] and nitroxide functionalized poly(propyleneimine) and poly(amido-amine) (PAMAM) dendrimers of the third and fourth generation have been developed as MRI contrast agents.[134] For the optical imaging of cartilage destruction, dipicolyamine was coupled to a fluorescent near infrared (NIR) dye, whereas cationic fluorophores without further conjugation were applied in vivo as contrast agent.[135]

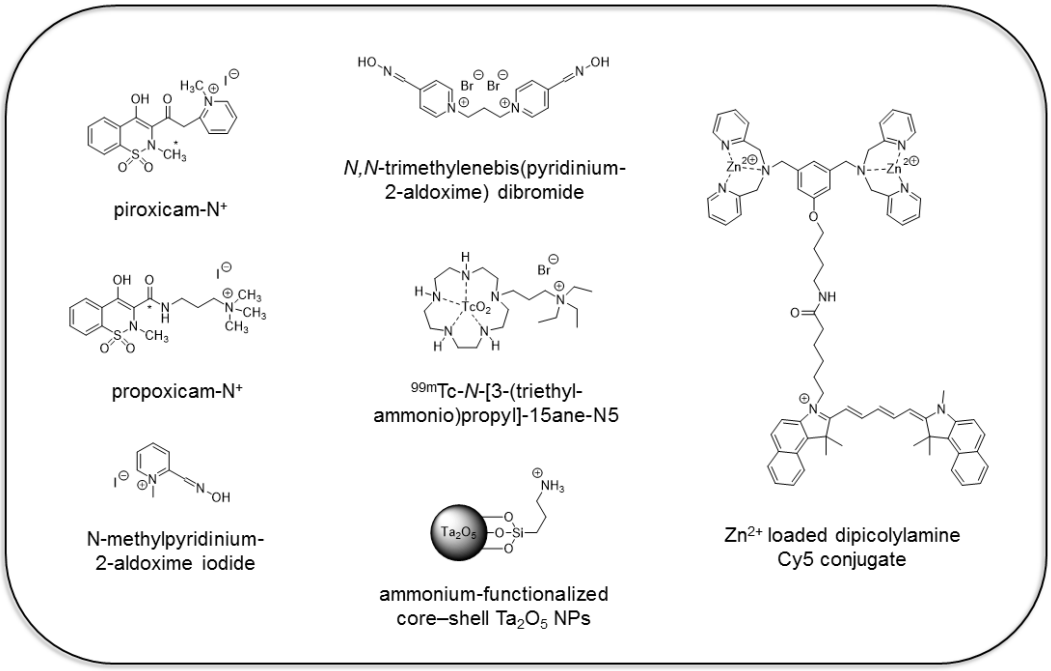

Figure 15. Overview of cationic compounds used for the imaging of cartilage tissue.

More sophisticated approaches utilize cartilage-specific peptide sequences (**Figure 16**).[136] WYRGRL, for instance, was identified as collagen type II-specific targeting ligand by a combined technique of phage display and affinity selection on sliced bovine cartilage.[136c] For poly(propylene sulfide) nanoparticles functionalized with this peptide, a size-dependent uptake into cartilage tissue was demonstrated.[136c] After intra-articular injection, particles with a size of 96 nm were exclusively found at the surface of the cartilage tissue, whereas smaller particles with a size of 38 nm entered the ECM and were uptaken by chondrocytes. Recently, Hu et al. conjugated the same peptide sequence along with pepstatin A to a DOTAM ligand in order to design a cartilage specific drug delivery system.[136d]

Jain and Bishnoi et al. applied a rather unusual approach for the delivery of therapeutic agents to cartilage tissue in a osteoarthritis rat model by utilizing chondroitin sulfate as the targeting unit on solid lipid nanoparticles (**Figure 16**).[137] Although anionic compounds are expected to have a lower efficacy in binding to cartilage due to their electrostatic repulsion by anionic components of the ECM, a significantly enhanced therapeutic effect in comparison to the free drug was observed. The higher efficacy of these particles is suggested to be a result of specific interactions with CD44, annexin, and leptin receptors expressed on chondrocytes and synovial fibroblasts.[137b, 138] The latter invade the ECM in inflammation-related joint diseases, which leads to cartilage destruction (see section 1.9). These results demonstrate that not only cationic but also anionic species can be used for cartilage targeting at least in

the diseased state since anionic proteoglycans might not be the only targets that can be addressed to achieve a higher affinity to the tissue. Especially in malfunctioning joints other binding partners might be present than in the healthy state, which emphasizes the need for more insights into molecular and cellular targets of applied compounds, in particular anions.

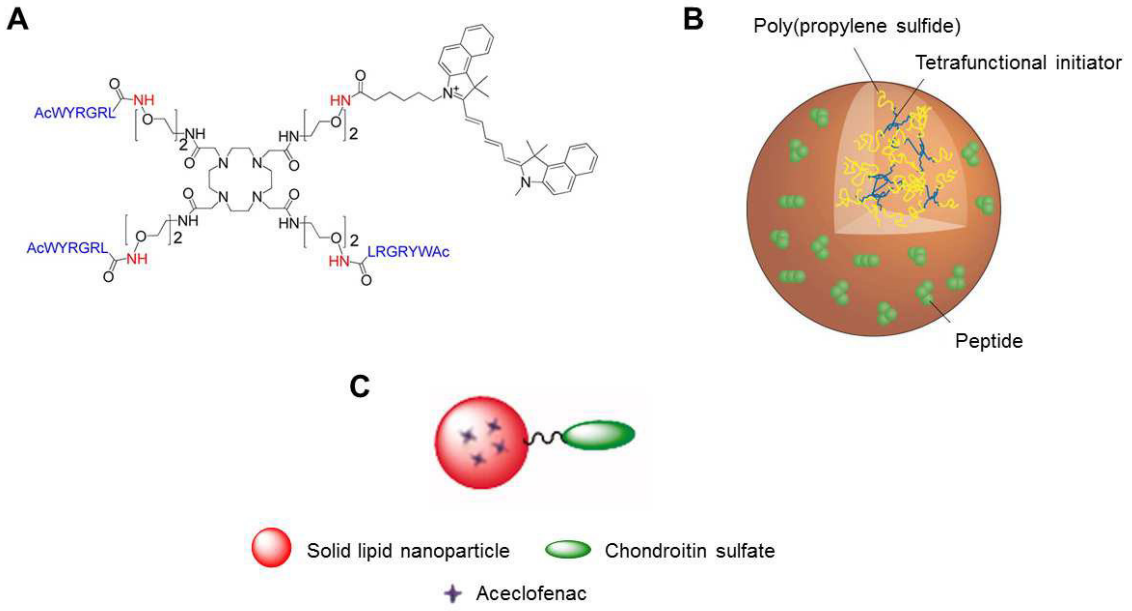

Figure 16. Architectures for the targeting of cartilage tissue (a) Chemical structure of a Cy5.5-labeled DOTAM ligand functionalized with the cartilage-specific peptide sequence WYRGL. Adapted with permission from: American Chemical Society: Hu et al. *Bioconjugate Chem.*,[136d] Copyright 2015. (b) WYRGL (green)-functionalized polymer nanoparticle consisting of poly(propylene sulfide) (yellow) and a tetrafunctional initiator (blue). The particle is covered with poly(ethylene glycol) (orange). Adapted with permission from: Macmillan Publishers Ltd.: Setton *Nat. Mater.*,[126] Copyright 2008. (c) Aceclofenac-loaded solid lipid nanoparticle covered with chondroitin sulfate as the targeting ligand. Modified with permission from Informa UK Ltd.: Bishnoi et al. *J. Drug Target.*,[137b] Copyright 2014.

As evident from the limited number of publications on this highly relevant topic, the development of cartilage specific architectures is still in its infancy. Most systems suffer from a lack of selectivity for the target, poor efficacy due to a fast renal or joint cavity clearance, insufficient enrichment in the ECM, or undesired side effects due to the accumulation in the reticuloendothelial system (RES), which could be defeated by modifying physiochemical properties. Polymeric architectures are ideal platforms to overcome these limitations, as they can be prepared in various sizes and with different functional groups by which renal excretion, toxicity, and recognition by the RES can be improved and optimized.[7, 139] Moreover, by using a multivalent approach, an increased binding affinity along with an enhanced selectivity can be achieved, which might lead to enhanced targeting properties.[14a]

With its multiple hydroxyl groups, dPG allows a versatile modification with different groups to obtain multivalent binding sites, while molecular weights, and sizes can be adjusted for the respective application. By implementation of cartilage-specific moieties, for instance, targeted drug delivery systems for arthritic diseases could be developed in order to prevent the ongoing cartilage destruction. In recent studies anionic dPGS showed a high affinity toward inflamed joints[69] (see Section 1.4), which might be related to the enrichment in cartilage due to interactions with receptors on chondrocytes or synovial fibroblasts, comparable to the chondroitin sulfate covered nanoparticles. However, to clarify these assumptions, more information about distribution patterns of dPGS within the joints, its interaction with proteins, cellular uptake, and insights into the uptake mechanism are needed. Moreover, it remains unclear whether also other anionic agents show an enhanced affinity toward inflamed joints and cartilage, in particular. In addition, targeting properties of a polymer could be altered by combining different anionic moieties and could lead to different targets. A mixed anion with bisphosphonates, for example, could also bind to calcified cartilage.

Therefore, the interaction of several dPG anions with cartilage and different ECM components was investigated in this thesis. The most promising candidates for the selective targeting of the tissue were spotted by incorporating fluorescent dyes and applying CLSM, while a series of binding assays was used to reveal molecular binding partners. Since arthritic diseases can lead to an advanced destruction of cartilage (see Section 1.9), leading to other molecular targets than in healthy tissue, binding affinities were also studied in the inflamed state.

1.9 Bone and Cartilage in Rheumatoid Arthritis

Rheumatoid arthritis (RA) is one of the most common systemic autoimmune diseases, which is primarily associated with joint destruction but also involves long-term complications such as cardiovascular disorders and osteoporosis, leading to a significant morbidity and increased mortality.[140] Although the cause of RA is not yet fully understood, a combination of environmental and genetic factors is thought to be involved in the development of the disease, which leads to the production of autoantibodies and a persistent immune response.[140a] The progress of RA is characterized by an inflammation and hyperplasia of the synovial membrane, cartilage destruction, and finally bone erosion (**Figure 17**).[141]

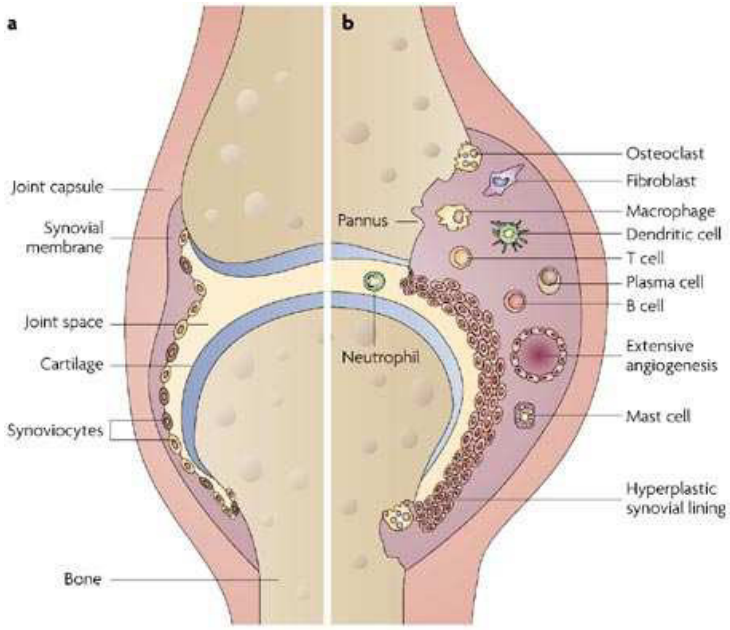

Figure 17. Schematic illustration of structural changes in joints within rheumatoid arthritis (RA). (a) Structure of a healthy joint. (b) In RA the synovial membrane is infiltrated by inflammatory immune cells and becomes hyperplastic. A pannus is formed which invades the articular cartilage and the underlying bone and degradation of the tissue proceeds. Adapted with permission from Nature Publishing Group: Strand et al. *Nature Rev. Drug Discov.*,[141] Copyright 2007.

