Freie Universität Berlin

Bioinert Polyglycerol Nano- and Microgels for the Encapsulation and Controlled Release of Proteins and Living Cells

DISSERTATION

Zur Erlangung des akademischen Grades des

Doktors der Naturwissenschaften (Dr. rer. nat.)

Eingereicht im Fachbereich Chemie, Biochemie und Pharmazie

der Freien Universität Berlin

vorgelegt von

M.Sc. Dirk Steinhilber

aus Mössingen

December 2013

Diese Arbeit wurde unter Anleitung von Prof. Dr. Rainer Haag im Zeitraum von Oktober 2008 bis Dezember 2013 am Institut für Chemie und Biochemie der Freien Universität Berlin angefertigt.

1. Gutachter: Prof. Dr. Rainer Haag

2. Gutachter: Priv.-Doz. Dr. Sebastian Seiffert

Disputation findet am 17.12.2013 statt

Acknowledgements

First and foremost I would like to thank Prof. Dr. R. Haag for his scientific support within the last years. Moreover, Priv.-Doz. Dr. Sebastian Seiffert is greatfully acknowledged for co-refereeing this thesis. All the members of the Haag-group are thanked for the great support and collaborations and scientific discussions.

Furthermore, the Caldéron-Group, Seiffert-Group and Tzschucke-Group are gratefully acknowledged for hosting me in their labs. Special thanks go to Prof. Dr. Steven Zimmerman and Dr. Andrew Zill for the research stay in Urbana-Champaign. I really learnt a lot about doing chemistry in these versatile research environments.

I thank Dr. Adam Lee Sisson for introducing me in the field of nanogels and for the great collaboration.

Dr. Pamela Winchester is especially thanked for the proofreading of the manuscripts. I would like to thank Jutta Hass for her steady support with all financial issues.

I would like to acknowledge my cooperation partners Prof. Dr. D. Weitz, Dr. Sebastian Seiffert, Dr. John Heyman, M.Sc. Torsten Rossow, Dr. Stefanie Wedepohl, Prof. Dr. Wolfgang Friess, Dr. Sarah Küchler, M.Sc. Madeleine Witting, Dr. Kai Licha, Dr. Pia Welker, Dr. Dorothea Mangoldt, Prof. Dr. Regine von Klitzing, and M.Sc. Marcel Richter.

Helmut Schlaad is thanked for the donation of KLE. Special thanks go to Andrea Schulz for the patient help with the electron microscope. Furthermore, Cathleen Schlesener, Tobias Becherer and Florian Paulus are gratefully acknowledged for MALDI-TOF and GPC measurements.

The following undergraduate- and graduate students that were working under my supervision for their lab courses and thesis are kindly acknowledged: M.Sc. Torsten Rossow, M.Sc. Florian Paulus, M.Sc. Mathias Dimde, M.Sc. Michael Staegemann, M.Sc. Sebastian Hackelbusch, and M.Sc. Kerstin Paul.

I appreciate all the research facilities in the Institute of Chemistry and Biochemistry, in particularly Dr. Andreas Schäfer, Dr. Andreas Springer, and their colleagues for their numerous assistances of NMR and MS measurements.

Financial support from the German Science Foundation in form of the SFB 765 and SFB 1112 as well as the Dahlem Research School is gratefully acknowledged.

Finally I want to thank my family for all the support throughout, patience and love throughout my education.

Contents

List of Abbreviations

FDA	US Food and Drug Administration
M_n	number average molecular weight
BSA	bovine serum albumin
EPO	erythropoietin
MSC	mesenchymal stem cell
CuAAc	copper-catalyzed azide-alkyne cycloaddition
SPAAC	strain promoted azide alkyne cycloaddition
TDAC	tetrazine inverse electron demand Diels-Alder cycloaddition
ATRP	atom transfer radical polymerization
PDS	pyridyldisulfide
DIFO	difluorinated cyclooctyne
PEG	poly(ethylene glycol)
PLGA	poly(lactic-co-glycolic acid)
PVA	poly(vinyl alcohol)
dPG	dendritic polyglycerol
lPG	linear polyglycerol
PDMS	polydimethylsiloxane
HA	Hyaluronate

1 Introduction

1.1 Biotherapeutics

Therapeutic proteins and cells which continuously produce and release therapeutic biomolecules have made an enormous impact in the biomedical field. Compared to small molecule drugs, therapeutic proteins and cells are superior because of their high specificity. Moreover, they are able to perform a variety of therapeutic functions and they can produce therapeutic agents continuously, thereby preventing multiple administrations. Additionally, cells have been used for tissue engineering applications to generate complex organs which are composed of hierarchical cell assemblies on the microscale embedded in extracellular matrix that is organized on the nanoscale. Living cells and proteins, however, are sensitive objects which need to be stabilized and protected from the immune system when being applied in vivo.

Hydrogels are the most frequently used scaffolds for the stabilization of cells and proteins because their highly hydrated microenvironment is similar to their native environment. Moreover, hydrogels provide a high degree of permeability for low-molecular mass nutrients and metabolites. The following sections will focus on applications of cell-laden microgels and protein-laden nanogels, as well as challenges with respect to their preparation and potential solutions to currently unsolved problems will be presented.

1.1.1 Microencapsulation of living cells for tissue engineering

In life science one of the most crucial problems is the insufficient number of donors for organ transplantations which leads to severe health issues of patients, suffering from organ failure or damage. The interdisciplinary field of tissue engineering aims to solve these problems, combining strategies from material and medical research. The capability of living organisms to arrange and assemble cells into tissues with interior cavities and multiple cell layers, that contain micro-patterned alignments of various cell types, has inspired many researchers in polymer science to develop new biomaterials for tissue engineering.[1] The development of economic procedures which provide hierarchical control of cell arrangement and density gradients under full retention of oxygen-, nutrient- and metabolite permeability is still an unsolved challenge.[2,3] Therefore, researches of various fields try to imitate of natural tissues, which are highly organized at the micro- and nanoscale; these hierarchical architectures regulate tissue and organ function.[4] Especially the building blocks of complex organs, such

as the nephrons in the kidney, the liver lobules, and pancreatic islets, are multicellular functional units on the microscale. Interestingly, the molecular interaction between cell types controls processes such as embryonic development, blood-brain barrier formation, stem cell differentiation,[5] and tumor angiogenesis.[6]

Thus, there is an urgent need for synthetic procedures to assemble different cell types into controlled architectures. Scaffold-free approaches have been developed to generate cell spheroids[7] and cell sheets[8] which have been assembled into multi-layered 3D constructs.[9,10] The assembling process using the cell surface directly, however, generates strong forces onto the cell membrane, which can have detrimental effects on cells and their extracellular matrix. Additionally, organization with hierarchical control on the microscale is difficult using these techniques.

To encapsulate the cells in a microgel scaffold is a promising alternative approach because the hydrogel shell can be used for self-assembling process, and additionally builds a protective cellular layer. Khademhosseini et al. reported the physical self-assembly of cell-laden square shaped microgels in non-polar liquids driven by the microgels' tendency to minimize their surface area[11] and recently published a DNA directed assembly.[12] A final free radical-cross-linking process stabilized the microgel assemblies. The use of hydrophobic liquids and free radicals, however, might cause cytotoxic effects. Furthermore, on-demand degradation of the microgel matrixes to liberate and harvest the cell assemblies remains an unsolved problem.

Additionally, microgels are excellent scaffolds to study the impact of matrix-elasticity on cellular behaviour. Interestingly, the growth of tissues and organs in the human body are mainly influenced by mechanical forces exerted on and by stem cells.[13] To understand the impact of forces on cellular behavior, cells were seeded on 2D naturally derived materials and encapsulated in 3D hydrogels made from natural polymers to find the influence of matrix stiffness on cellular behavior.[14–16] Cell behavior was directed by the mechanical properties of the substrates in both cases. To get a detailed understanding of these cell-matrix interactions, however, defined synthetic need to be developed.

1.1.2 Microencapsulation of living cells for therapeutic applications

Microencapsulated cells have also been applied as therapeutics for diabetes, heart- and neurological diseases, bone and cartilage defects and cancer. Shaw et al. applied therapeutic stem cells encapsulated in biodegradable hydrogels for the treatment of brain cancer. Interestingly, the authors could show that the encapsulation of stem cells increased their

retention in the tumor, therefore permitting tumor-selective migration and release of diagnostic and therapeutic proteins in vivo. As a result, tumor growth was delayed and the survival of mice was significantly increased.[17]

Diabetes is a metabolic disorder which is generated from defects in insulin secretion, insulin action or both. The transplantation of cells which continuously produce insulin, the so called islets of Langerhans, has been proposed as a promising therapeutic approach to keep the insulin level constantly high.[18] Limited availability of cell-donating human tissues and the need for lifelong immune-suppression which results in severe side effects makes the widespread application of this therapy difficult. In 1980 Lim and Sun implanted micro-encapsulated islet cells into rats and the microencapsulated islets corrected the diabetic state for several weeks.[19] Since this groundbreaking study, numerous promising results have been reported in several animal models including rodents,[20] dogs[21] and monkeys.[22] Furthermore, clinical trials have been performed by Elliott et al.,[23] Calafiore et al.,[24] and Tuch et al.[25] In summary, these clinical trials have reported insulin secretion, however, long term correction of blood sugar control, remains as an unsolved challenge. Due to the low biocompatibility of the matrix material the foreign body response resulted in bio-film formation on the capsules that hindered the diffusion of oxygen and nutrients, and lead to cell death of encapsulated islets. To this end, improvements on long-term viability, risk of immune reactions, together with the development of highly biocompatible polymeric membranes, with sufficient permeability, should be addressed to further explore their possible clinical applications.