The synovial membrane is the main target in arthritic diseases, lining the inner capsule surface of diarthrodial joints. It consists of an outer subintimal and an inner intimal layer, which is in contact with the synovial fluid.[142] Normally, the membrane is hypocellular, containing a low amount of macrophages and synovial fibroblasts in the intima and few inflammatory cells in the subintima.[142] However, shortly after the onset of RA, immune cells such as macrophages, dendritic cells, and mast cells infiltrate the subintima and synovial fibroblasts start to proliferate, which leads to the thickening of the lining layer.[143] Induced by an insufficient lymphangiogenesis and local hypoxic

conditions, the formation of new blood vessels is observed along with an increased permeability, which allows an enhanced recruitment of immune cells and plasma proteins.[127a, 140a] Macrophages, resident in the synovial membrane, release ROS, RNS, matrix-degrading enzymes, and cytokines such as TNFα or interleukins and thereby promote the persistent inflammation of the membrane (synovitis).[140a, 142] The occurring microenvironmental changes related to synovitis lead to a reduced production of lubricin, a glycoprotein responsible for the lubrication of the joints, which alters the protein-binding properties of the cartilage surface, enables the invasion of synovial fibroblasts into the ECM, and causes a loss of function.[144] Infiltrating synovial fibroblasts induce the degradation of the collagen type II network and aggrecan by secretion of matrix metalloproteinases (MMPs) and other matrix enzymes (e.g. ADAMTS), which can lead to a higher porosity (**Figure 18**).[140b, 145] In addition, chondrocytes undergo apoptosis due to the release of IL-1, IL-17, and reactive nitrogen intermediates, which further promotes the destruction of articular cartilage.[140a]

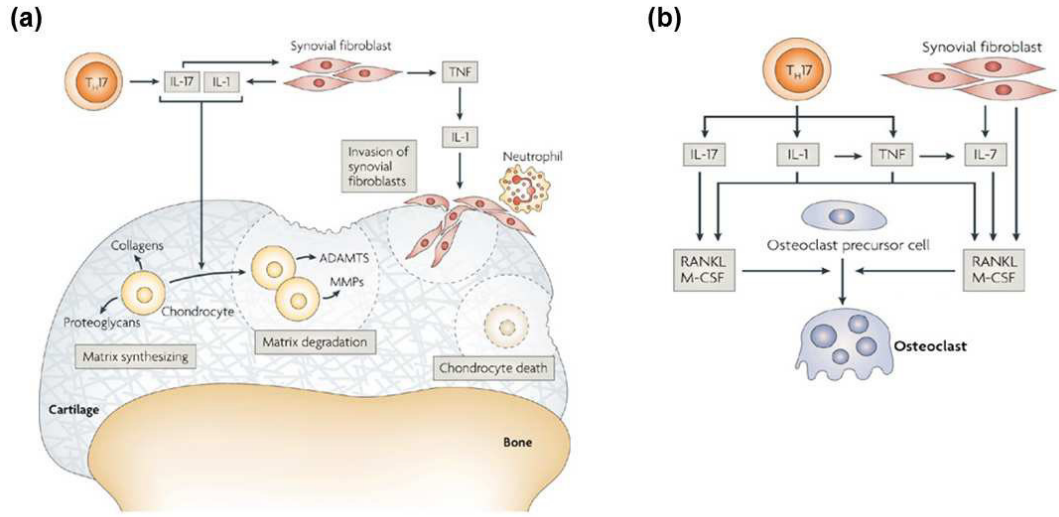

Figure 18. Schematic illustration of pathways for the destruction of cartilage and bone during rheumatoid arthritis. (a) Cartilage degradation is mediated by the release of matrix metalloproteinases (MMPs) and other matrix-degrading enzymes like aggrecanases (ADAMTS). T helper cells or synovial fibroblasts induce matrix destruction by secretion of interleukins (IL-1 and IL-17). Activated by tumor necrosis factor (TNF) and IL-1, synovial fibroblasts invade the extracellular matrix and release matrix degrading enzymes. In addition, chondrocytes undergo apoptosis mediated by IL-1, IL-17, and reactive nitrogen intermediates, which further promotes cartilage destruction. (b) Bone erosion is promoted by osteoclasts which differentiate from osteoclast precursor cells by the release of receptor activator of nuclear factor-κB ligand (RANKL) and macrophage colony-stimulating factor (M-CSF). Differentiation is achieved by the secretion of IL-1, IL-7, IL-17, and TNF which are produced by T helper cells or synovial fibroblasts. Adapted with permission from Nature Publishing Group: McInnes at al. *Nature Rev. Immunol.*,[140b] Copyright 2007.

Regulated by osteoclasts (see Section 1.5), bone erosion was found to rapidly occur in RA and is highly associated with synovitis.[146] Cortical bone is traversed by microvessels that connects the outer synovial membrane with the inner bone marrow.[146] During RA, an inflammation of the bone marrow is observed, which

stimulates the recruitment of osteoclast precursor cells to the hypertrophied synovium (pannus), that invades articular cartilage and bone (**Figure 17**).[146-147] The differentiation and activation of these precursor cells is mediated by inflammatory cytokines such as macrophage colony-stimulating factor (M-CSF) and RANKL, expressed by T helper cells and synovial fibroblasts (**Figure 18**).[140b] In addition to the upregulation of bone-resorbing osteoclasts, tissue erosion at the pannus interface is promoted by the inhibition of osteoblast differentiation from mesenchymal precursor, due to the production of signaling factors such as dickkopf-1, frizzled-related protein 1, and sclerostin.[146]

Since bone erosion occurs in early stages of RA and an advanced cartilage destruction is observed soon after the onset of the disease, both related to synovitis, the efficient targeting of inflammation is a promising approach to avoid tissue destruction by inhibiting inflammatory processes. In addition, a targeted drug delivery system could support the healing process and could lead to an improved quality of life. With ongoing joint degradation, other molecular binding partners than in healthy tissue can be present, which is why the affinity of dendritic polyanions toward bone and cartilage was also evaluated in the inflammatory state.

2 Motivation and Objective

Within the emerging field of nanomedicine, soft-matter nanoparticles based on dendritic polyglycerol (dPG) are of high interest for therapeutic and diagnostic applications due to their tunable chemical and physiochemical properties, which can be tailored for the respective purpose. Specificity and affinity for a certain target, for instance, can be accomplished by functionalizing the outer periphery of the polymer, while pharmacokinetics can be altered by modifying the scaffold, polymer size, and by implementing cleavable linkers. The aim of this thesis is to develop and characterize stable and degradable polyglycerol anions with target-specific properties toward inflammation, bone, and cartilage. *In vitro* and *in vivo* studies will be conducted with dye labeled compounds to gain more information about potential binding partners in order to unravel underlying molecular and biological mechanisms and interactions with biological systems. The main objectives are depicted in **Figure 19**.

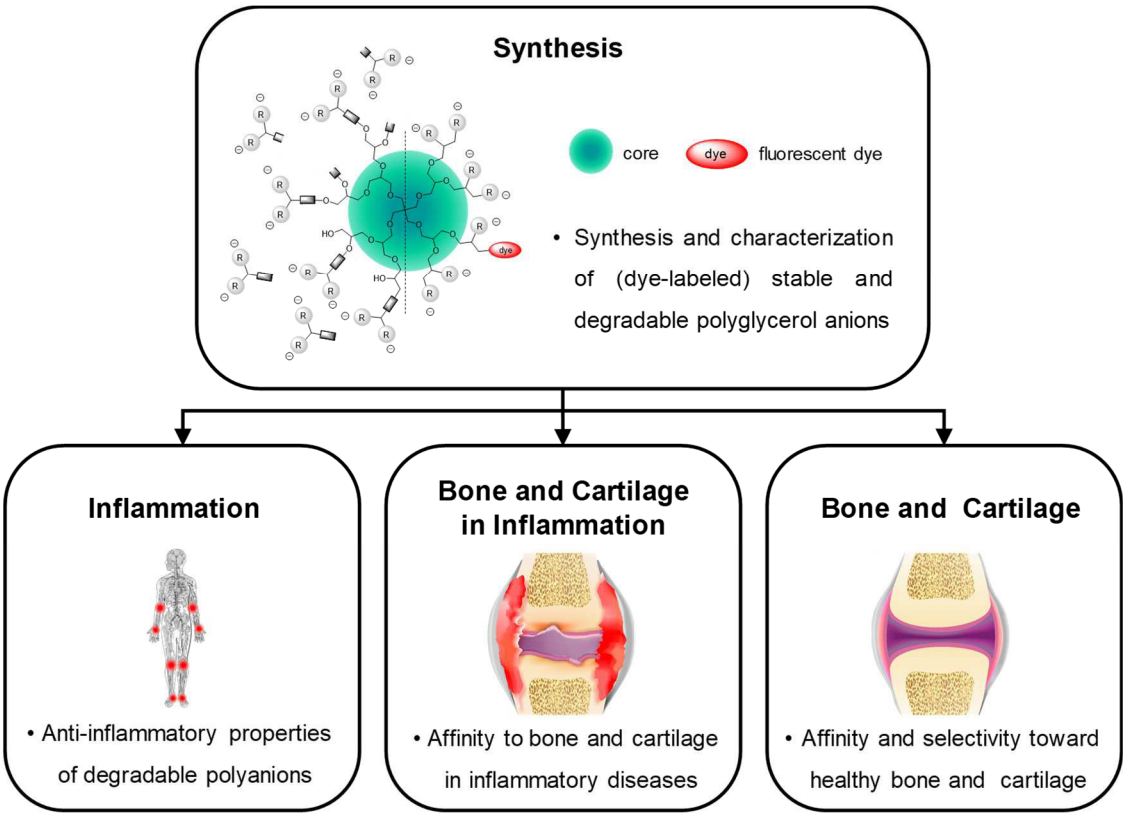

Figure 19. Overview of the main objectives of this PhD thesis.

Dendritic polyglycerol sulfate (dPGS) is known to efficiently block L- and P-selectin and to inhibit the activation of the complement system, which makes it a highly potent therapeutic and diagnostic agent for inflammatory related diseases. However, biodistribution studies have revealed its accumulation in organs of the RES, which

diminishes its use in clinical trials and points to the need for comparable cleavable systems. Therefore, shell degradable polysulfates based on dendritic polyglycerol will be developed and evaluated regarding their anti-inflammatory and anti-coagulant potential. To assess distinct degradation profiles, different types of enzymatically and hydrolytically cleavable linker will be introduced.

In the past, anionic compounds were successfully applied as drug delivery systems for bone and cartilage in order to prevent tissue destruction, a central feature of diverse joint diseases. Along with the strong accumulation of dPGS in inflamed joints in a rheumatoid arthritis model, an enhanced affinity to bone or cartilage is also suggested. Hence, dendritic polyglycerol anions will be investigated for their interaction with both tissues, in order to provide a detailed insight into their targets on the molecular, cellular, and tissue level. Moreover, changes in binding affinities in inflammatory diseases will be studied and the most promising candidates for the selective targeting of bone and cartilage will be identified.

3 Publications and Manuscripts

In the following section the published articles are listed and the contributions of the author are specified.

3.1 Shell Cleavable Dendritic Polyglycerol Sulfates Show High Anti-Inflammatory Properties by Inhibiting L-Selectin Binding and Complement Activation

Sabine Reimann, Dominic Gröger, Christian Kühne, Sebastian B. Riese, Jens Dernedde, Rainer Haag* *Advanced Healthcare Materials* **2015**, *4*, 2154 - 2162.[148]

http://dx.doi.org/10.1002/adhm.201500503

A new class of fully synthetic shell cleavable multivalent polysulfates is prepared by introducing degradable linkers into a stable biocompatible dendritic polyglycerol scaffold and subsequent sulfation. The sulfated polymers show different degradation profiles, low anticoagulant and high anti-inflammatory properties, are able to efficiently bind to L-selectin and inhibit the complement activation at very low concentrations in vitro.

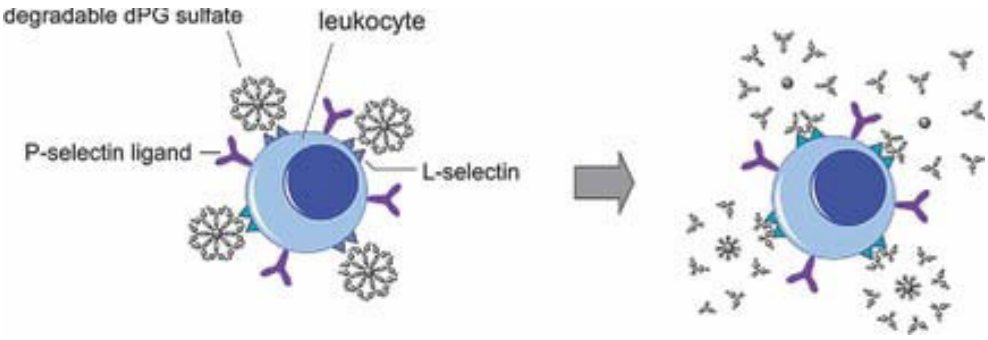

Figure 20. Adapted with permission from Reimann et al.[148] Copyright 2015 Wiley.

In this publication the author contributed to the concept, preparation, and characterization of the polymers, conducted the stability experiments, evaluated the degradation profiles, and composed the manuscript.

3.2 Selectivity in Bone Targeting with Multivalent Dendritic Polyanion Dye Conjugates

Dominic Gröger, Michael Kerschnitzki, Marie Weinhart, **Sabine Reimann**, Tobias Schneider, Benjamin Kohl, Wolfgang Wagermaier, Gundula Schulze-Tanzil, Peter Fratzl, Rainer Haag* *Advanced Healthcare Materials* **2014**, *3,* 375 - 385. [149]

http://dx.doi.org/10.1002/adhm.201300205

Fully synthetic, multivalent polyanion dye conjugates are applied for selective bone targeting. The in vitro binding affinity toward hydroxyapatite and collagen is strongly dependent on the anionic moiety. Polyglycerol-based polyelectrolytes are shown to be potent candidates for selective, bone-targeted imaging applications, tissue engineering, or drug delivery.

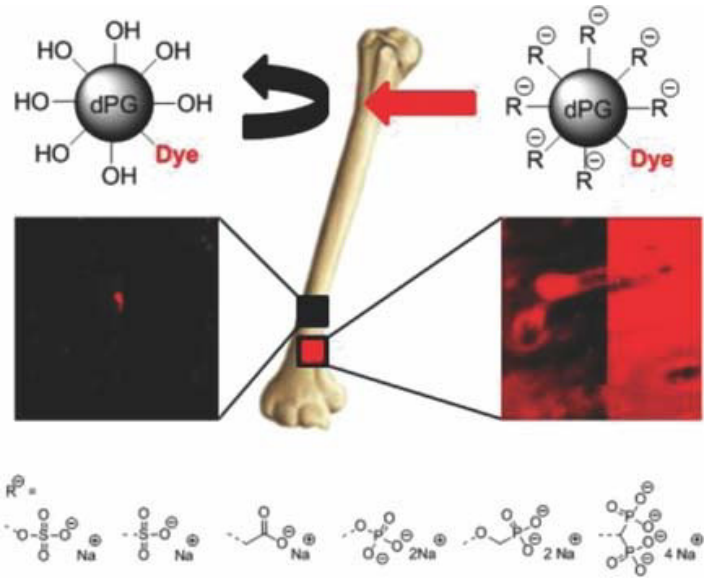

Figure 21. Adapted with permission from Gröger et al.[149] Copyright 2013 Wiley.

In this publication the author contributed to parts of the synthesis, characterization, and size and zeta potential measurements of the polymers.

3.3 Dendritic Polyglycerol Anions for the Selective Targeting of Native and Inflamed Articular Cartilage

S. Reimann, T. Schneider, P. Welker, F. Neumann, K. Licha, G. Schulze-Tanzil, W. Wagermaier, P. Fratzl,* and R. Haag* *Journal of Materials Chemistry B* **2017**, *5*, 4754 - 4767.[150]

http://dx.doi.org/10.1039/C7TB00618G

Dye-conjugated polyanions show high affinities toward native and inflamed cartilage dependent on the anionic moiety and the condition of the tissue.

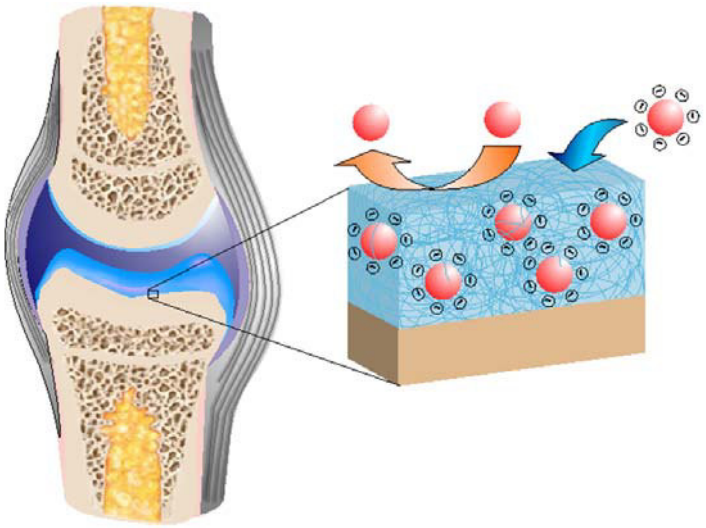

Figure 22. Adapted with permission from Reimann et al.[150] Copyright 2017 Royal Society of Chemistry.

In this publication the author contributed to the concept, preparation and characterization of the polymers, conducted the binding affinity assay, carried out SEM and CLSM imaging, and composed the manuscript.

4 Summary and Conclusion

The aim of this thesis was to develop stable and degradable anionic polymers with a high target-specificity toward inflammation, bone, and cartilage and additionally gain more information about molecular and cellular binding partners of the polyanions in order to unravel the underlying interactions with biological systems.

Previous studies revealed the high affinity of dendritic polyglycerol sulfate toward L- and P-selectin. However, an undesired accumulation of dPGS in the liver and spleen was observed after intravenous administration. Therefore, shell cleavable polysulfates were synthesized, characterized, and investigated regarding their anti-inflammatory activity in the first part of this thesis. The degradable anions were prepared with similar molecular weights and numbers of sulfate groups by introducing enzymatically or hydrolytically cleavable moieties into a stable dPG backbone and subsequent sulfation. The highly water-soluble particles contained either only ester groups (dPG-thioglyceryl pentanoatyl sulfate, dPG-TPS) or additional carbamate (dPG-thioglyceryl methylpropanoatyl sulfate, dPG-TMPS) or amide (dPG-amidoglyceryl succinyl sulfate, dPG-ASuS) functionalities. By applying a concentration-dependent, competitive, SPR-based L-selectin binding assay, the anti-inflammatory activity of the polysulfates was determined *in vitro*. IC_{50} values decreased in the order dPG-ASuS < dPG-TPS < dPG-TMPS from low nanomolar to high picomolar range and were comparable to the inhibitory effect of dPGS. All cleavable polyanions exhibited a minor influence on blood coagulation up to a concentration of 100 nM in an activated partial thromboplastin time (APTT) clotting assay. Moreover, dPG-TMPS and dPG-TPS strongly inhibited the activation of the complement system in human serum already at a concentration of 250 nM, as determined by an ELISA-based method. These results indicate that not only the number of anionic moieties on a polymer defines its interaction with biological systems, but also the nature of the linker, in particular, its length, flexibility, and hydrophobicity. The elemental analysis of dPGS revealed its high stability over 4 weeks under neutral (pH 7.4) and acidic (pH 5.0) conditions as well as upon treatment with carboxylesterase I. In contrast, NMR analysis showed that the cleavable systems underwent ester hydrolysis at different rates. dPG-ASuS slowly decomposed under all tested conditions. dPG-TMPS and dPG-TPS degraded much faster, whereby approximately half of the linkers cleaved within 1 week. However, none of the sulfates showed complete decomposition. Surprisingly, the condition itself had no noticeable effect on the degradation behavior. Apparently, the selected enzyme did not contribute to the decomposition, which might have been due to electrostatic repulsion by the anionic groups, inhibition of the enzyme by the polysulfates, or the tightly packed

structure of the polymers may have shielded the enzyme from acting. On the other hand, the breakdown of the polysulfates under neutral conditions suggests that they might not reach the inflammation site fully intact, due to premature decomposition when applied intravenously. This could eventually result in a lower affinity to inflamed tissue *in vivo*. In addition, other enzymes than the carboxylesterase I, present in the bloodstream, could reduce the target efficacy of the cleavable polyanions. However, to clarify these assumptions, *in vivo* studies investigating the binding affinity toward inflamed tissue would be needed along with more insight into the anti-inflammatory activity of lower sulfate-functionalized particles. Moreover, the potential risks of the cleavage products have to be evaluated including their biocompatibility and toxicity, since they could trigger an immune response themselves and could lead to severe bleeding or activation of the complement. In addition, biodistribution studies have to be conducted in order to determine the excretion pathways of the cleavable polysulfates and demonstrate the suggested improved pharmacokinetics. Although it is assumed that the compounds are eliminated from the liver and spleen after their complete breakdown, leaving the neutral polymer, there is no evidence that the dPG scaffold is indeed excreted once it entered the organs. To overcome these problems, core-degradable polysulfates based on Boltorn-type polyesters or copolymers of glycerol and glycidyl methacrylate,[151] for instance, could be applied. In this case the toxicity and immunologic response of the cleavage products are especially interesting, since a variety of different low- and high-molecular weight compounds can be produced during the breakdown of the polymer, which would have to be examined in detail.