1.1.3 Nanoencapsulation of therapeutic proteins

Therapeutic proteins such as antibodies, cytokines, growth factors, and enzymes are playing an increasing role in the treatment of viral, malignant, and autoimmune diseases.[26,27] Since human insulin entered the market 30 years ago, the number of pharmaceutical proteins has increased significantly.[28] With more than 130 FDA (US Food and Drug Administration) approved products and many more in development, protein therapeutic proteins are a strong building block in virtually every field of medicine, like cancer treatment, inflammatory diseases, vaccines, and diagnostics.[29] Therapeutic proteins are advantageous over small molecule drugs because they are highly specific and they can perform a big number of therapeutic functions. They can catalyze a variety of biochemical reactions, form membrane receptors and channels, transport molecules within a cell or from one organ to another, and support intracellular and extracellular scaffolding; these modes of action can hardly be tackled by small synthetic compounds. Therapeutic proteins are produced using bacteria, yeast,

mammalian cells, and transgenic plants. By this means proteins can be obtained which are less immunogenic, as compared to animal-extracted proteins. Therapeutic proteins generate their mode of action from their amino acid-based primary, secondary, and tertiary structure.[30] The same time, however, the complex three-dimensional structure generates the main limitations which are the poor stability, due to proteolytic and chemical degradation as well as physical unfolding and aggregation.[31,32] This instability result in a loss of activity and often initiates an immune response.[33] Additionally, due to the harsh conditions in the stomach, oral administration is not feasible. Furthermore, the proteins' hydrophilic nature hinders the transport across biological membranes, which makes oral and transdermal administrations ineffective. As a result, therapeutic proteins usually need to be administered intravenously. By the use of this administration route, however, the fast renal clearance and consequently short half-lives are obtained, which lead to frequent injections and limit the practicability of this therapy.[34]

Additionally therapeutic proteins often suffer from insufficient stability and shelf-life, costly production, immunogenic and allergic potential, as well as poor bioavailability and sensitivity towards proteases.[35] An elegant method to overcome most of these problems is the conjugation of poly(ethyleneglycol) (PEG) chains onto the surface of the protein.[36,37] Covalent PEGylation of the native protein increases its molecular weight and as a result prolongs the half-life in vivo.[38] By the molecular weight elevation passive targeting to solid tumors can be achieved according to the enhanced permeation and retention effect.[39,40] As a result, PEGylated proteins have been approved by the food and drug administration (FDA) and four products already entered the market.

In recent studies, however, antibodies were formed upon administration of a PEGylated drugs which led to a therapy failure in some patients.[41] This was a surprising result because PEG-protein conjugates have been considered as drugs without immunogenic responses for a long time.[42,43] Additionally, PEGylation of proteins may lead to a loss of biological activity.[44] In conclusion, PEGylated therapeutic agents need to be re-examined by investigating PEG antibodies. Additionally, new biocompatible polymers need to enter the biomedical field, which can circumvent these limitations.

1.1.3.1 Protein encapsulation into PLGA nanoparticles

Nanoparticles are promising scaffolds for proteins because they can be injected and furthermore, they can release their payloads over a prolonged period of time. The most studied synthetic polymers for protein encapsulation are aliphatic polyesters, polyanhydrides,

polyorthoesters, polyphosphazenes, and poly(amino acids).[45,46] Interestingly, several formulations of poly(lactic-co-glycolic acid) (PLGA) achieved to enter the market.[47,48] Although PLGA showed some first promising results, the hydrophobicity, acidic degradation products, which generate very low local pH values down to 1.5[49] caused protein denaturations and aggregations.[50] Additionally, incomplete release and chemical reactions between proteins and polymers were obtained.[51] As a result, new delivery scaffolds need to be developed to further improve protein therapy.

1.1.3.2 Protein encapsulation into liposomes

Liposomes, that are composed of phospholipid bilayers with an aqueous core, are established delivery systems in nanomedicine including therapeutic protein drugs.[52] For the delivery of therapeutic proteins the guests can be encapsulated in the core of the liposome, surrounded by a protective lipid bilayer.[53] These delivery vesicles are considered to be non-toxic when the used phospholipids are found in mammalian cells. Despite these encouraging results problems with protein formulation and stability as well as low encapsulation efficiency, incomplete release, and poor control over release kinetics limit the practical use of these protein delivery systems.

1.1.3.3 Protein encapsulation into nanogels

These problems can be circumvented, when proteins are encapsulated non-covalently into nanogels.[54,55] Physical entrapment was employed for the incorporation of insulin in cholesterol-modified pullulan nanogels.[56] Fréchet and co-workers reported an antigen presentation in vitro by the use of pH responsive microparticles.[57–59] The authors showed that the incubation of these nanogels loaded with ovalbumin with dendritic cells derived from bone marrow resulted in enhanced presentation of ovalbumin derived peptides. Kiyono and co-workers developed nanogels for intranasal antibody delivery and vaccination. These nanogels were cross-linked by hydrophobic interactions various proteins were encapsulated.[60] Although these are encouraging results bioorthogonal encapsulation, which is required for the stabilization of sensitive enzymes, has not been achieved.

1.2 Encapsulation strategies

Within the past few years, two strategies have been developed for the encapsulation of living cells and proteins into hydrogel networks. They can be encapsulated after gel formation by the diffusion of the guest into the network due to specific interactions of the guest with the gels.[61] Strong interactions with the gel matrix, however, might cause denaturation of the encapsulated payloads and diffusion limitations lead to low encapsulation efficiencies. Additionally, the diffusion of cells into preformed hydrogels is often restricted, since the average mesh size of most hydrogels is much smaller than the diameter of a living cell. Another strategy entraps the encapsulated payloads in-situ to the hydrogel formation process, which ensures high encapsulation efficiencies and a homogenous distribution of the guest within the entire gel particle.[62] Additionally, the encapsulated payloads can be embedded very tightly in the gel matrix by tuning the degree of cross-linking. Thus, the guest can be transported to the target site without any loss of payload by leaching (Figure 1).

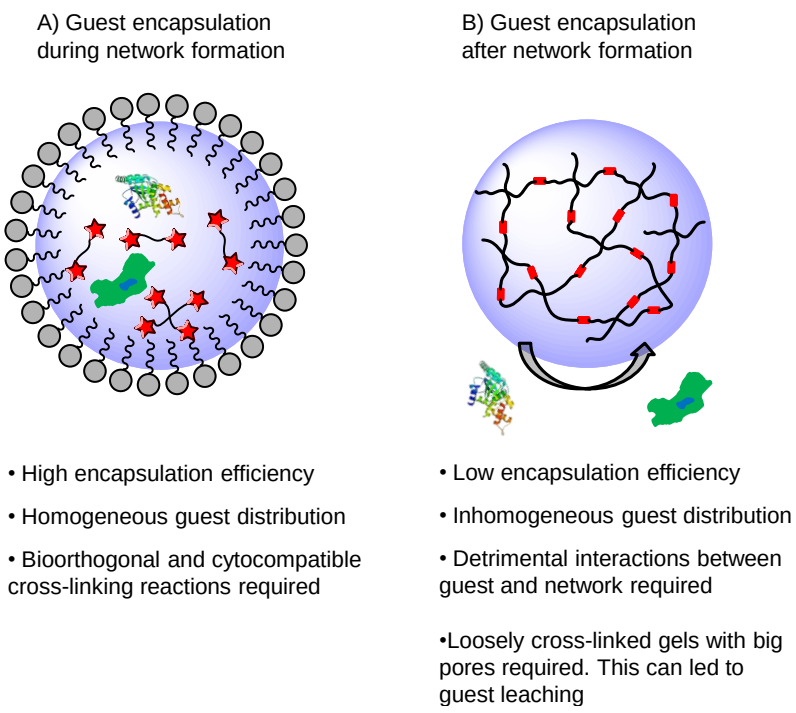


Figure 1. Comparison of the most common protein and cell encapsulation strategies. (A) Encapsulation during the hydrogel formation yields high encapsulation efficiencies and homogenous guest distributions. Bioorthogonal and cytocompatible cross-linking reactions, however, are necessary. (B) Encapsulation into pre-formed hydrogels requires strong host-guest interactions which may be detrimental to the guests. Additionally, hydrogels need to be soft and loosely cross-linked to generate big pores to allow the guest to diffuse in. This may lead to guest leaching under dilute conditions.

1.3 Biocompatible polymers as hydrogel building blocks

Hydrogels for the encapsulation of living cells and proteins have been prepared using a variety of polymeric materials, which can be divided into natural or synthetic polymers. Natural polymers such as alginate and hyaluronic acid are prominent gel matrix materials, because they are the main components of the natural extracellular matrix. These polymers, however, are mixtures which show uncontrolled interactions with biomolecules and living cells. Additionally, they are difficult to be functionalized, have poor mechanical properties and do not allow the control of degradation rates.[63] Therefore, synthetic analogues have entered the biomedical field, which generate high control of extracellular matrix ligand conjugation, mechanical properties, and pore sizes.

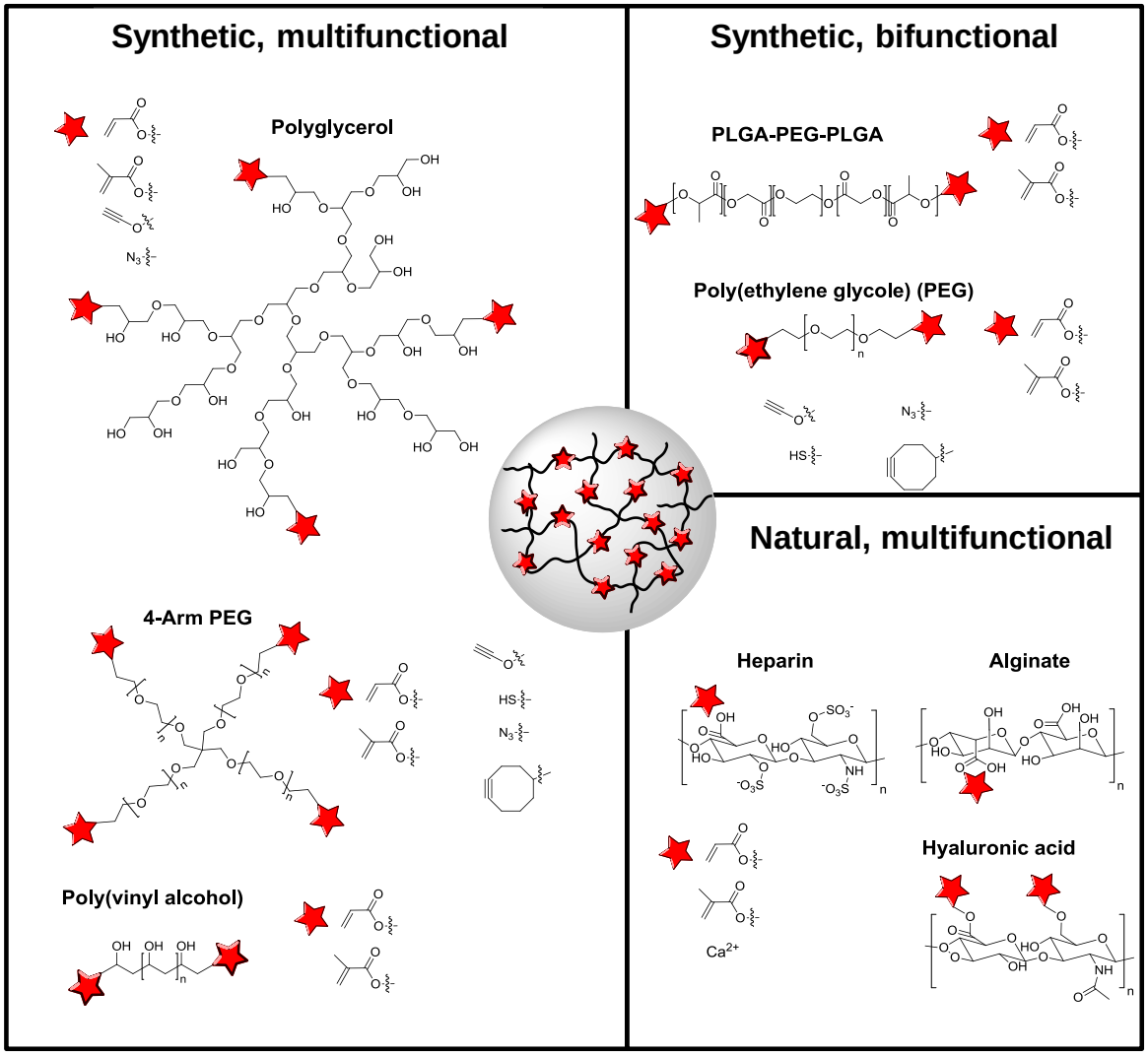


Figure 2. Classification of polymers that are used as building blocks for hydrogels according to their origin and degree of functionalization. Red stars indicate reactive functional groups for cross-linking.