In the second part, different types of dPG polyanions were developed and investigated regarding their targeting properties toward bone and cartilage. The nature of the applied polymers is based on the knowledge about the chemical structure of common hydroxyapatite-binding moieties and the interaction of anionic particles with receptors that are expressed on cells like leukocytes, chondrocytes, and synovial fibroblasts. The conjugation of a dye by a sequential one-pot click approach allowed the investigation of binding affinities toward organic and mineralized bone compartments. While dPG sulfate and sulfonate (dPGSn) showed stronger binding to the collagen type I matrix, the phosphate- (dPGP), phosphonate- (dPGPn), and bisphosphonate-functionalized (dPGBP) particles expressed a higher affinity to mineralized parts. These results were confirmed by applying confocal laser scanning microscopy (CLSM) after incubation of native and demineralized bone sections with the polyanions and in a quantitative UV-based hydroxyapatite binding assay. Investigating normalized penetration profiles with native bone, an exceptionally strong binding of

dPGP was found. The high affinity of dPGS and dPGSn toward demineralized bone might be related to their binding to collagen but has to be further evaluated in a collagen type I binding assay to exclude, i.e., electrostatic interactions with other matrix components such as proteins. Up to a concentration of 10 µM, none of the polymers showed a significant influence on the cell viability of murine fibroblasts, although all anions were rapidly uptaken. However, a reduction of cell viability to 50 - 20% was noticed at a concentration of 10 mM for the phosphorus containing particles and the dPG carboxylate. Hence, the development of anions with a lower degree of functionalization might be a promising approach to achieve improved biocompatibility along with high target specificity.

Related to the binding affinity of sulfur-containing particles to the collagenous matrix of demineralized bone, several dye-labeled dPG anions were evaluated for their interaction with cartilage and different matrix components *in vitro*. By applying CLSM, the neutral polymer and low functionalized dPGBP (dPGBP$_{7\%}$) were identified as inert compounds, whereas an enhanced binding was found for dPGS, dPGP, and dPGBP with a high degree of functionalization. Surprisingly, a mixed anion equipped with BP and sulfate groups (dPGS/BP$_{7\%}$) showed an exceptionally high affinity to the ECM, which is related to its strong interaction with collagen type II, as shown by a normalized fluorescence-based binding assay. For all other anions, further studies have to be conducted to identify their molecular targets. In order to mimic inflamed tissue, cartilage was treated with IL-1β, which led to a higher accumulation of the anions within the matrix. Either molecular binding partners other than in normal tissue are present or the matrix components are more accessible for the polyanions in inflamed cartilage. In addition, no significant influence on the viability of chondrocytes was found up to a polymer concentration of 10 µM, although all polyanions, except dPGBP$_{7\%}$, were rapidly taken up by the cells. This indicates that a minimal number of anionic groups is needed for cell recognition and targeting of cartilage. Moreover, it became evident that the interaction with the ECM is highly dependent on the anionic moiety and the condition of the tissue. Additionally, the studies revealed that the targeting properties of polymers can be altered by introducing different anionic moieties.

Based on the strong binding of BPs to bone along with a minor affinity for cartilage and a suggested lower toxicity, dye-labeled dPGBP$_{7\%}$ was applied in a CIA rat model for the selective targeting of bone *in vivo* in order to visualize severe cases of RA where bone erosion occurs. NIR fluorescence imaging revealed an increasing affinity of dPGBP$_{7\%}$ toward inflamed joints with higher clinical scores although the compound accumulated a lot more slowly than dPGS. Histological examinations of inflamed joints showed the high affinity of dPGBP$_{7\%}$ for mineralized compartments and its uptake by

osteoblasts. By combining CLSM and SEM images, an enhanced enrichment in non-calcified cartilage with increasing scores was found, while calcified cartilage was not addressed. Compared to this, dPGS did not interact with bone but strongly accumulated in chondrocytes and cartilage, independent of the CIA score, which underlines once again its high sensitivity for inflammatory and degenerative processes at early stages of joint diseases.

In conclusion, investigating the targeting properties of dPG based polyanions, sulfur-containing compounds are the scaffolds of choice when it comes to addressing inflammation or a collagenous matrix - like cartilage. In contrast, phosphorous-containing anions are more powerful for targeting mineralized compartments. However, binding affinities are highly dependent on the nature and number of the anionic groups as well as the condition of the target. Although interactions of polyanions with biological systems were elucidated to a certain extent, still a lot of open questions remain, especially concerning the molecular binding partners of the respective polymer.

5 Outlook

The development of the above-mentioned shell cleavable polysulfates is considered a successful first proof of concept. However, degradation patterns of future systems have to be improved in order to avoid the breakdown of the particles before reaching the target and ensure a high selectivity along with a high anti-inflammatory effect. This might be realized by implementing more stable functionalities like disulfide bonds into the dPG backbone, which can be cleaved within the reducing environment of cells. Alternatively, core degradable systems could be applied, which might decompose into smaller fragments that might be cleared much faster *in vivo*. Preliminary results with a sulfated Boltorn-type polyester showed promising results regarding its targeting properties toward inflammation *in vivo* in a CIA rat model after intravenous injection. However, further investigations are needed to explore the assumed degradability of the compound. In order to get more insight into the biodistribution and pharmacokinetics of degradable compounds, labeling methods such as radiolabeling are needed that allow quantitative data. However, the introduction of radiolabels into degradable systems is not trivial, since each additional synthetic step bears the risk of particle breakdown. At least in the case of shell cleavable polymers, this problem could be circumvented by implementing the sulfated linker as the final step. Nevertheless, the development of low-molecular-weight sulfates is highly challenging due to difficulties in their purification or the lack of full conversion of all functional groups into sulfates.[152] In addition, more information about potential molecular binding partners and a detailed understanding concerning the influence of surface charge density and size on the *in vivo* behavior of a particle is needed. First experiments have already been conducted to identify protein interactions with dPGS by applying qualitative techniques such as electrophoretic mobility shift assay (EMSA) as well as quantitative methods such as SPR, microscale thermophoresis (MST), isothermal titration calorimetry (ITC), or quartz crystal microbalance (QCM). However, the underlying mechanisms, which lead to the undesired organ accumulation of dPGS, including the role of physiochemical properties, are still not fully understood.

Polyglycerol anions have also been found to express differently pronounced affinities toward bone and cartilage depending on the number and nature of the anionic moiety. These variations in binding could be used to address compartments that show features of both tissues like calcified cartilage, for instance, which might be realized by hybrid structures that contain different anionic groups. This thesis proved the synthetic feasibility of this approach by developing a randomly distributed mixed anion. However, a more sophisticated agent with spatially divided functional groups might show

improved targeting properties, since the amount of each anionic group could be adjusted, although the optimal ratio might be time consuming to find. The introduction of a fluorescent dye between both anionic moieties would lead to a highly defined compound and would allow the verification of successful binding. Preliminary experiments to develop such a spatially divided bifunctional anion have been carried out using several different synthetic routes, but the final coupling step failed.

6 Abstract und Kurzzusammenfassung

In this thesis, stable and degradable polyanions and their dye-labeled counterparts were synthesized and investigated concerning their targeting properties toward inflammation, bone, and cartilage. In the first part, sulfated shell cleavable systems derived from dendritic polyglycerol were established. These particles showed improved degradation profiles *in vitro* in comparison to dPGS and a high anti-inflammatory potential in a L-selectin binding assay. In addition, only a minor anticoagulant effect and a strong inhibition of the complement activation were observed.

In the second part, dye-labeled dendritic polyglycerol anions were evaluated for their interaction with bone and cartilage in various quantitative and qualitative *in vitro* assays. While a strong enrichment in mineralized compartments in bone was demonstrated for phosphorous-containing anions, sulfated and sulfonated particles bound with higher affinity to the organic collagen matrix. The target specificity of the polyanions was further validated with native and inflamed cartilage. Highly functionalized dPG bisphosphonate, sulfate, and phosphate showed moderate binding affinities toward native cartilage, which increased by IL-1β treatment of the tissue. In contrast to this, an exceptionally strong enrichment in cartilage was found for a mixed anion containing sulfate and bisphosphonate groups, which is related to its high affinity to collagen type II. In a CIA model, low functionalized dPGBP demonstrated a strong accumulation in mineralized compartments of inflamed joints and an increasing affinity to non-calcified cartilage with higher clinical scores, as determined by histological examinations. In contrast, dPGS did not show any interaction with bone but a strong binding to cartilage independent of the score, which emphasizes once again the high sensitivity of sulfated compounds for inflammatory processes at early stages.

This thesis demonstrates how specificity toward a certain target can be achieved by variation of the anionic moiety and the ligand density on a polymer scaffold. Moreover, it was revealed that interactions of polyanions with biological systems are related with the condition of the tissue and the nature of the polymer architecture.

Abstract und Kurzzusammenfassung

In dieser Arbeit wurden stabile und bioabbaubare Polyanionen sowie ihre Farbstoff-funktionalisierten Analoga synthetisiert und bezüglich ihrer Targeting-Eigenschaften gegenüber Entzündungen, Knochen und Knorpel untersucht. Im ersten Teil wurden sulfatierte Schale-spaltbare Systeme, basierend auf dendritischem Polyglycerol, etabliert. Diese zeigten verbesserte Abbauprofile *in vitro* im Vergleich zu dPGS, sowie eine hohe anti-inflammatorische Aktivität in einem L-Selectin Bindungsassay. Darüber hinaus wurden sowohl eine geringe antikoagulante Wirkung sowie eine starke Inhibition der Komplementsystem-Aktivierung beobachtet.

Im zweiten Teil wurden Farbstoff-markierte dendritische Polyglycerolanionen bezüglich ihrer Interaktion mit Knochen und Knorpel in mehreren quantitativen and qualitativen *in vitro* Assays evaluiert. Während für phosphorhaltige Anionen eine starke Anreicherung in mineralisierten Teilen des Knochens demonstriert wurde, konnte für sulfatierte und sulfonierte Partikel eine stärke Bindung an die organische Kollagenmatrix festgestellt werden. Die Target-Spezifität wurde zudem für nativen und entzündeten Knorpel validiert. Hoch funktionalisiertes dPG Bisphosphonat, Sulfat und Phosphat zeigten moderate Bindungsaffinitäten gegenüber nativem Knorpel, die durch die Behandlung des Gewebes mit IL-1β zunahmen. Für ein gemischtes Anion hingegen, welches sowohl Sulfat als auch Bisphosphonat Gruppen aufwies, wurde eine außergewöhnlich starke Anreicherung im Knorpel gefunden, was in Zusammenhang mit dessen hohen Affinität zu Kollagen Typ II steht. In einem rheumatoiden Arthritis Model wurde für gering funktionalisiertes dPG Bisphosphonat mittels histologischer Untersuchung eine starke Akkumulation in mineralisierten Kompartimenten entzündeter Gelenke sowie mit höherem klinischen Score eine zunehmende Affinität zu nicht-kalzifiziertem Knorpel nachgewiesen. Im Vergleich dazu zeigte dPGS keinerlei Interaktion mit Knochen, jedoch eine starke Bindung an Knorpel unabhängig vom Score, was abermals die hohe Sensitivität sulfatierter Verbindungen gegenüber entzündlichen Prozessen in früher Stadien hervorhebt.

Diese Arbeit demonstriert wie die Spezifität gegenüber einem bestimmten Target durch Variation der anionischen funktionellen Gruppe und Ligandendichte eines Polymer erreicht werden kann. Zudem wurde gezeigt, dass die Interaktionen zwischen Polyanionen und biologischen Systemen vom Zustand des Gewebes und der Beschaffenheit der Architektur eines Polymers abhängig sind.

7 References

[1] J. Shi, P. W. Kantoff, R. Wooster, O. C. Farokhzad, *Nat Rev Cancer* **2017**, *17*, 20-37.

[2] E. Taylor, T. J. Webster, *International Journal of Nanomedicine* **2011**, *6*, 1463-1473.

[3] R. Mehendale, M. Joshi, V. B. Patravale, **2013**, *30*, 1-49.

[4] a) K. Chaudhury, V. Kumar, J. Kandasamy, S. RoyChoudhury, *International Journal of Nanomedicine* **2014**, *9*, 4153-4167; b) L. Y. Rizzo, B. Theek, G. Storm, F. Kiessling, T. Lammers, *Current opinion in biotechnology* **2013**, *24*, 1159-1166.