1.3.1.1 Alginates

Alginate is a hydrophilic, cationic polysaccharide, which is obtained from brown algae and often consists of irregular residues. Alginate hydrogels are typically cross-linked by the inonic interactions between the carboxylic groups, located in the backbone of the polymer, and divalent cations such as calcium. The degree of cross-linking in such supramolecular gels, however, is highly concentration dependent and dynamic. Under high dilutions, which are typically present in drug delivery and tissue engineering applications, the cross-links brake in a non-controlled manner and the gel dissolves. Covalent gelation with various crosslinkers, such as adipic acid dihydrazide and lysine, can be employed to overcome this uncontrolled degradation. A lack of cell-specific interactions, however, can limit the use of alginate hydrogels in bioengineering applications. Moreover, biodegradation products of alginates are typically above the renal clearance threshold of the kidney.[64] Mooney and co-workers, however, found that partially oxidized alginate, which undergoes hydrolytic biodegradation, can be utilized to overcome these limitations.[65] Alginate microgels were loaded with various mammalian cell lines under high retention of cell viability.[66,67] The formation of secondary particles during the ionic cross-linking process, however, generated alginate microgels with increased polydispersity.[68]

1.3.1.2 Hyaluronic acid

Hyaluronate (HA) is a biodegradable polysaccharide which decomposes in the presence of hyaluronidase and can be oxidized. HA is one of the main extracellular matrix building blocks and plays an important role in various biological processes, including wound healing, angiogenesis, and activation of various signaling pathways that direct cell adhesion, cytoskeletal rearrangement, migration, proliferation, and differentiation.[69] Batch-to-batch variation and the possibility of contamination with endotoxins and pathogenic factors, however, limit the biomedical applicability of this material. Furthermore, the rapid and uncontrolled degradation of HA in the presence of hyaluronidase can hinder its usefulness in certain applications. Nevertheless, HA-based hydrogels have been used for tissue regeneration and sustained therapeutic delivery.[70]

1.3.2 Synthetic polymers

1.3.2.1 Poly(ethylene glycol)

Poly(ethylene glycol) (PEG), is a hydrophilic and uncharged polymer, which is well known for its protein resistance. PEG is traditionally prepared by the anionic polymerization of ethylene oxide. By the initiator selection homo- and heterofunctional linear polymers can be prepared. Additionally, PEGs can be post-functionalized via their hydroxyl end groups to obtain numerous homofunctional or heterofunctional terminal groups, including thiols,[71] vinyl sulfones,[72] maleimides,[73] acrylates,[74] allyls.[75] As a result, the PEG hydrogels have been widely used for cell- and protein encapsulation.[76,77] The excellent biocompatibility and low toxicity of PEG-based hydrogels make them ideal candidates for various biomedical applications, and PEG-containing formulations have been approved by the FDA for several medical applications, such as therapeutic protein conjugates.[78] PEG, however, induces immunogenic body reactions which make its wide use in biomedical applications questionable (section 1.1.3).

1.3.2.2 Poly(vinyl alcohol)

Poly(vinyl alcohol) (PVA) is commercially available and prepared by partial or complete hydrolysis of poly(vinyl acetate). PVA is a multifunctionl polymer with pendant hydroxyl groups in the backbone that can be used for bioconjugation. Due to its low protein adsorption and excellent biocompatibility, PVA has been applied for the preparation of soft contact lenses, eye drops, tissue adhesion barriers, and cartilage replacement applications.[79,80] Incomplete hydrolysis of acetate groups in the polymer backbone, however, very often created solubility problems, which limited the widespread applicability of this synthetic polymer for many biomedical applications. These problems, however, have been overcome recently and PVA might become one of the most promising polymeric materials for biomedical applications.

1.3.2.3 Polyglycerol

The polyglycerols belong to the class of a non-cytotoxic polymers which possesses excellent properties for many biomedical applications.[81,82] Polyglycerols are well suited to be used as hydrogel materials because of their protein resistance.[83,84] Minimal interaction with the polymer matrix provides maximal protein stabilization. Additionally, the rigidity of dendritic macromolecules generates high diffusion barriers for the encapsulated proteins, thereby

facilitating stable transport behavior. The polymerization process has been theoretically modeled which provides high control over molecular weight, molecular weight distribution and degree of branching of the targeted polymers.[85,86] Interestingly, polyglycerols posses multivalent hydroxyl functionality that allows its surface to be modified with bioactive substances, while the polymer remains water soluble.[52,81,87] Furthermore, the degree of branching can be freely adjusted by the choice of polymerization conditions.[88–90] As a result, dendritic (dPG) and linear polyglycerol (lPG) can be obtained, which are in combination very useful hydrogel building blocks. Hydrogel parameters such as degree of cross-linking and degree of functionalization can easily be adjusted. Moreover, Brooks and co-workers found that dPG and lPG are even less cytotoxic than the structurally similar, well established and FDA-approved PEG.[91–93] dPG is traditionally prepared by anionic, ring-opening multibranching polymerization of glycidol under slow monomer addition. These conditions yield polymers with a narrow polydispersity and a number average molecular weight M_n of up to 20 kDa.[90] Recently, Brooks and co-workers reported a molecular weight extension of dPG up to 1 MDa, which corresponds to a hydrodynamic diameter of 10 nm by the use of an emulsion type polymerization.[94] In addition, Hennink and colleagues used dPG macromonomers to prepare cell-laden hydrogels by a free-radical photo-polymerization.[95,96] The same polymerization method was also applied in soft- and photolithography as well as in micromolding to prepare microparticles.[97] However, despite their utility for some applications, dPG macro- and microgels prepared by these methods exhibit a low degree of swelling, which is unfavorable for many biomedical purposes. Hence, new methods are required for the preparation of defined dPG-networks on multiple length scales.

1.4 Crosslinking chemistry

Because ideal loading efficacy and homogenous guest distribution in the gel network can only be achieved when the gelation reaction is performed in presence of the guest, the reactions need to fulfill many requirements. In an ideal case these reactions are fast at 37 °C, catalyst free and bioorthogonal. Although ionic cross-linking is also a very useful gelation strategy, this section focuses on covalent cross-linking, since the poor stability of ionic and supramolecular cross-links under physiological conditions remains challenging.

1.4.1 Bioorthogonality in chemistry

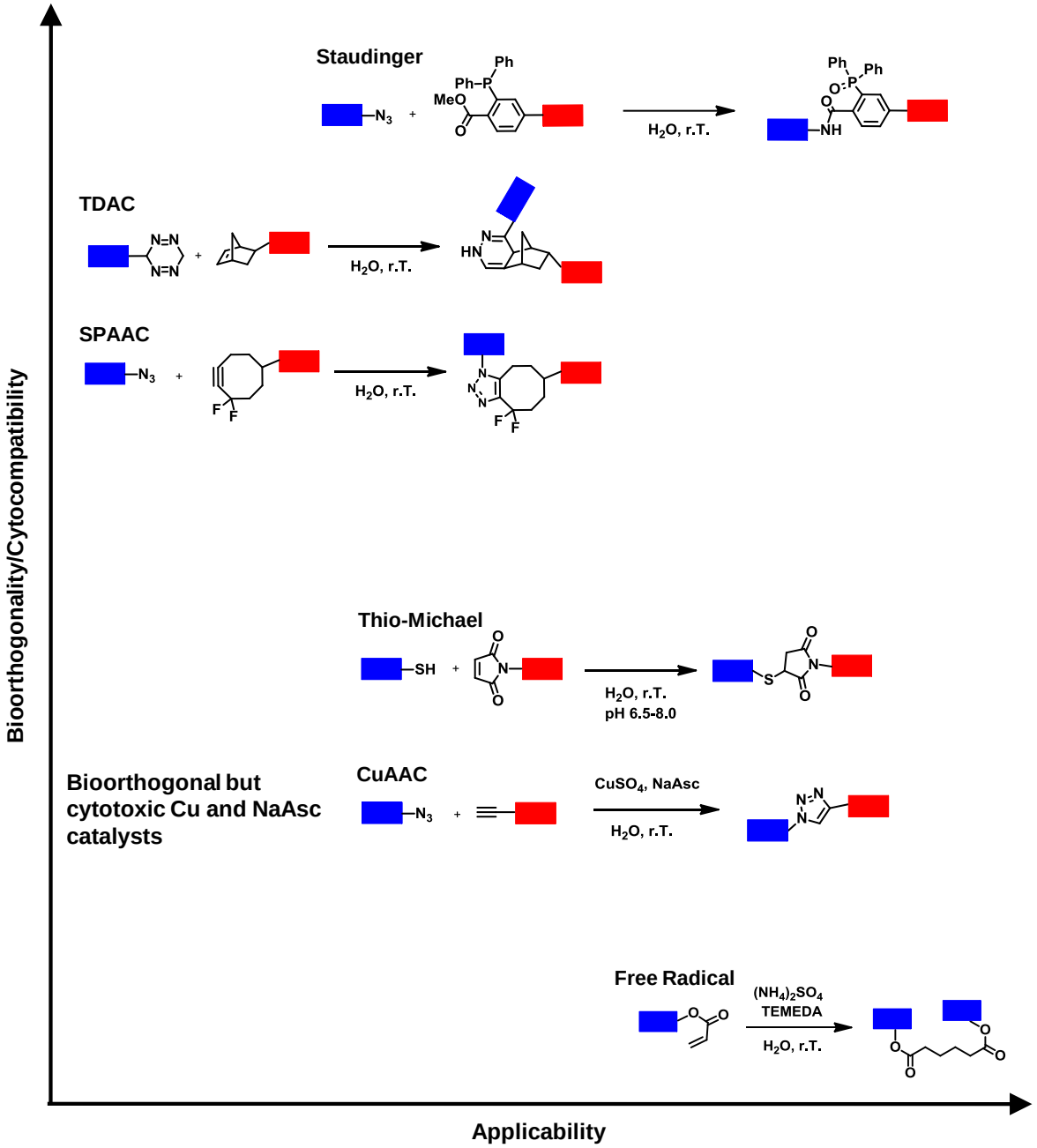
The concept of bioorthogonal reactions has been introduced by Carolyn Bertozzi in 2003. Bioorthogonal reactions are defined as reactions that do not react and interact with functional groups in biological systems. Additionally, the functional groups that participate in the bioorthogonal reaction, must selectively react with each other under conditions, which are non-toxic to cells and organisms. Furthermore, the reactive groups should be small to prevent unwanted physical interaction with the biological system. A prerequisite for a reaction to be bioorthogonal is that this reaction does not occur in living systems. Additionally, the stability of reactants and products in water, the reaction kinetics, unwanted side reactivity with biofunctionalities, and the possibility to install the functional groups synthetically must be critically addressed.[98–100]