[5] D. Bobo, K. J. Robinson, J. Islam, K. J. Thurecht, S. R. Corrie, *Pharm Res* **2016**, *33*, 2373-2387.

[6] W. Hannah, P. B. Thompson, *Journal of Environmental Monitoring* **2008**, *10*, 291-300.

[7] A. E. Nel, L. Madler, D. Velegol, T. Xia, E. M. V. Hoek, P. Somasundaran, F. Klaessig, V. Castranova, M. Thompson, *Nat Mater* **2009**, *8*, 543-557.

[8] M. E. Fox, F. C. Szoka, J. M. J. Fréchet, *Accounts of Chemical Research* **2009**, *42*, 1141-1151.

[9] C. D. Walkey, W. C. W. Chan, *Chemical Society Reviews* **2012**, *41*, 2780-2799.

[10] E. Fröhlich, *International Journal of Nanomedicine* **2012**, *7*, 5577-5591.

[11] A. Varki, *Glycobiology* **1993**, *3*, 97-130.

[12] M. Mammen, S.-K. Choi, G. M. Whitesides, *Angewandte Chemie International Edition* **1998**, *37*, 2754-2794.

[13] a) J. Rao, J. Lahiri, L. Isaacs, R. M. Weis, G. M. Whitesides, *Science* **1998**, *280*, 708; b) A. Mulder, J. Huskens, D. N. Reinhoudt, *Organic & Biomolecular Chemistry* **2004**, *2*, 3409-3424; c) J. Huskens, *Current Opinion in Chemical Biology* **2006**, *10*, 537-543.

[14] a) C. Fasting, C. A. Schalley, M. Weber, O. Seitz, S. Hecht, B. Koksch, J. Dernedde, C. Graf, E.-W. Knapp, R. Haag, *Angewandte Chemie International Edition* **2012**, *51*, 10472-10498; b) B. Bouvier, *Journal of Chemical Information and Modeling* **2016**, *56*, 1193-1204; c) J. Hall, P. A. Karplus, E. Barbar, *Journal of Biological Chemistry* **2009**, *284*, 33115-33121; d) H.-J. Schneider, *Angewandte Chemie International Edition* **2009**, *48*, 3924-3977.

[15] C. A. Hunter, H. L. Anderson, *Angewandte Chemie International Edition* **2009**, *48*, 7488-7499.

[16] S. I. Rudnick, G. P. Adams, *Cancer Biotherapy & Radiopharmaceuticals* **2009**, *24*, 155-161.

[17] V. M. Krishnamurthy, L. A. Estroff, G. M. Whitesides, in *Fragment-based Approaches in Drug Discovery*, Wiley-VCH Verlag GmbH & Co. KGaA, **2006**, pp. 11-53.

[18] J. R. Williamson, *Nat Chem Biol* **2008**, *4*, 458-465.

[19] W. Jiang, K. Nowosinski, N. L. Löw, E. V. Dzyuba, F. Klautzsch, A. Schäfer, J. Huuskonen, K. Rissanen, C. A. Schalley, *Journal of the American Chemical Society* **2012**, *134*, 1860-1868.

[20] G. K. Ackers, M. L. Doyle, D. Myers, M. A. Daugherty, *Science* **1992**, *255*, 54.

[21] a) G. D. Glick, J. R. Knowles, *Journal of the American Chemical Society* **1991**, *113*, 4701-4703; b) G. D. Glick, P. L. Toogood, D. C. Wiley, J. J. Skehel, J. R. Knowles, *Journal of Biological Chemistry* **1991**, *266*, 23660-23669.

[22] K. Ley, C. Laudanna, M. I. Cybulsky, S. Nourshargh, *Nature Reviews Immunology* **2007**, *7*, 678-689.

[23] U. Weiss, *Nature* **2008**, *454*, 427-427.

[24] A. Jain, S. G. Huang, G. M. Whitesides, *Journal of the American Chemical Society* **1994**, *116*, 5057-5062.

[25] P. I. Kitov, H. Shimizu, S. W. Homans, D. R. Bundle, *Journal of the American Chemical Society* **2003**, *125*, 3284-3294.

[26] a) S.-K. Choi, M. Mammen, G. M. Whitesides, *Journal of the American Chemical Society* **1997**, *119*, 4103-4111; b) M. Mammen, G. Dahmann, G. M. Whitesides, *Journal of Medicinal Chemistry* **1995**, *38*, 4179-4190; c) W. J. Lees, A. Spaltenstein, J. E. Kingery-Wood, G. M. Whitesides, *Journal of Medicinal Chemistry* **1994**, *37*, 3419-3433; d) G. B. Sigal, M. Mammen, G. Dahmann, G. M. Whitesides, *Journal of the American Chemical Society* **1996**, *118*, 3789-3800; e) J. E. Kingery-Wood, K. W. Williams, G. B. Sigal, G. M. Whitesides, *Journal of the American Chemical Society* **1992**, *114*, 7303-7305; f) I. Papp, C. Sieben, A. L. Sisson, J. Kostka, C. Böttcher, K. Ludwig, A. Herrmann, R. Haag, *ChemBioChem* **2011**, *12*, 887-895; g) J. D. Reuter, A. Myc, M. M. Hayes, Z. Gan, R. Roy, D. Qin, R. Yin, L. T. Piehler, R. Esfand, D. A. Tomalia, J. R. Baker, *Bioconjugate Chemistry* **1999**, *10*, 271-278.

[27] a) G. Kretzschmar, U. Sprengard, H. Kunz, E. Bartnik, W. Schmidt, A. Toepfer, B. Hörsch, M. Krause, D. Seiffge, *Tetrahedron* **1995**, *51*, 13015-13030; b) G. Baisch, R. Öhrlein, *Angewandte Chemie International Edition in English* **1996**, *35*, 1812-1815; c) S. Enders, G. Bernhard, A. Zakrzewicz, R. Tauber, *Biochimica et Biophysica Acta (BBA) - General Subjects* **2007**, *1770*, 1441-

1449; d) K. Shailubhai, S. Z. Abbas, G. S. Jacob, *Biochemical and Biophysical Research Communications* **1996**, *229*, 488-493; e) I. Papp, J. Dernedde, S. Enders, R. Haag, *Chemical Communications* **2008**, 5851-5853.

[28] a) E. Fan, Z. Zhang, W. E. Minke, Z. Hou, C. L. M. J. Verlinde, W. G. J. Hol, *Journal of the American Chemical Society* **2000**, *122*, 2663-2664; b) P. I. Kitov, J. M. Sadowska, G. Mulvey, G. D. Armstrong, H. Ling, N. S. Pannu, R. J. Read, D. R. Bundle, *Nature* **2000**, *403*, 669-672; c) M. Mourez, R. S. Kane, J. Mogridge, S. Metallo, P. Deschatelets, B. R. Sellman, G. M. Whitesides, R. J. Collier, *Nat Biotech* **2001**, *19*, 958-961.

[29] S.-K. Choi, M. Mammen, G. M. Whitesides, *Chemistry & Biology* **1996**, *3*, 97-104.

[30] T. H. Mourey, S. R. Turner, M. Rubinstein, J. M. J. Frechet, C. J. Hawker, K. L. Wooley, *Macromolecules* **1992**, *25*, 2401-2406.

[31] K. L. Wooley, J. M. J. Fréchet, C. J. Hawker, *Polymer* **1994**, *35*, 4489-4495.

[32] V. Kottisch, D. T. Gentekos, B. P. Fors, *ACS Macro Letters* **2016**, *5*, 796-800.

[33] a) B. Nandy, S. Saurabh, A. K. Sahoo, N. M. Dixit, P. K. Maiti, *Nanoscale* **2015**, *7*, 18628-18641; b) Y.-H. Jiang, P. Emau, J. S. Cairns, L. Flanary, W. R. Morton, T. D. McCarthy, C.-C. Tsai, *AIDS Research and Human Retroviruses* **2005**, *21*, 207-213; c) C. F. Price, D. Tyssen, S. Sonza, A. Davie, S. Evans, G. R. Lewis, S. Xia, T. Spelman, P. Hodsman, T. R. Moench, A. Humberstone, J. R. A. Paull, G. Tachedjian, *PLoS ONE* **2011**, *6*, e24095.

[34] a) W. Tao, C. Richards, D. Hamer, *AIDS Research and Human Retroviruses* **2008**, *24*, 925-929; b) V. Pirrone, B. Wigdahl, F. C. Krebs, *Antiviral Research* **2011**, *90*, 168-182.

[35] A. Sunder, R. Hanselmann, H. Frey, R. Mülhaupt, *Macromolecules* **1999**, *32*, 4240-4246.

[36] F. Paulus, R. Schulze, D. Steinhilber, M. Zieringer, I. Steinke, P. Welker, K. Licha, S. Wedepohl, J. Dernedde, R. Haag, *Macromolecular Bioscience* **2014**, *14*, 643-654.

[37] H. Jin, W. Huang, X. Zhu, Y. Zhou, D. Yan, *Chemical Society Reviews* **2012**, *41*, 5986-5997.

[38] a) D. Steinhilber, S. Seiffert, J. A. Heyman, F. Paulus, D. A. Weitz, R. Haag, *Biomaterials* **2011**, *32*, 1311-1316; b) D. Steinhilber, M. Witting, X. Zhang, M. Staegemann, F. Paulus, W. Friess, S. Küchler, R. Haag, *Journal of Controlled Release* **2013**, *169*, 289-295; c) D. Steinhilber, A. L. Sisson, D. Mangoldt, P. Welker, K. Licha, R. Haag, *Advanced Functional Materials* **2010**, *20*, 4133-4138.

[39] M. Weinhart, D. Gröger, S. Enders, J. Dernedde, R. Haag, *Biomacromolecules* **2011**, *12*, 2502-2511.

[40] S. Roller, H. Zhou, R. Haag, *Mol Divers* **2005**, *9*, 305-316.

[41] M. A. Quadir, R. Haag, *Journal of Controlled Release* **2012**, *161*, 484-495.

[42] R. Medzhitov, *Nature* **2008**, *454*, 428-435.

[43] P. Libby, *Nutrition Reviews* **2007**, *65*, S140.

[44] C. R. Parish, *Nat Immunol* **2005**, *6*, 861-862.

[45] L. M. Coussens, Z. Werb, *Nature* **2002**, *420*, 860-867.

[46] E. J. Kunkel, E. C. Butcher, *Nat Rev Immunol* **2003**, *3*, 822-829.

[47] J. Bestebroer, C. J. C. de Haas, J. A. G. van Strijp, *FEMS Microbiology Reviews* **2010**, *34*, 395.

[48] M. Kirschfink, *Immunopharmacology* **1997**, *38*, 51-62.

[49] T. W. Du Clos, C. Mold, in *Clinical Immunology (Third Edition)* (Ed.: R. R. R. A. F. T. S. W. S. J. F. M. Weyand), Mosby, Edinburgh, **2008**, pp. 305-325.

[50] a) N. Kaila, B. E. Thomas, *Expert Opinion on Therapeutic Patents* **2003**, *13*, 305-317; b) S. R. Barthel, J. D. Gavino, L. Descheny, C. J. Dimitroff, *Expert Opinion on Therapeutic Targets* **2007**, *11*, 1473-1491.

[51] a) R. P. McEver, C. Zhu, *Annual Review of Cell and Developmental Biology* **2010**, *26*, 363-396; b) K. Ley, C. Laudanna, M. Cybulsky, S. Nourshargh, *Nature Reviews Immunology* **2007**, *7*, 678-689.

[52] D. J. Mitchell, P. Li, P. H. Reinhardt, P. Kubes, *Blood* **2000**, *95*, 2954-2959.

[53] E. E. Simanek, G. J. McGarvey, J. A. Jablonowski, C.-H. Wong, *Chemical Reviews* **1998**, *98*, 833-862.

[54] W. S. Somers, J. Tang, G. D. Shaw, R. T. Camphausen, *Cell* **2000**, *103*, 467-479.

[55] A. Koenig, R. Jain, R. Vig, K. E. Norgard-Sumnicht, K. L. Matta, A. Varki, *Glycobiology* **1997**, *7*, 79-93.

[56] D. J. Lefer, *Annual Review of Pharmacology and Toxicology* **2000**, *40*, 283-294.

[57] D. M. Lewinsohn, R. F. Bargatze, E. C. Butcher, *The Journal of Immunology* **1987**, *138*, 4313-4321.

[58] P. Mehta, R. D. Cummings, R. P. McEver, *Journal of Biological Chemistry* **1998**, *273*, 32506-32513.

[59] R. E. Bruehl, F. Dasgupta, T. R. Katsumoto, J. H. Tan, C. R. Bertozzi, W. Spevak, D. J. Ahn, S. D. Rosen, J. O. Nagy, *Biochemistry* **2001**, *40*, 5964-5974.

[60] A. Varki, *Proceedings of the National Academy of Sciences of the United States of America* **1994**, *91*, 7390-7397.

[61] I. Capila, R. J. Linhardt, *Angewandte Chemie International Edition* **2002**, *41*, 390-412.

[62] M. Bernfield, M. Götte, P. W. Park, O. Reizes, M. L. Fitzgerald, J. Lincecum, M. Zako, *Annual Review of Biochemistry* **1999**, *68*, 729-777.

[63] T. E. Warkentin, A. Greinacher, *The Annals of Thoracic Surgery* **2003**, *76*, 638-648.

[64] H. Türk, R. Haag, S. Alban, *Bioconjugate Chemistry* **2004**, *15*, 162-167.

[65] M. Calderón, M. A. Quadir, S. K. Sharma, R. Haag, *Advanced Materials* **2010**, *22*, 190-218.

[66] a) J. Dernedde, A. Rausch, M. Weinhart, S. Enders, R. Tauber, K. Licha, M. Schirner, U. Zügel, A. von Bonin, R. Haag, *Proceedings of the National Academy of Sciences* **2010**, *107*, 19679-19684; b) M. Weinhart, D. Gröger, S. Enders, S. B. Riese, J. Dernedde, R. K. Kainthan, D. E. Brooks, R. Haag, *Macromolecular Bioscience* **2011**, *11*, 1088-1098.

[67] a) K. Oishi, Y. Hamaguchi, T. Matsushita, M. Hasegawa, N. Okiyama, J. Dernedde, M. Weinhart, R. Haag, T. F. Tedder, K. Takehara, H. Kohsaka, M. Fujimoto, *Arthritis & Rheumatology* **2014**, *66*, 1864-1871; b) D. Maysinger, D. Gröger, A. Lake, K. Licha, M. Weinhart, P. K. Y. Chang, R. Mulvey, R. Haag, R. A. McKinney, *Biomacromolecules* **2015**, *16*, 3073-3082.

[68] S. Biffi, S. Dal Monego, C. Dullin, C. Garrovo, B. Bosnjak, K. Licha, P. Welker, M. M. Epstein, F. Alves, *PloS one* **2013**, *8*, e57150.

[69] K. Licha, P. Welker, M. Weinhart, N. Wegner, S. Kern, S. Reichert, I. Gemeinhardt, C. Weissbach, B. Ebert, R. Haag, M. Schirner, *Bioconjugate Chemistry* **2011**, *22*, 2453-2460.

[70] Y. Zhong, M. Dimde, D. Stöbener, F. Meng, C. Deng, Z. Zhong, R. Haag, *ACS Applied Materials & Interfaces* **2016**, *8*, 27530-27538.

[71] a) D. Gröger, F. Paulus, K. Licha, P. Welker, M. Weinhart, C. Holzhausen, L. Mundhenk, A. D. Gruber, U. Abram, R. Haag, *Bioconjugate Chemistry* **2013**, *24*, 1507-1514; b) C. Holzhausen, D. Gröger, L. Mundhenk, P. Welker, R. Haag, A. D. Gruber, *Nanomedicine: Nanotechnology, Biology and Medicine* **2013**, *9*, 465-468.

[72] S. Staufenbiel, C. Weise, R. H. Müller, *Macromolecular Symposia* **2014**, *345*, 42-50.

[73] D. Wang, S. C. Miller, P. Kopečková, J. Kopeček, *Advanced Drug Delivery Reviews* **2005**, *57*, 1049-1076.

[74] K. K. Sivaraj, R. H. Adams, *Development* **2016**, *143*, 2706.

[75] P. Fratzl, H. S. Gupta, E. P. Paschalis, P. Roschger, *Journal of Materials Chemistry* **2004**, *14*, 2115-2123.

[76] a) M. Dermience, G. Lognay, F. Mathieu, P. Goyens, *Journal of Trace Elements in Medicine and Biology* **2015**, *32*, 86-106; b) J. D. Currey, *Journal of Materials Science* **2012**, *47*, 41-54.

[77] J. D. Currey, *Journal of Biomechanics* **2003**, *36*, 1487-1495.

[78] B. Clarke, *Clinical Journal of the American Society of Nephrology* **2008**, *3*, S131-S139.

[79] a) D. R. Carter, D. M. Spengler, *Clinical Orthopaedics and Related Research* **1978**, *135*; b) D. M. L. Cooper, C. E. Kawalilak, K. Harrison, B. D. Johnston, J. D. Johnston, *Current Osteoporosis Reports* **2016**, *14*, 187-198.

[80] E. A. Zimmermann, E. Schaible, H. Bale, H. D. Barth, S. Y. Tang, P. Reichert, B. Busse, T. Alliston, J. W. Ager, R. O. Ritchie, *Proceedings of the National Academy of Sciences* **2011**, *108*, 14416-14421.

[81] E. A. Zimmermann, R. O. Ritchie, *Advanced Healthcare Materials* **2015**, *4*, 1287-1304.

[82] N. A. Sims, C. Vrahnas, *Archives of Biochemistry and Biophysics* **2014**, *561*, 22-28.

[83] C. V. B. d. Gusmão, W. D. Belangero, *Revista Brasileira de Ortopedia* **2009**, *44*, 299-305.

[84] a) L. J. Gibson, *Journal of Biomechanics* **1985**, *18*, 317-328; b) T. M. Keaveny, E. F. Morgan, G. L. Niebur, O. C. Yeh, *Annual Review of Biomedical Engineering* **2001**, *3*, 307-333.

[85] J. A. Buckwalter, M. J. Glimcher, R. R. Cooper, R. Recker, *The Journal of Bone & Joint Surgery* **1995**, *77*, 1276-1289.

[86] S. A. Low, J. Kopeček, *Advanced Drug Delivery Reviews* **2012**, *64*, 1189-1204.

[87] W. Wagermaier, H. S. Gupta, A. Gourrier, M. Burghammer, P. Roschger, P. Fratzl, *Biointerphases* **2006**, *1*, 1-5.

[88] T. A. Franz-Odendaal, B. K. Hall, P. E. Witten, *Developmental Dynamics* **2006**, *235*, 176-190.

[89] H. Chen, T. Senda, K.-y. Kubo, *Medical Molecular Morphology* **2015**, *48*, 61-68.

[90] S. A. Gittens, G. Bansal, R. F. Zernicke, H. Uludağ, *Advanced Drug Delivery Reviews* **2005**, *57*, 1011-1036.

[91] J. R. Neale, N. B. Richter, K. E. Merten, K. G. Taylor, S. Singh, L. C. Waite, N. K. Emery, N. B. Smith, J. Cai, W. M. Pierce, *Bioorganic & medicinal chemistry letters* **2009**, *19*, 680-683.

[92] S. Lan, Q. Ming-Cai, W. Ling-Ling, Z. Hu, L. Jing-Sheng, *Zhongguo Yao Li Xue Bao* **2000**, *21*, 200-204.

[93] a) J. Ishizaki, Y. Waki, T. Takahashi-Nishioka, K. Yokogawa, K.-i. Miyamoto, *Journal of Bone and Mineral Metabolism* **2009**, *27*, 1-8; b) O. Liang, H. Wencai, H. Gu, G. Li, *Letters in Organic Chemistry* **2009**, *6*, 272-277; c) K. Yokogawa, K. Miya, T. Sekido, Y. Higashi, M. Nomura, R. Fujisawa, K. Morito, Y. Masamune, Y. Waki, S. Kasugai, K.-i. Miyamoto, *Endocrinology* **2001**, *142*, 1228-1233; d) T. Sekido, N. Sakura, Y. Higashi, K. Miya, Y. Nitta, M. Nomura, H. Sawanishi, K. Morito, Y. Masamune, S. Kasugai, K. Yokogawa, K.-I. Miyamoto, *Journal of Drug Targeting* **2001**, *9*, 111-121.

[94] D. Wang, S. Miller, M. Sima, P. Kopečková, J. Kopeček, *Bioconjugate Chemistry* **2003**, *14*, 853-859.

[95] a) J. D. Bergstrom, R. G. Bostedor, P. J. Masarachia, A. A. Reszka, G. Rodan, *Archives of Biochemistry and Biophysics* **2000**, *373*, 231-241; b) J. E. Fisher, M. J. Rogers, J. M. Halasy, S. P. Luckman, D. E. Hughes, P. J. Masarachia, G. Wesolowski, R. G. G. Russell, G. A. Rodan, A. A. Reszka, *Proceedings of the National Academy of Sciences* **1999**, *96*, 133-138; c) A. I. Idris, J. Rojas, I. R. Greig, R. J. van't Hof, S. H. Ralston, *Calcif Tissue Int* **2008**, *82*, 191-201.

[96] a) J. C. Frith, J. Mönkkönen, S. Auriola, H. Mönkkönen, M. J. Rogers, *Arthritis & Rheumatism* **2001**, *44*, 2201-2210; b) J. C. Frith, J. Mönkkönen, G. M. Blackburn, R. G. G. Russell, M. J. Rogers, *Journal of Bone and Mineral Research* **1997**, *12*, 1358-1367.

[97] a) L. I. Plotkin, R. S. Weinstein, A. M. Parfitt, P. K. Roberson, S. C. Manolagas, T. Bellido, *Journal of Clinical Investigation* **1999**, *104*, 1363-1374; b) L. I. Plotkin, S. C. Manolagas, T. Bellido, *Bone* **2006**, *39*, 443-452; c) J. H. Tobias, J. W. M. Chow, T. J. Chambers, *Bone* **1993**, *14*, 619-623.

[98] R. G. G. Russell, *Pediatrics* **2007**, *119*, 150-162.

[99] K. Hochdörffer, K. Abu Ajaj, C. Schäfer-Obodozie, F. Kratz, *Journal of Medicinal Chemistry* **2012**, *55*, 7502-7515.

[100] H. Pan, M. Sima, S. C. Miller, P. Kopečková, J. Yang, J. Kopeček, *Biomaterials* **2013**, *34*, 6528-6538.

[101] a) T. Kowada, J. Kikuta, A. Kubo, M. Ishii, H. Maeda, S. Mizukami, K. Kikuchi, *Journal of the American Chemical Society* **2011**, *133*, 17772-17776; b) A. Arkader, C. D. Morris, *Cancer Imaging* **2008**, *8*, 131-134; c) P. Anderson, *Expert Opinion on Pharmacotherapy* **2006**, *7*, 1475-1486.