1.4.2 Free radical

Hydrogel formation by free-radical cross-linking offers several advantages over other cross-linking strategies and is therefore the most frequently used crosslinking strategy.[101] Reactive groups that can be cross-linked by free radical polymerization such as acrylates and acrylamides can be introduced into monomers or macromonomrs using convenient ester and amide coupling strategies. Furthermore, the free radical gelation reaction can be performed by the use of various initiation triggers including light, temperature and redox-conditions. Photoinitiation is most suitable for hydrogel formation in presence of biological systems because reaction kinetics and radical concentration can be controlled by the light intensity. As a result, the formation of hydrogels with spatiotemporal control over cross-link density and bio-functionalization, which is known to influence the fate of biological system, can be achieved.[102]

Radicals, however, are highly reactive species which might be transferred to biological systems and therefore cause damage to them. Additionally, free-radical reactions are highly exothermic thereby potentionally leading to a local increase in temperature, which can cause protein denaturations and light scattering causes low conversions and cross-link density in deep hydrogel layers.[103] Furthermore, acrylates are strong Michael-acceptors which might react with thiols from terminal cysteins located on the cell membrane. Lin et al. showed that the radical cross-linking reaction is not bioorthogonal because the gelation reaction was the cause of incomplete bovine serum albumin (BSA) release from PEG hydrogels due to covalent grafting during the gelation reaction.[104] Furthermore, Encinas et al. found, that BSA

can act as a chain transfer reagent during radical cross-linking.[105] In a very elegant study, Dhert et al. studied the viability and proliferation of mesenchymal stem cells (MSCs) monolayers after exposure to UV-light in the presence of a photo-initiator. The authors found severe effects of photo-polymerization on viability, proliferation and reentry into the cell cycle of the exposed cells in monolayers.[106]



Scheme 1. Classification of gelation reactions according to their bioorthogonality/cytotoxicity and commercial availability.

Nevertheless many cell lines including human MSCs,[107–109] NIH-3T3 fibroblasts[110,111] cardiomyocytes[112] and proteins such as horseradish peroxidase[113], lysozyme[114], BSA and alkaline phosphatase[115] have been encapsulated into hydrogels retaining high viabilities and activities by the use of free-radical chemistry.

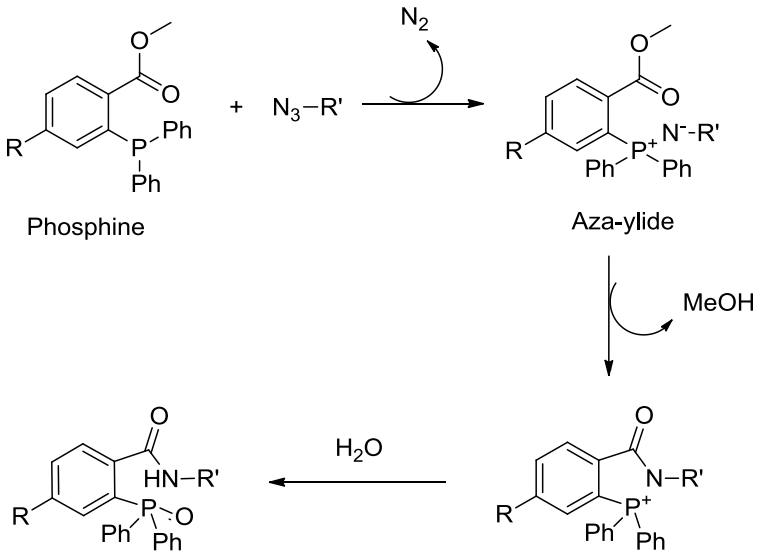
In summary, radical gelation reactions were intensively used for the encapsulation of living cells and proteins into hydrogels because of the commercial abundance and convenient reaction conditions. To satisfy the future challenges in drug delivery and tissue engineering, however, more bioorthogonal, cyto- and, protein compatible cross-linking chemistries need to be applied.

1.4.3 Thio-Michael addition

Thio-Michael addition reactions can be conducted in aqueous medium, at room temperature, and at physiological pH, which makes them well suited for the preparation of cell- and protein encapsulated hydrogels. This reaction is based on the nucleophilic addition of a thiol to a Michael-acceptor usually an acrylate, maleimide or a vinylsulfone. Within the last years the thio-Michael reaction is appearing as a promissing cross-linking chemistry due to the high chemoselectivity of Michael acceptors for thiols and convenient chemistries to install the cross-linkable groups. Additionally, Michael-type additions generate more regular networks than gels prepared by free-radical cross-linking due to the reduced amount of side reactions.

Thiols and Michael acceptors such as acrylates and maleimides, however, can react with thiols located on cell- and protein surfaces[116,117] and are therefore not bioorthogonal. Nevertheless, this type of cross-linking reaction that has been pioneered by Hubbell and coworkers and lead to various hydrogels using acrylates,[118,119] and vinyl sulfones[120,121] as Michael acceptors. Although the high reactivity of the Michael-acceptors showed cross-reactivity with terminal cysteins,[121] this chemistry has been used to prepare cell- and protein laden hydrogels. Erythropoietin (EPO), which is a therapeutic protein, has been encapsulated into PEG hydrogels by thio-Michael chemistry showing activity both in vivo and in vitro.[122,123] Additionally, C2C12 murine myoblasts,[124] hMSCs[125,126] into PEG-based hydrogels has been under retention of term viability using this chemistry. In conclusion cross-linking by thio-Michael addition has emerged as a powerful tool for cell- and protein encapsulation. For applications, however, where side reactions between the gel building blocks and the living cells or proteins need to be prevented, more bioorthogonal reactions have to be established.

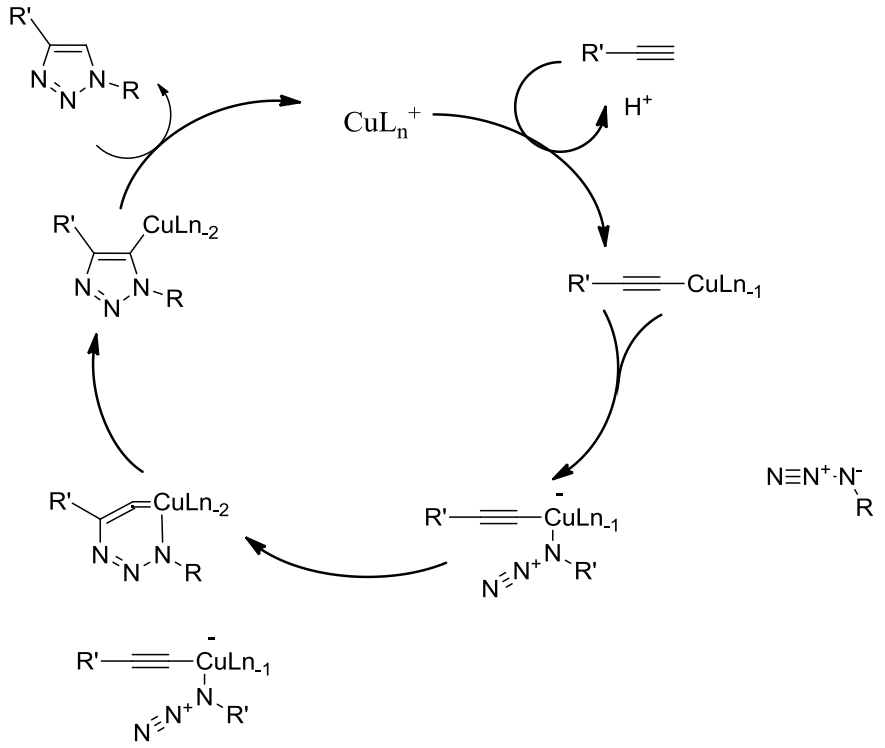
1.4.4 Staudinger ligation



Scheme 2. Schematic representation of the Staudinger ligation.

The Staudinger reaction, which has been introduced by Hermann Staudinger in 1919[127] is the reaction between organic phosphines and azides. This conversion is particularly useful because both functional groups are absent in living systems, therefore they fulfill the criteria of being bioorthogonal. Additionally azides are inert in biological systems, and easy to be installed synthetically. The first step of this reaction is the nucleophilic attack of the phosphine at the azide in an highly bioorthogonal manner thereby forming a phosphorous nitrogen bond called aza-ylide bond. Because this bond is not stable under physiological conditions and quickly hydrolyzes, Bertozzi et al. modified the phosphine groups with an ester group in close proximity. As a result, the nitrogen nucleophile immediately attacks the ester to form a stable amide bond which enabled the authors to selectively label cell surfaces with functional phosphines in vitro[128] and in vivo.[129] Although this reaction appears as useful tool for various bioconjugations applications[130–134] only a few hydrogels have been prepared using the Staudinger reaction as gelation chemistry.[135–137] Downsides of this reaction are the slow reaction kinetics and the enzymatic phosphine oxidation.

1.4.5 Cu-catalyzed azide-alkyne cycloaddition



Scheme 3. Cu catalyzed azide-alkyne cycloaddition.

The Huisgen 1,3-dipolar cycloaddition between azides and alkynes to form a triazole bond is one of the most explored organic reactions.[138] Slow reaction kinetic, however, limited the applicability of this reaction in chemical biology until the research groups of Meldal and Sharpless independently discovered the copper-catalyzed azide-alkyne cycloaddition (CuAAC), which proceeded at room temperature.[139,140] Sharpless et al. selected the CuAAC as one of the most perfect reactions which they defined as click chemistry. Such reactions must be high in yield, generate no- or inoffensive by-products, high thermodynamic driving force, readily available starting materials, and a benign solvent such as water.[141] Because azides and alkynes are functional groups which are not present in living organisms and are inert in biological media, CuAAC also meets the criteria of being bioorthogonal.[142] However, these procedures are critical for the encapsulation of sensitive biomolecules, because cytotoxic copper ions can damage them.[143,144] Apart from that, copper contaminations may induce oligonucleotide[145] and polysaccharide degradation.[146] Furthermore, alkyne homo-coupling reactions were reported thus limiting the application of this reaction in the polymer science field.[147] Nevertheless, CuAAC has been extensively used for bioconjugation reactions, polymer and dendrimer synthesis[148,149] and also for the encapsulation of yeast cells[150] into 3D hydrogels and for 2D cell culture on top of

hydrogels.[151] In summary, the CuAAC possesses high potential as cross-linking reaction for the encapsulation of biological systems into hydrogels. Toxic copper-contaminations, however, severely limit its applicability in the biomedical field when purifications such as chromatography are not possible.

1.4.6 Strain promoted azide-alkyne cycloaddition

In 1961 Krebs and co-workers found that the cycloaddition between phenylazide and cyclooctyne proceeds as a highly exothermic and fast reaction at room temperature.[152] The reaction rate enhancement of cyclic alkynes compared to the rates obtained using linear alkynes can be explained by the ring strain which is generated, when alkynes are located in an 8-membered ring. As a result, the activation energy for the reaction is significantly reduced thereby strongly accelerating the reaction without the need of a catalyst. Bertozzi and co-worker realized the high potential of this strain promoted azide-alkyne cycloaddition (SPAAC) and screened the reaction rates of several cyclooctynes.[153] They found that the SPAAC of cyclooctynes with two fluorine atoms next to the alkyne (DIFO) is about 15 times faster than the Staudinger reaction, therefore improving the shortcomings of this reaction. Interestingly, the authors were now able to fluorescently label cell membranes in vitro[154] and in vivo.[155]

The broad application of metal-free cycloaddition in biomedical applications, however, is limited by tedious synthetic routes for preparation of cyclooctynes. The synthesis of a second generation DIFO, for example, requires eight synthetic steps.[156] van Delft and co-workers reduced the amount of steps to 4 in a very elegant work. The same time they increased the cyclooctyne reactivity towards azides by introducing additional ring-strain by incorporation of a cyclopropane ring to the cyclooctyne. Additionally, the lipophilicity of this new class of cyclooctynes, which is known to reduce bioorthogonality, was low compared to the cyclooctynes prepared by Bertozzi and co-workers.[157] Cross-reactivity of these highly reactive cyclooctynes with thiols has been observed,[158,159] which prevents complete bioorthogonality of this reaction because many proteins and cells contain free thiols on their surface.[117] Because the reaction between azides and cyclooctynes is much faster than the addition of thiols to cycloooctynes, however, SPAAC is well suited for the encapsulation of cells[160,161] and proteins into hydrogels. Anseth and co-workers were the first to apply SPAAC for hydrogel formation in presence of fibroblasts and obtained good cell viabilities of more than 90%, which was observed 24 h after encapsulation.[162]

1.4.7 Tetrazine inverse electron demand Diels-Alder cycloaddition

Tetrazine inverse electron demand Diels-Alder cycloaddition (TDAC) has first been reported by Carboni and Lindsey who found that tetrazines reacted rapidly at room temperature with a variety of unsaturated compounds without the use of a catalyst.[163] The reaction starts with the [4+2] Diels-Alder cycloaddition between tetrazines and numerous dienophiles to form the cycloadduct that immediately undergoes an irreversible retro Diels-Alder step under the release of nitrogen.[164]

Sauer and co-workers screened the cycloaddition reaction kinetics of various tetrazines with numerous dienophiles such as alkynes, alkenes, donor-substituted and unsubstituted cycloalkenes.[165–167] Interestingly, they reported that the dienophile mainly influences the reaction rate constant, which can vary over 9 orders of magnitude. Internal olefins react slowly with tetrazines, which is an important requirement to prevent the side reaction with cis-alkenes of the cell membranes. Sauer reported a rate constant of 12700 $M^{-1}\cdot sec^{-1}$ for the highly strained olefin *trans*-cyclooctene.[167] In contrast, *cis*-cyclooctene was reported to react with a rate constant of 0.03 $M^{-1}sec^{-1}$ under the same conditions. Additionally, the accessibility of cis-alkenes in cell membranes is hindered; therefore the selectivity of TDAC is increased. Although TDAC is not completely bioorthogonal, the big difference in the rates of main- and side reaction makes TDAC well suited for the encapsulation of proteins and living cells into nano- and microgels. Anseth and co-workers were again the first to prepare stem cell-laden PEG-hydrogels using TDAC as cross-linking chemistry. Furthermore, TDAC has been applied for in vitro- and in vivo imaging[168–170]

1.5 Preparation of nano- and microgels by gelation in nano- and microreactors

For the preparation of nano- and microgels reactive monomers and macromonomers are loaded into nano- and microreactors, which are usually, emulsion droplets or cavities generated by soft-lithography.[171] After cross-linking the macromonomers inside of these nano- and micro-templates gel particles are formed which have the same size and shape as the template. Additionally, self assembly of the macromonomers can be used to prepare nano-and microgels. The choice of the templation method is crucial for the encapsulation of sensitive biological systems such as living cells and proteins, because strong mechanical forces might rupture cell membranes and the complex 3D structure of proteins might be destroyed. Additionally, cytotoxic solvents and other harmful additives, such as surfactants, should not

get into direct contact with encapsulated guests to avoid detrimental effects. Furthermore, the choice of templation method directly influences the properties of the prepared gels, such as particle size, degree of cross-linking and distribution of degree of cross-linking. Therefore, a careful selection of the templation method needs to be performed.

1.5.1 Mini- and microemulsion droplet gelation

Nanogels are usually prepared by the templation of reactive monomers on the nanometer scale and subsequent crosslinking of the templates to obtain hydrogel nanoparticles. The most frequently used methods are templations in mini[172–175]- and microemulsion droplets[176–178]. High energy input by ultrasonication, which is required for the formation of miniemulsions, makes the encapsulation of labile compounds difficult using this technique. The formation of microemulsions requires high surfactant loadings, which lead to purification problems and thereby also limit applications of this technique. Due to the fact that for a lot of applications nanogels with a defined size and monodisperse size-distribution are crucial the concept of nanoreactors was established, where small nano-sized droplets, well separated from each other, serve as reactors for chemical reactions. The size of the created nanoparticles are generally 1:1 copies of the dispersed droplets.[179,180] Stable nanoreactors can be achieved by suppressing all processes which destabilize emulsions. These processes are coalescence and Ostwald ripening. Preventing coalescence by collision of the dispersed droplets can be achieved by appropriate surfactants, which are normally in the direct case ionic surfactants (electrostatic stabilization) and in the inverse case block-co-polymers (steric stabilization).[181] The Ostwald ripening, depending on droplet size, polydispersity and solubility of dispersed-in continuous phase, describes the molecular diffusion from small to big droplets due to the higher Laplace pressure in the small droplets.[182] It was reported that the Ostwald ripening can be suppressed by addition of small amounts of a third component, which mostly dissolves in the dispersed phase and balances the Laplace pressure by the osmotic pressure created in the dispersed droplets. Emulsions which consist of two immiscible liquids, a surfactant and an osmotic pressure agent, are called miniemulsions.[183] Under intense shearing of the biphasic system, monodisperse and stable dispersed droplets are created in which the Laplace pressure and osmotic pressure are counter-balanced (Figure 3).[184]

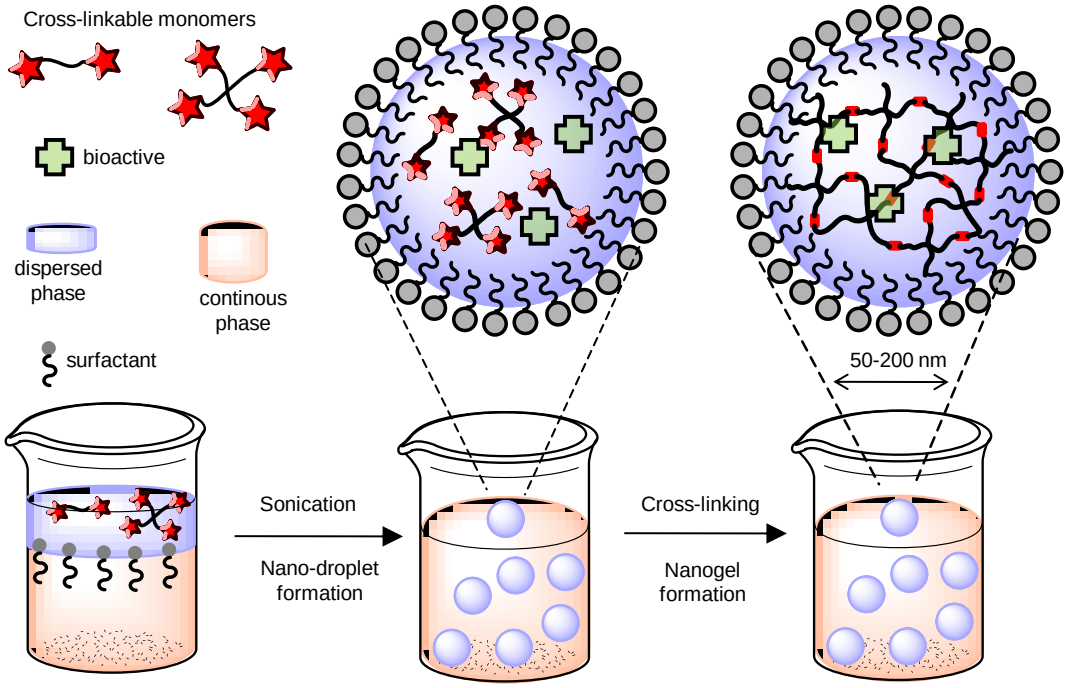


Figure 3. Schematic representation of the preparation of nanogels by miniemulsion droplet gelation.