[102] M. McClung, S. T. Harris, P. D. Miller, D. C. Bauer, K. S. Davison, L. Dian, D. A. Hanley, D. L. Kendler, C. K. Yuen, E. M. Lewiecki, *The American Journal of Medicine* **2013**, *126*, 13-20.

[103] W. Gu, C. Wu, J. Chen, Y. Xiao, *International Journal of Nanomedicine* **2013**, *8*, 2305-2317.

[104] C. Clementi, K. Miller, A. Mero, R. Satchi-Fainaro, G. Pasut, *Molecular Pharmaceutics* **2011**, *8*, 1063-1072.

[105] a) H. Pan, M. Sima, P. Kopečková, K. Wu, S. Gao, J. Liu, D. Wang, S. C. Miller, J. Kopeček, *Molecular Pharmaceutics* **2008**, *5*, 548-558; b) K. Miller, R. Erez, E. Segal, D. Shabat, R. Satchi-Fainaro, *Angewandte Chemie International Edition* **2009**, *48*, 2949-2954.

[106] H. Chen, G. Li, H. Chi, D. Wang, C. Tu, L. Pan, L. Zhu, F. Qiu, F. Guo, X. Zhu, *Bioconjugate Chemistry* **2012**, *23*, 1915-1924.

[107] M. Salerno, E. Cenni, C. Fotia, S. Avnet, D. Granchi, F. Castelli, D. Micieli, R. Pignatello, M. Capulli, N. Rucci, A. Angelucci, A. D. Fattore, A. Teti, N. Zini, A. Giunti, N. Baldini, *Current Cancer Drug Targets* **2010**, *10*, 649-659.

[108] Y. Wen, S. Pan, X. Luo, X. Zhang, W. Zhang, M. Feng, *Bioconjugate Chemistry* **2009**, *20*, 322-332.

[109] A. Swami, M. R. Reagan, P. Basto, Y. Mishima, N. Kamaly, S. Glavey, S. Zhang, M. Moschetta, D. Seevaratnam, Y. Zhang, J. Liu, M. Memarzadeh, J. Wu, S. Manier, J. Shi, N. Bertrand, Z. N. Lu, K. Nagano, R. Baron, A. Sacco, A. M. Roccaro, O. C. Farokhzad, I. M. Ghobrial, *Proceedings of the National Academy of Sciences* **2014**, *111*, 10287-10292.

[110] E. J. Carbone, K. Rajpura, B. N. Allen, E. Cheng, B. D. Ulery, K. W. H. Lo, *Nanomedicine: Nanotechnology, Biology and Medicine* **2017**, *13*, 37-47.

[111] L. Wachsmuth, S. Söder, Z. Fan, F. Finger, T. Aigner, *Histol. Histopathol.* **2006**, *21*, 477-485.

[112] J. A. Buckwalter, H. J. Mankin, *The Journal of Bone & Joint Surgery* **1997**, *79*, 612-632.

[113] Z. Piao, M. Takahara, M. Harada, H. Orui, M. Otsuji, M. Takagi, T. Ogino, *Plast. Reconstr. Surg.* **2007**, *119*, 830-836.

[114] K. E. Kuettner, *Clinical Biochemistry* **1992**, *25*, 155-163.

[115] J. Clouet, C. Vinatier, C. Merceron, M. Pot-vaucel, Y. Maugars, P. Weiss, G. Grimandi, J. Guicheux, *Drug Discovery Today* **2009**, *14*, 913-925.

[116] T. Aigner, A. Sachse, P. M. Gebhard, H. I. Roach, *Advanced Drug Delivery Reviews* **2006**, *58*, 128-149.

[117] T. Iwakura, A. Inui, A. H. Reddi, *Arthritis and rheumatism* **2013**, *65*, 408-417.

[118] J. A. Buckwalter, H. J. Mankin, A. J. Grodzinsky, *Instructional course lectures* **2005**, *54*, 465-480.

[119] T. Aigner, J. Stöve, *Advanced Drug Delivery Reviews* **2003**, *55*, 1569-1593.

[120] R. W. Haines, A. Mohuiddin, *Journal of Anatomy* **1968**, *103*, 527-538.

[121] C. D. Hoemann, C.-H. Lafantaisie-Favreau, V. Lascau-Coman, G. Chen, J. Guzmán-Morales, *J Knee Surg* **2012**, *25*, 085-098.

[122] P. G. Bullough, A. Jagannath, *Journal of Bone & Joint Surgery, British Volume* **1983**, *65-B*, 72-78.

[123] K. P. Arkill, C. P. Winlove, *Osteoarthritis and Cartilage* **2008**, *16*, 708-714.

[124] S. Frisenda, C. Perricone, G. Valesini, *Autoimmunity Reviews* **2013**, *12*, 591-598.

[125] a) X. Ayral, *Best Practice & Research Clinical Rheumatology* **2001**, *15*, 609-626; b) D. S. Pisetsky, E. St.Clair, *JAMA* **2001**, *286*, 2787-2790.

[126] L. Setton, *Nat Mater* **2008**, *7*, 172-174.

[127] a) J. R. Levick, *Arthritis & Rheumatism* **1981**, *24*, 1550-1560; b) M. Bottini, K. Bhattacharya, B. Fadeel, A. Magrini, N. Bottini, N. Rosato, *Nanomedicine: Nanotechnology, Biology and Medicine* **2016**, *12*, 255-268.

[128] a) A. Maroudas, P. Bullough, *Nature* **1968**, *219*, 1260-1261; b) C. H. Evans, *BioDrugs* **2005**, *19*, 355-362.

[129] N. Gerwin, C. Hops, A. Lucke, *Advanced Drug Delivery Reviews* **2006**, *58*, 226-242.

[130] T. Vos, A. D. Flaxman, M. Naghavi, R. Lozano, C. Michaud, M. Ezzati, K. Shibuya, J. A. Salomon, S. Abdalla, V. Aboyans, J. Abraham, I. Ackerman, R. Aggarwal, S. Y. Ahn, M. K. Ali, M. A. AlMazroa, M. Alvarado, H. R. Anderson, L. M. Anderson, K. G. Andrews, C. Atkinson, L. M. Baddour, A. N. Bahalim, S. Barker-Collo, L. H. Barrero, D. H. Bartels, M.-G. Basáñez, A. Baxter, M. L. Bell, E. J. Benjamin, D. Bennett, E. Bernabé, K. Bhalla, B. Bhandari, B. Bikbov, A. B. Abdulhak, G. Birbeck, J. A. Black, H. Blencowe, J. D. Blore, F. Blyth, I. Bolliger, A. Bonaventure, S. Boufous, R. Bourne, M. Boussinesq, T. Braithwaite, C. Brayne, L. Bridgett, S. Brooker, P. Brooks, T. S. Brugha, C. Bryan-Hancock, C. Bucello, R. Buchbinder, G. Buckle, C. M. Budke, M. Burch, P. Burney, R. Burstein, B. Calabria, B. Campbell, C. E. Canter, H. Carabin, J. Carapetis, L. Carmona, C. Cella, F. Charlson, H. Chen, A. T.-A. Cheng, D. Chou, S. S. Chugh, L. E. Coffeng, S. D. Colan, S. Colquhoun, K. E. Colson, J. Condon, M. D. Connor, L. T. Cooper, M. Corriere, M. Cortinovis, K. C. de Vaccaro, W. Couser, B. C. Cowie, M. H. Criqui, M. Cross, K. C. Dabhadkar, M. Dahiya, N. Dahodwala, J. Damsere-Derry, G. Danaei, A. Davis, D. De Leo, L. Degenhardt,

R. Dellavalle, A. Delossantos, J. Denenberg, S. Derrett, D. C. Des Jarlais, S. D. Dharmaratne, et al., *The Lancet* **2012**, *380*, 2163-2196.

[131] a) G. D. Olsen, E. M. Chan, W. K. Riker, *Journal of Pharmacology and Experimental Therapeutics* **1975**, *195*, 242-250; b) H. Garrigue, J. C. Maurizis, J. C. Madelmont, C. Nicolas, J. M. Meyniel, A. Louvel, P. Demerseman, H. Sentenac-Roumanou, A. Veyre, *Xenobiotica* **1991**, *21*, 583-595.

[132] a) J.-C. Maurizis, M. Rapp, C. Nicolas, M. Ollier, M. Verny, J.-C. Madelmont, *Drug Metabolism and Disposition* **2000**, *28*, 418-422; b) C. Nicolas, M. Verny, I. Giraud, M. Ollier, M. Rapp, J.-C. Maurizis, J.-C. Madelmont, *Journal of Medicinal Chemistry* **1999**, *42*, 5235-5240; c) M. Ollier, J.-C. Maurizis, C. Nicolas, J. Bonafous, M. de Latour, A. Veyre, J.-C. Madelmont, *Journal of Nuclear Medicine* **2001**, *42*, 141-145.

[133] J. D. Freedman, H. Lusic, B. D. Snyder, M. W. Grinstaff, *Angewandte Chemie International Edition* **2014**, *53*, 8406-8410.

[134] C. S. Winalski, S. Shortkroff, E. Schneider, H. Yoshioka, R. V. Mulkern, G. M. Rosen, *Osteoarthritis and Cartilage* **2008**, *16*, 815-822.

[135] a) X. Hu, Q. Wang, Y. Liu, H. Liu, C. Qin, K. Cheng, W. Robinson, B. D. Gray, K. Y. Pak, A. Yu, Z. Cheng, *Biomaterials* **2014**, *35*, 7511-7521; b) H. Hyun, E. A. Owens, H. Wada, A. Levitz, G. Park, M. H. Park, J. V. Frangioni, M. Henary, H. S. Choi, *Angewandte Chemie International Edition* **2015**, *54*, 8648-8652.

[136] a) J. Cabanas-Danes, C. Nicosia, E. Landman, M. Karperien, J. Huskens, P. Jonkheijm, *Journal of Materials Chemistry B* **2013**, *1*, 1903-1908; b) H.-Y. Hu, N.-H. Lim, H.-P. Juretschke, D. Ding-Pfennigdorff, P. Florian, M. Kohlmann, A. Kandira, J. Peter von Kries, J. Saas, K. A. Rudolphi, K. U. Wendt, H. Nagase, O. Plettenburg, M. Nazare, C. Schultz, *Chemical Science* **2015**, *6*, 6256-6261; c) D. A. Rothenfluh, H. Bermudez, C. P. O'Neil, J. A. Hubbell, *Nat Mater* **2008**, *7*, 248-254; d) H.-Y. Hu, N.-H. Lim, D. Ding-Pfennigdorff, J. Saas, K. U. Wendt, O. Ritzeler, H. Nagase, O. Plettenburg, C. Schultz, M. Nazare, *Bioconjugate Chemistry* **2015**, *26*, 383-388; e) Y. Pi, X. Zhang, J. Shi, J. Zhu, W. Chen, C. Zhang, W. Gao, C. Zhou, Y. Ao, *Biomaterials* **2011**, *32*, 6324-6332.

[137] a) A. Jain, S. K. Mishra, P. R. Vuddanda, S. K. Singh, R. Singh, S. Singh, *Nanomedicine: Nanotechnology, Biology and Medicine* **2014**, *10*, 1031-1040; b) M. Bishnoi, A. Jain, P. Hurkat, S. K. Jain, *Journal of Drug Targeting* **2014**, *22*, 805-812.

[138] a) S. Fuchs, B. Dankbar, G. Wildenau, W. Goetz, C. H. Lohmann, C. O. Tibesku, *Journal of Orthopaedic Research* **2004**, *22*, 774-780; b) E. F. Morand,

P. Hall, P. Hutchinson, Y. H. Yang, *Mediators of Inflammation* **2006**, *2006*, 73835.

[139] H. Ringsdorf, *Journal of Polymer Science: Polymer Symposia* **1975**, *51*, 135-153.