With this system an almost 1:1 copy of the dispersed droplets and the created nanoparticles can be obtained and reactions only proceed via droplet nucleation in the nanoreactors without any mass transport.[185] Both direct and inverse miniemulsions can be prepared with water as polar phase but also polar aprotic organic liquids can be used[186] The preparation of miniemulsions starts from a biphasic mixture which is first rapidly stirred and then treated to strong mechanical stress, whereby tip sonicators are used for lab scale and high pressure homogenizer for industrial scale. This treatment causes constant fusion and fission of the dispersed droplets and the particle size distribution decreases until an equilibrium size is reached.[187] The high stability of miniemulsion was demonstrated by the suppression of the formation of Prussian blue inside dispersed droplets. Separately two inverse miniemulsions were prepared, one having $FeCl_3$ and the other $K_4[Fe(CN)_6]$ dissolved in the droplets. The two miniemulsions were mixed and there was no blue colour change on the timescale of most chemical reactions obtainable which proves that miniemulsions have the characteristics of perfect nanoreactors. Sonication of the emulsion induced mixing of the nanodroplets and the blue colour appeared immediately.[188]

Matyjaszewski and co-workers atom transfer radical polymerization (ATRP) in inverse minemulsion droplets to encapsulate various molecules including such as gold nanoparticles, bovine serum albumin, rhodamine B isothiocyanate-dextran, or fluoresceine isothiocyanate-dextran into PEG-based nanogels.[189] Although this is a promising approach, the use of free

radicals, heavy metal catalysts and strong energy input by ultrasonication might have prevented the encapsulation of sensitive therapeutic proteins.

If the surfactant concentration of a macroemulsion is greatly increased, a thermodynamically stable microemulsion,[190] consisting of a polar phase, nonpolar phase and a surfactant which form discrete nanodroplets or sponge-like phases, is created. Droplet diameters in microemulsions are extremely small and usually lay in between 10 and 100 nm. The small droplets scatter the light only to a tiny amount and microemulsions appear as optically transparent or translucent. The droplets are completely covered with surfactant molecules which cause a surface tension close to zero and thermodynamic stability. For the preparation of microemulsions often a co-surfactant (alcohols of middle chain length) is used which localizes in between the dispersed and continuous phase and further decreases the surface tension.[191] Direct- (oil dispersed in water) and indirect (water dispersed in oil) microemulsions can be used for polymerizations to produce hydrophobic and hydrophilic polymeric nanoparticles respectively. The diameter of these particles can be varied between 5 and 100 nm with narrow polydispersities.[192] Because of the thermodynamic stability, (unlike macro- and miniemulsion) the initial state before polymerization depends only on composition and temperature and not on the preparation method.

In the reaction kinetics of microemulsion polymerizations, the constant rate interval, as compared to macroemulsions, is missing and particles are generated continuously by homogenous- and micellar nucleation.[193] In the micellar nucleation mechanism an initiator molecule starts the polymerization in a monomer swollen micelle. The reaction proceeds by monomer diffusion from uninitiated monomer swollen micelles. That's why the created nanogels are much bigger as compared to the monomer swollen micelles. Because this process is diffusion controlled, rather polydisperse nanogels are generated. DeSimone et al. reported an inverse microemulsion co-polymerization of 2-acryloxyethyl-trimethyl-amoniumchloride, 2-hydroxyethylacrylate and poly(ethylene glycol) diacrylate to form monodisperse and biocompatible nanogels as vectors for DNA and oligonucleotides. The size of the formed nanogels was manipulated by varying the crosslinker concentration over a wide range (30-80 nm).[176] For the preparation of microemulsions, however, large quantities of surfactant are required, which hinders this technique to be applied for the encapsulation of sensitive biomacromolecules into nanogels.

1.5.2 Soft lithography

Particle Replication in non-wetting templates is a powerful approach to prepare nano- and microgels which has been by the De Simone group[194] This technique is based on the preparation of a master template by soft lithography[171] Then a liquid fluoropolymer is poured on the surface of the master template and photochemically cross linked, and peeled away. By this means precise molds are generated that have micro- or nanoscale cavities which are filled with macromonomer solutions. Due to the high surface tension between the perfluorinated scaffold and the macromonomer solution, the cavities are filled only. Then the macromonomers are cross-linked by external triggers such as UV-light. The array of particles can be removed by bringing the mold in contact with a harvesting film. Finally, free flowing particles can be obtained by separating the harvesting film from the particles.[194] Using this technique, deSimone et al. prepared hemoglobin loaded microgels which mimic human red blood cells[195] and BSA loaded microgels that release their payloads in reducing environments.[196] Hennink et al. recently described the synthesis of dPG microgels by utilizing micromolding, soft micromolding and photolithography and final photocuring of dPG methacrylate monomers.[197] Soft lithography techniques, however, require cross-linking reactions that are initiated by external triggers. The reactions involve very often cytotoxic radicals which are therefore not suitable for the encapsulation of sensitive biomolecules.

1.5.3 Cross-linked micelles

Cross-linked micelles are prepared by the use of the controlled self-assembly behavior of amphiphilic block copolymers whereby they tend to aggregate on the nanometer scale (Figure 4). To improve the concentration-dependent stability of these nano-aggregates, chemical cross-linking is carried out within the polymer chains to generate stable nanogels. Since the aggregation is performed in water, this nanogel preparation proceeds under mild conditions which enable the entrapment of labile, therapeutically relevant agents into the gel network.
In an very interesting work van Hest et al have recently shown, that micelle shape changed from rod-like to spherical upon micelle gelation by strain promoted azide-alkyne cycloaddition reactions.[198] Structurally, these aggregates can be broadly classified as either core cross-linked[199–205] or shell cross-linked micelles.[206–208] Thayumanavan and coworkers prepared random copolymers that contained oligo(ethylene glycol) and pyridyldisulfide (PDS) units as side-chain functionalities[201].

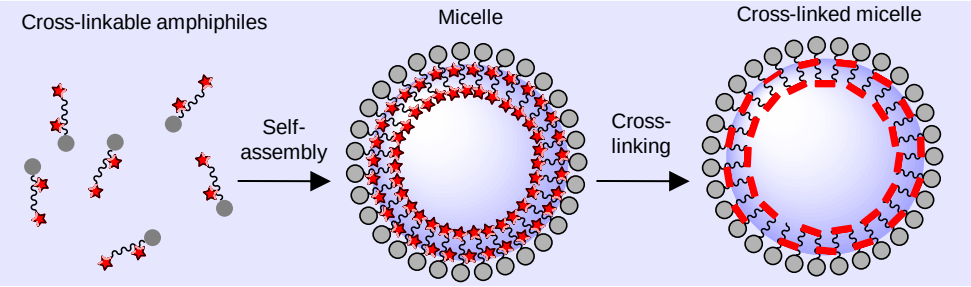


Figure 4. Nanogel formation by micelle gelation.

These copolymers self-assembled in aqueous solution and the PDS cores were cross-linked by the addition of a catalytic amount of dithiotreitol. Interestingly, the authors showed that encapsulated guests increased their retention time within the micelle structure with increasing cross-linking density. Even though this approach is often called “surfactant free” in literature, amphiphilic polymers are required which might interact and denature the encapsulated payloads. Additionally, material parameters like size, elasticity and shape are difficult to influence. The aggregation behavior, however, of amphiphilic block copolymers is difficult to manipulate and makes the control of nanogel material properties, like variation of size, shape, and elasticity, difficult.

1.5.4 Nanoprecipitation

Polymeric nanoparticles can be fabricated by nanoprecipitation, which is a facile, mild, and low energy input process. Nanoparticle formation via nanoprecipitation starts from the nucleation of small aggregates of macromolecules and is followed by aggregation of these nuclei, which stops as soon as the colloidal stability is reached. Dinegar et al. have proposed a theory, which describes the nucleation in supersaturated solutions into nanodispersions.[209] Entanglements between the polymer chains, which prevent nanoparticle formation need to be suppressed. This can be achieved by the use of low molecular weight and branched polymers. The resulting nanoparticle size at the end of aggregation correlates with the polymer concentration. There is a critical concentration, where the polymer solution is sufficiently dilute to be metastable resulting in nucleation of the polymers, is the so-called ‘‘ouzo’’ region. This effect is named by the Greek aperitif that becomes cloudy by spontaneous emulsification of water and anethole.[210]

The nanoprecipitation technique has only be applied for the preparation of hard and non-polar nanoparticles built from polystyrene,[211] poly(methyl methacrylate) [212,213], and PLGA.[211,214] These materials, however, are not suitable for the encapsulation of labile

bioactives, because they lead to denaturations of the encapsulated guests. Therefore, new procedures need to be developed, which allow the preparation of protein loaded nanogels.

1.5.5 Microfluidic droplet gelation

Microfluidic emulsion-droplet templating is a powerful approach to prepare monodisperse cell-laden microgels.[215–218] For droplet microfluidics either glass capillary devices can be used or devices made by lithography techniques, commonly consisting of polydimethylsiloxane (PDMS).[219] PDMS devices are advantageous when aqueous solutions need to be emulsified because soft lithography complex flow channels, which are required for cell encapsulation, can be fabricated. Moreover, by the use of lithography a large number of devices can be made.[220] Monodisperse droplets can be generated by the use of a flow focusing microfluidic device, into which aqueous macromonomer precursor solutions and the cells are injected separately. Because the cells are injected into the middle inlet this stream can act as a diffusion barrier for the reactive macromonomers and therefore prevents pre-gelation in the channels. At the first cross-junction, these three fluids formed a laminar coflowing stream in the microchannel. This stream is broken to form monodisperse pre-microgel droplets at the second cross-junction by flow focusing with immiscible paraffin oil. The droplet formation induces a rapid mixing of all the components inside the droplets which leads to a subsequent cross-linking of the macromonomers. As a result, cell encapsulated microgels with the same size and spherical shape as the droplets are generated.

Takeuchi et al. prepared mammalian cell-laden alginate microgels without loss of cell-viability using microfluidic droplet gelation. Interestingly, the authors could show that their approach generated microgels with high uniformity, and also a higher control over size and shape of the microbeads compared to other techniques was achieved.[221] Kumacheva et al. reported a microfluidic approach for the encapsulation of two mouse embryonic stem cell lines into agarose microgels, which had different mechanical properties.[222] Synthetic cell-laden microgels were also prepared by Doyle et al.; free radical cross-linking chemistry, however, reduced the cell-viability of the encapsulated yeast cells significantly.[223] Therefore more cytocompatible cross-linking procedures need to be developed for the encapsulation of cells with long-term viability.

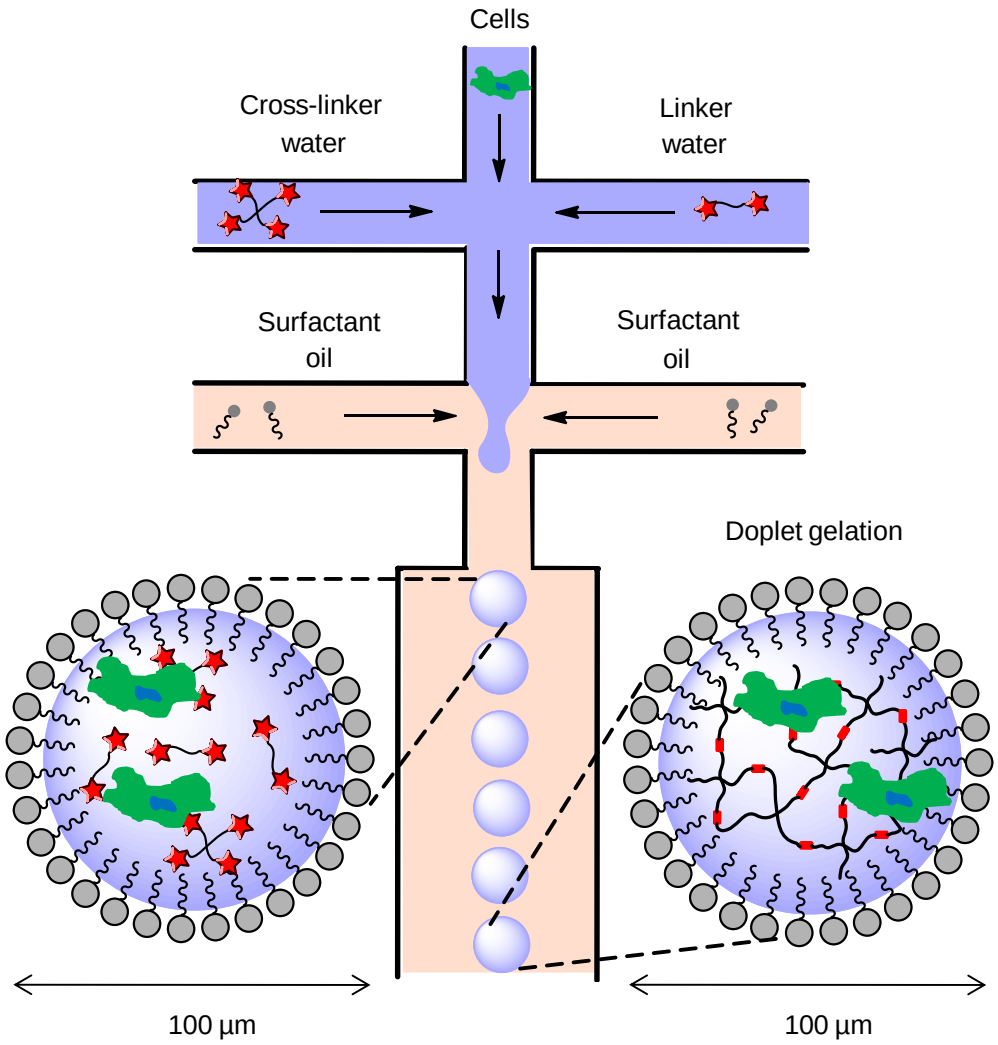


Figure 5. Schematic representation of microgel formation by microfluidic droplet gelation.

1.6 Degradable gel particles

Stimuli-responsive hydrogels are materials which change their properties in response to environmental stimuli.[224] These smart materials can swell, shrink, degrade, or undergo a sol−gel phase transition upon exposure to physical or chemical triggers such as, changes in pH, temperature, solvent, pressure, ionic strength, light, and concentration of biomolecules.[225,226] This responsive behavior can be used for the controlled release of actives from hydrogels which is often required for applications in which the hydrogel scaffold shields the guest in deactivated form during transport. Once the target is reached, the gel matrix degrades, releases the guest and the therapeutic function of the guest is activated. Additionally, in tissue engineering applications, degradation provides space for proliferating cells and allows infiltration of blood vessels.[227] Moreover, hydrogel degradation is known to

have direct influence cell proliferation, migration, and differentiation. In controlled drug and gene delivery applications,

To achieve this goal, hydrogel degradation by cleavage of chemical bonds located in the constituent polymer network, followed by gel dissolution and release of encapsulated guests is a promising strategy. When the hydrogel mesh size is smaller than the hydrodynamic radius of the cargo molecule release kinetics can be tuned by the bond cleavage kinetics. Additionaly, the generated low molecular weight degradation fragments can be cleared by the kidneys, which reduces the possibility of long term toxicity due to organ accumulation.[36] degradation permits spatiotemporal control of the release of cargo molecules

Degradable hydrogels can be designed to cleave via hydrolysis, enzymatic hydrolysis, photolytic cleavage or a combination of these mechanisms with varying degrees of control and desired degradation rates depending on the application. Ideally, degradation products are biocompatible without inducing any side effects, such as cytotoxicity, inflammation, or immunological and foreign body responses. Therefore, cleavable groups need to be carefully selected, according to the 1) tuneability of cleavage rates, 2) compatibility of degradation products and the 3) bioavailability and compatibility of corresponding stimuli. Various chemical bonds such as disulfides,[228–233], phosphate esters,[234] silyl ethers,[235] and esters[236] have been introduced into hydrogel networks which are cleaved in response to specific biological stimuli including pH or reductive environments (Table 1).[237]

1.6.1 Enzymatic degradation

Enzymatic cleavage is of particular importance for the degradation of hydrogels composed of natural polymers, proteins, or peptide linkages. This type of degradation is diffusion controlled because enzymes need to access the cleavable site and the diffusion of enzymes in hydrogels with small mesh sizes is hindered. As a result, enzymatic degradation of gel particles at the surface of the gel particles can be significant (Figure 6). As a result, release rates migt be difficult to be controlled and are often slow. Enzymatic degradation generates lower molecular weight fragments that are functionalized with carboxyl-and hydroxyl groups in case of an ester cleavage and carboxyl and amine groups for a cleaved amide bond. These degradation products are usually non-cytotoxic if the amine loading is kept low. In an interesting study, Patterson and Hubbell introduced protease-sensitive peptides into PEG hydrogels using Michael-type addition reactions. When incubated with proteases, the hydrogel samples degraded via enzymatic hydrolysis with variable rates depending upon the

peptide sequence used. Interestingly, encapsulated fibroblasts showed increased spreading and proliferation when cultured within hydrogels using more rapidly degrading peptides.[238]

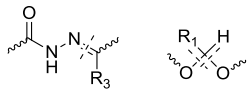
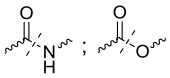
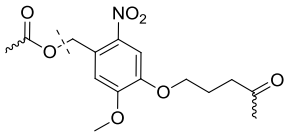
	Trigger	Degradation rate	Control over degradation rate	Biomedical targets
pH-Controlled hydrolysis e.g. acetals 	protons	fast	linker chemistry pH value	extracellular space in tumors an inflamations (pH 6-6.5) intracellular organelles (endosomes pH 5.5, lysosomes pH 4.5)
Enzymatic ester- amide hydrolysis 	enzymes	slow	diffusion control	enzymes overexpressed in tumors
Disulfide reduction 	glutathione	medium	glutathione concentration	cytoplasma
Photolytic cleavage 	hv	fast	light intensity	

Table 1. Comparison of cleavable linkers according to their degradation trigger, rate, control and biomedical target.

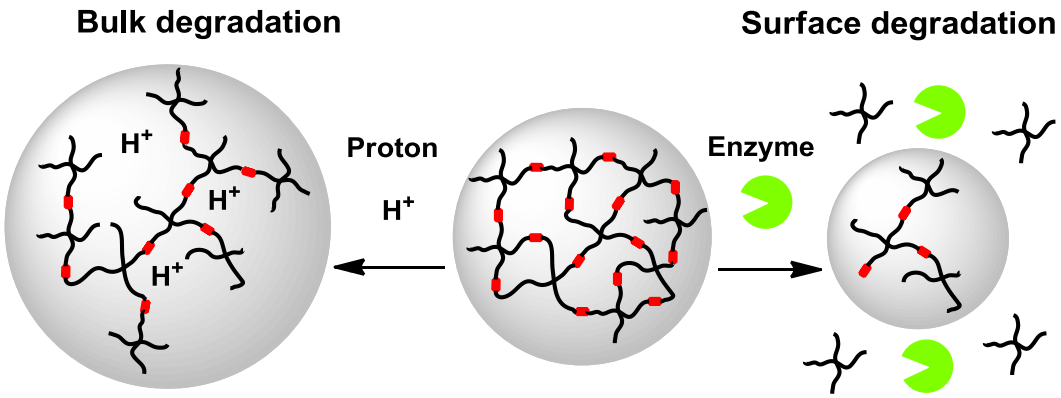


Figure 6. Schematic representation of bulk- and surface degradation mechanisms. Enzymatic surface degradation occurs when the enzyme diameter is significantly larger than the mesh size of the hydrogel, which prevents enzyme diffusion into the hydrogel. Because in this thesis enzymes should be encapsulated in hydrogels without leaching, for these hydrogels surface degradation can be expected.

1.6.2 Photolytic degradation

Photolabile monomers and polymers engineered to cleave under irradiation conditions and allow the spatial and temporal control of hydrogel degradation. By illuminating specific areas of the hydrogol with long wavelength UV, visible, or two-photon IR light, hydrogel degradation is induced. Photodegradation, however, generates free radicals[71] which are very often cytotoxic, denature proteins, and influence cellular behavior.[95] Additionally, light scattering causes low conversions and cross-link density in deep hydrogel layers and makes therefore a controlled degradation difficult.[103] Anseth and coworkers pioneered the development of photodegradable hydrogels for cell culture by creating an acrylated nitrobenzyl ether-derived moiety with a pendant carboxylic acid that could be attached to PEG and act as a cleavable crosslinker. Externally triggered degradation has been performed to release encapsulated hMSCs upon demand.[239] Additionally, photodegradable nanogels were prepared which released a BSA model protein upon illumination.[115] Almutairi and coworkers recently reported synthesis of photodegradable nanoparticles which erode under illumination by a self immolative pathway.[240]

1.6.3 Reductive degradation

There is a significant difference in redox potential between the extracellular and the intracellular environment(~100–1000 fold).[241] This effect is strongly related to the intra- and extracellular reduced-glutathione concentration, which is enzymatically controlled. Based on redox-gradient researchers have designed hydrogels, containing disulfides within the constituent polymer network. Disulfides are stable in the extracellular space, however, once they reach intracellular cytosol, dislufides are reduced to the corresponding thiols and the hydrogel degrades.[242] Additionally, a reductive activity has been evidenced in intracellular endosomes and lysosomes.[243] As a result, many groups introduced disulfide linkages into their nanogel networks to achieve an intracellular drug release.[228–233] Matyjaszewski et al. prepared biodegradable nanogels cross-linked with disulfide linkages by inverse miniemulsion atom transfer radical polymerization (ATRP). The authors were able to encapsulate and release small molecule drug doxorubicin in vitro.[172] Although this is a promising approach, reductions rates are often slow, difficult to be manipulated, and intracellular residence times of nanogels can be short.