[140] a) I. B. McInnes, G. Schett, *New England Journal of Medicine* **2011**, *365*, 2205-2219; b) I. B. McInnes, G. Schett, *Nat Rev Immunol* **2007**, *7*, 429-442.

[141] V. Strand, R. Kimberly, J. D. Isaacs, *Nat Rev Drug Discov* **2007**, *6*, 75-92.

[142] M. D. Wechalekar, M. D. Smith, *World Journal of Orthopedics* **2014**, *5*, 566-573.

[143] A. Katrib, H. P. McNeil, P. P. Youssef, *Inflamm. Res.* **2002**, *51*, 170-175.

[144] D. K. Rhee, J. Marcelino, M. Baker, Y. Gong, P. Smits, V. Lefebvre, xE, ronique, G. D. Jay, M. Stewart, H. Wang, M. L. Warman, J. D. Carpten, *The Journal of Clinical Investigation* **2005**, *115*, 622-631.

[145] V. C. Mow, A. Ratcliffe, A. Robin Poole, *Biomaterials* **1992**, *13*, 67-97.

[146] G. Schett, E. Gravallese, *Nature reviews. Rheumatology* **2012**, *8*, 656-664.

[147] S. M. Jung, K. W. Kim, C.-W. Yang, S.-H. Park, J. H. Ju, *Journal of Immunology Research* **2014**, *2014*, 15.

[148] S. Reimann, D. Gröger, C. Kühne, S. B. Riese, J. Dernedde, R. Haag, *Advanced Healthcare Materials* **2015**, *4*, 2154-2162.

[149] D. Gröger, M. Kerschnitzki, M. Weinhart, S. Reimann, T. Schneider, B. Kohl, W. Wagermaier, G. Schulze-Tanzil, P. Fratzl, R. Haag, *Advanced Healthcare Materials* **2014**, *3*, 375-385.

[150] S. Reimann, T. Schneider, P. Welker, F. Neumann, K. Licha, G. Schulze-Tanzil, W. Wagermaier, P. Fratzl, R. Haag, *Journal of Materials Chemistry B* **2017**, *5*, 4754-4767.

[151] M. Hu, M. Chen, G. Li, Y. Pang, D. Wang, J. Wu, F. Qiu, X. Zhu, J. Sun, *Biomacromolecules* **2012**, *13*, 3552-3561.

[152] R. A. Al-Horani, U. R. Desai, *Tetrahedron* **2010**, *66*, 2907-2918.

8 Appendix

8.1 Publications, Patents, and Conference Contributions

Publications

1. D. Gröger, M. Kerschnitzki, M. Weinhart, **S. Reimann**, T. Schneider, B. Kohl, W. Wagermaier, G. Schulze-Tanzil, P. Fratzl, R. Haag, *Selectivity in Bone Targeting with Multivalent Dendritic Polyanion Dye Conjugates,* Advanced Healthcare Materials **2014**, *3,* 375-385.
2. **S. Reimann**, D. Gröger, C. Kühne, S. B. Riese, J. Dernedde, and R. Haag, *Shell Cleavable Dendritic Polyglycerol Sulfates Show High Anti-Inflammatory Properties by Inhibiting L-Selectin Binding and Complement Activation,* Advanced Healthcare Materials **2015**, *4*, 2154–2162.
3. **S. Reimann**, T. Schneider, P. Welker, F. Neumann, K. Licha, G. Schulze-Tanzil, W. Wagermaier, P. Fratzl, and R. Haag *Dendritic Polyglycerol Anions for the Selective Targeting of Native and Inflamed Articular Cartilage*, Journal of Materials Chemistry B **2017**, *5*, 4754-4767.
4. K. Pant, J. Pufe, K. Zarschler, R. Bergmann, J. Steinbach, **S. Reimann**, R. Haag, J. Pietzsch, H. Stephan *Surface charge and particle size determine the in vitro and in vivo behavior of dendritic polyglycerols*, Nanoscale **2017**, *9*, 8723-8739.

Publications in preparation

1. P. Welker, M. Schirner, **S. Reimann**, A.-M. Laube, D. Mangoldt, J. Dernedde, I. Gemeinhardt, R. Haag, K. Licha *Sulfated dendritic polymers with improved inflammation-specific accumulation and clearance in fluorescence imaging of collagen-induced rheumatoid arthritis*.
2. H. Juch, L. Nikitina, **S. Reimann**, M. Gauster, G. Dohr, B. Obermayer-Pietsch, R. Haag *Dendritic polyglycerol nanoparticles show charge dependent bio-distribution in early human placental explants and alter hCG secretion*.

Appendix

Patents

R. Haag, **S. Reimann**, J. Dernedde, DE 2015-102015206819, WO 2016166317 A1 20161020 *Polyglycerol derivative and a method for manufacturing the same*.

Conference Contributions

1. BSRT Symposium: The Show of Regeneration: Easy to See, Hard to Foresee, Berlin (Germany), December 2012, poster presentation: S. Reimann, D. Gröger, S. B. Riese, J. Dernedde, R. Haag, *Stability of Dendritic Polyanions and their In Vitro Biological Evaluation.*
2. BSRT Symposium: Regeneration is Communication: Fireside Chats between Cells & Matrices, Berlin (Germany), December 2013, short oral and poster presentation: S. Reimann, D. Gröger, M. Kerschnitzki, N. H. Lim, K. Licha, P. Welker, H. Nagase, P. Fratzl, R. Haag, *Multivalent Dendritic Polyanions and their Selectivity Toward Bone and Cartilage*.
3. The European Calcified Tissue Society Symposium, Prague (Czech Republic), May 2014, poster presentation: S. Reimann, D. Gröger, N. H. Lim, K. Licha, P. Welker, T. Schneider, H. Nagase, P. Fratzl, R. Haag, *Selective Targeting of Bone and Cartilage with Multivalent Dendritic Polyanions*.
4. International Dendrimer Symposium, Montreal (Canada), July 2015, short oral and poster presentation: S. Reimann, D. Gröger, C. Kühne, S. B. Riese, J. Dernedde, R. Haag, *Biodegradable Dendritic Polyglycerol Sulfates Efficiently Inhibit L-Selectin Binding and Complement Activation.*
5. EuCHeMS Chemistry Congress, Seville (Spain), September 2016, poster presentation: S. Reimann, K. Pant, C. Kühne, H. Stephan, J. Pietzsch, J. Dernedde, R. Haag, *About the Anti-Inflammatory Activity and Biodistribution of Shell Cleavable Dendritic Polyglycerol Sulfates.*

8.2 Curriculum Vitae

Der Lebenslauf ist aus Gründen des Datenschutzes nicht enthalten.

8.3 Abbreviations

ΔG_N^{multi}	Total free energy difference of multivalent interactions
ΔG_{avg}^{multi}	Total free energy difference of multivalent interactions
K_N^{multi}	Binding constant of multivalent interactions
K_{avg}^{multi}	Average binding constant of multivalent interactions
K^{mono}	Binding constant of monovalent interactions
ΔG^{mono}	Total free energy difference of monovalent interactions
ΔS^{conf}	Conformational entropy
ΔS^{mono}	Total entropy of monovalent interactions
ΔG	Free energy difference
AC	Articular cartilage
ADAMTS	A disintegrin and metalloproteinase with thrombospondin motifs
APTT	Activated partial thromboplastin time
Arg	Arginine
ATP	Adenosine triphosphate
BPs	Bisphosphonates
CAM	Cell adhesion molecule
CIA	Collagen induced arthritis
CLSM	Confocal laser scanning microscopy
DLS	Dynamic light scattering
DOTAM	1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid amide
dPG	Dendritic polyglycerol
dPG-ASuS	Dendritic polyglycerol amidoglyceryl succinyl sulfate
dPGBP	Dendritic polyglycerol bisphosphonate, highly functionalized
$dPGBP_{7\%}$	Dendritic polyglycerol bisphosphonate, low functionalized
dPGP	Dendritic polyglycerol phosphate
dPGPn	Dendritic polyglycerol phosphonate
dPGS	Dendritic polyglycerol sulfate
$dPGS/BP_{7\%}$	Dendritic polyglycerol functionalized with bisphosphonate and sulfate groups
dPGSn	Dendritic polyglycerol sulfonate
dPG-TMPS	Dendritic polyglycerol thioglyceryl methylpropanoatyl sulfate
dPG-TPS	Dendritic polyglycerol thioglyceryl pentanoatyl sulfate
ECM	Extracellular matrix
ELISA	Enzyme linked immunosorbent assay

EMSA	Electrophoretic mobility shift assay
EPR	Enhanced permeability and retention
FDA	US food and drug administration
FGF-23	Fibrogrowth factor 23
Fuc	Fucose
GABA	Gamma-aminobutyric acid
GAG	Glycosaminoglycan
Gal	Galactose
GlcA	β-D-glucuronic acid
GlcNAc	2-deoxy-2-acetamido-α-D-glucopyranosyl
GlcNS	2-deoxy-2-sulfamido-α-D-glucopyranosyl
GlcNS(3S,6S)	2-deoxy-2-sulfamido-α-D-glucopyranosyl-3,6-di-O-sulfate
GlcNS(6S)	2-deoxy-2-sulfamido-α-D-glucopyranosyl-6-O-sulfate
HA	Hydroxyapatite
His	Histidine
HIT	Heparin-induced thrombocytopenia
HIV-1	Human immunodeficiency virus-1
HPMA	Poly(2-hydroxypropyl methacrylate)
HS	Heparan sulfate
i.v.	Intra venous
IC_{50}	Half maximal inhibitory concentration
ICAM-1	Intracellular adhesion molecule-1
IdoA	α-L-iduronic acid
IdoA(2S)	2-*O*-sulfo-α-L-iduronic acid
IL-1	Interleukin-1
ITC	Isothermal titration calorimetry
K_d	Dissociation constant
Le^a	Nonsialylated Lewis^X
Le^x	Nonsialylated Lewis^X
MAC	Membrane attack complex
M-CSF	Macrophage colony-stimulating factor
MMPs	Matrix metalloproteinases
MST	Microscale thermophoresis
N	Number of individual ligand-receptor interactions
nab	Nanoparticle albumin-bound
NIR	Near infrared
NMR	Nuclear magnetic resonance

NPs	Nanoparticles
NS	Number of sulfate groups
NSAIDs	Non-steroidal anti-inflammatory drugs
NTA	Nanoparticle tracking analysis
PAMAM	Poly(amido-amine)
PDI	Polydispersity index
PEG	Polyethylene glycol
PLGA	Poly(lactide-co-glycolide)
PLL	Poly-L-lysine
PRINT	Particle replication in non-wetting template
PSGL-1	P-selectin glycoprotein ligand-1
QCM	Quartz crystal microbalance
RA	Rheumatoid arthritis
RANKL	Receptor activator of nuclear factor kappa-B ligand
RES	Reticuloendothelial system
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
SEM	Scanning electron microscopy
SERMs	Selective estrogen receptor modulators
Sia	Sialic acid
siRNA	Small interfering RNA
sLe^a	Sialylated Lewis^a
sLe^x	Sialylated Lewis^X
SPR	Surface plasmon resonance
SZP	Superficial zone protein
TNFα	Tumor necrosis factor α
TRAP	Tartrate-resistant acid phosphatase
VCAM-1	Vascular cell adhesion molecule-1
β	Enhancement factor
K	Binding constant
α	Degree of cooperativity