1.6.4 pH-Controlled degradation

Hydrolysis at acidic pH is very promising for controlled release applications. Many in vivo medical targets possess different unique pH values such as 7.4 in the bloodstream and healthy tissues and 6.5 in inflamed and cancerous tissues. In intracellular endosomes and lysosomes the pH drops to 5.5 and 4.5, respectively.[36] Additionally, protons are known to play a major role in cellular communication, as a result, encapsulated cells may actively degrade their hydrogel matrix, which has direct impact on cell differentiation,[244] proliferation, and migration.[245] In contrast to enzymatic degradation, which is diffusion controlled,[246] acidic hydrolysis shows no diffusion limitation due to the small size of proton catalysts. Thus, the degradation kinetics only depends on the chemical bond cleavage and can therefore be precisely controlled. Acid cleavable hydrogels have been prepared by free radical crosslinking[14] and copper(I)- catalysed azide-alkyne cycloaddition (CuAAC).[15] However, these procedures are not suitable for the encapsulation of sensitive biomolecules, because the generation of free radicals and heavy metal catalysts can damage them.

2 Scientific goals

As illustrated in Figure 7 the four main challenges for the nano-encapsulation of therapeutic proteins and micro-encapsulation of living cells are the A) synthesis of bioinert cross-linkers, B) mild formation of cross-linker and guest loaded nano- and micro-droplet templates, C) cross-linking reactions that are orthogonal to the functional groups of living cells and enzymes, and D) controlled release of the guests at the target site by network degradation and dissolution. In thesis the author focused on these points to improve existing procedures from literature to obtain nano- and microgels for the encapsulation and controlled release of proteins and living cells.

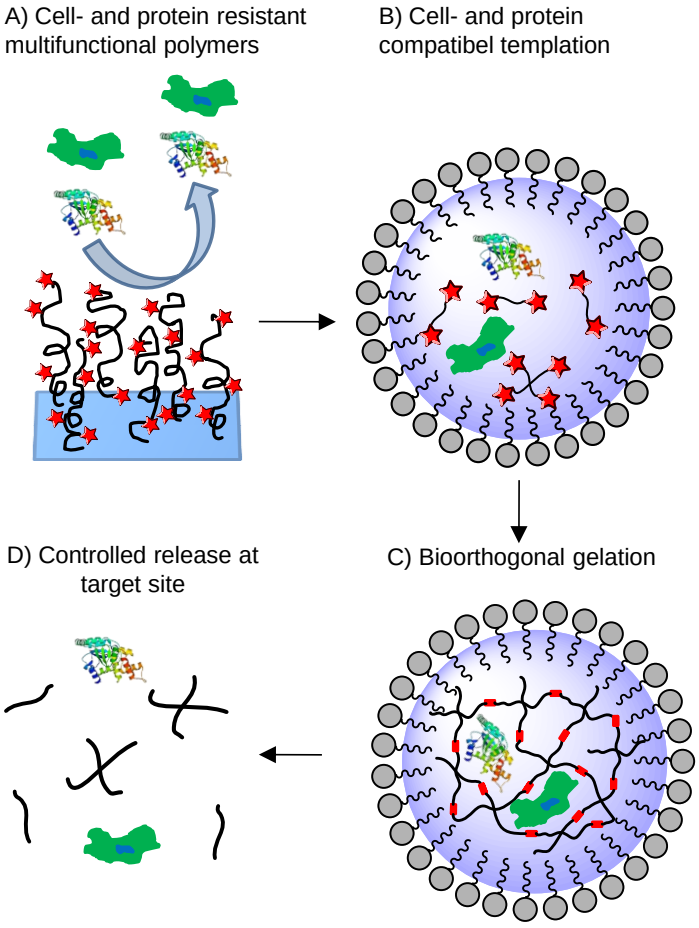


Figure 7. Schematic representation of the four main challenges in nanoencapsulation of proteins into nanogels and the microencapsulation of living cells into microgels. (A) Preparation of cell- and protein resistant polymers which are functionalized with reactive groups for cross-linking. (B) Nano- and microparticle templation have to be performed under mild conditions to prevent guest damage and deactivation. (C) Cross-linking and gel formation reactions need to be orthogonal to the functional

groups present on the biological systems. (D) After the guest has reached its biomedical target a controlled release with precise control over release rate is required. Hydrogel degradation by cleavage of chemical bonds located in the constituent polymer network, followed by gel dissolution and release of encapsulated guests is a promising strategy.

A) Dendritic polyglycerol (dPG) and linear PEG were selected as bioinert hydrogel building blocks.[81] These materials are highly cell- and protein resistant which is a prerequisite to prevent detrimental hydrogel–cell and hydrogel-enzyme interactions.[83,247] Furthermore, dPG is a multifunctional and hydrophilic polymer that contains 138 OH-groups at a molecular weight M_w = 10 kDa. As a result, dPG shall be used as basic building block for the preparation of hydrogel particles. Synthetic procedures will be developed to obtain novel polyglycerol monomers and macromonomers that can be cross-linked.

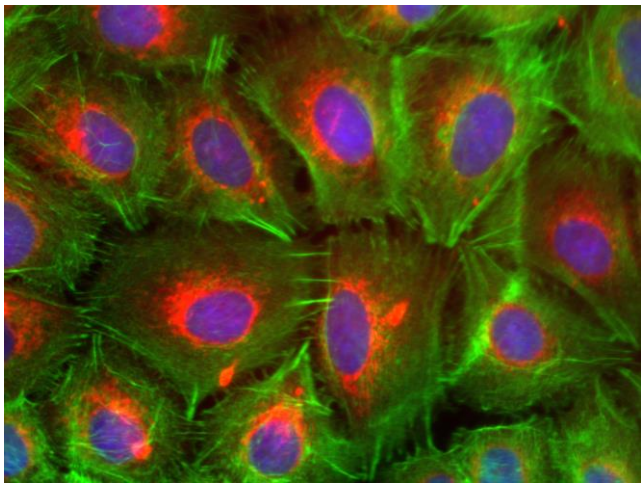
B) For the preparation of nano- and microgels reactive monomers or macromonomers and the bioactive guest need to be templated in enclosed nano- and microreactors. Because these procedures often require harsh conditions, in this thesis various templation methods will be applied and their suitability for the encapsulation of therapeutic proteins and living cells will be studied. Additionally, new methodologies will be developed that can be performed under cyto- and protein compatible conditions.

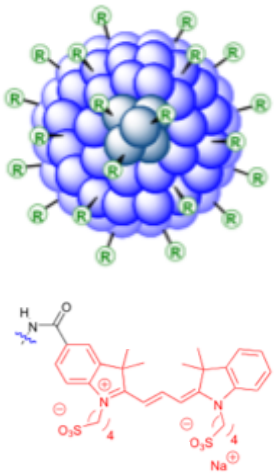
C) To achieve homogenous and efficient encapsulation of cells and proteins in a hydrogel network the guests need to be loaded during the gelation reaction. Hence, mild cross-linking conditions are required to be (i) orthogonal to the functional groups of the biomolecules, (ii) non cytotoxic, and (iii) fast at 37 °C. Additionally, the synthetic effort for the preparation of the cross-linkable macromonomers should be kept as low as possible because big polymer amounts are required for the specific applications. Therefore, in this thesis different cross-linking chemistries will be performed and evaluated according to their cyto- and protein compatibility and towards their synthetic efficacy.

D) Many applications require a controlled release of the encapsulated guests under physiological conditions. The goal of this thesis is prepare linkers that degrade upon a specific biological stimulus such as pH and the reductive intracellular environment and therefore release the guest. Degradation rate shall be precisely controlled by chemical modifications of the linkers.

3 Publications

3.1 Biocompatible functionalized polyglycerol microgels with cell penetrating properties





A. L. Sisson, **D. Steinhilber**, T. Rossow, P. Welker, K. Licha, R. Haag
a) *Angew. Chem.* **2009**, *121*, 7676-7681.
b) *Angew. Chem. Int. Ed.* **2009**, *48*, 7540-7545.
DOI: 10.1002/anie.200901583
http://onlinelibrary.wiley.com/doi/10.1002/anie.200901583/abstract

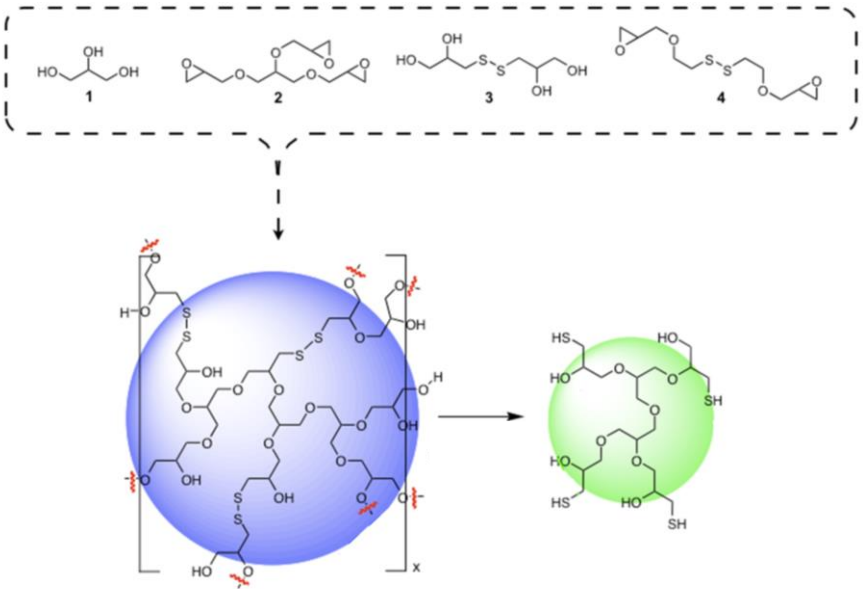
Author contributions

D. Steinhilber: Miniemulsion polymerisation, characterisation, fluorescence labelling, preparation of the manuscript.
A. L. Sisson: Supervison, correction of the manuscript.
T. Rossow: Miniemulsion polymerisation, fluorescence labelling.
P. Welker, K. Licha: Cellular studies.
R. Haag: Supervision, correction of the manuscript, scientific discussion of the data.

Abstract

In manuscript a facile and versatile approach for the synthesis of polyglyerol nanogels had been designed with dimensions between 20 and 80 nm were previously unobtainable. Beneficially, such nanoparticles have a narrow size distribution and can be readily functionalized with a wide range of groups by click chemistry. The biocompatible nature of polyglycerol materials is very promising for future applications as delivery vehicles for drugs and dyes. Owing to the size of these nanogels they rapidly and harmlessly internalize into cells by an endocytotic pathway.

3.2 Synthesis, reductive cleavage and cellular interaction studies of biodegradable, polyglycerol nanogels



D. Steinhilber, A. L. Sisson, D. Mangoldt, P. Welker, K. Licha, R. Haag
Adv. Funct. Mater. **2010**, *20*, 4133-4138.
DOI: 10.1002/adfm.201000410
http://onlinelibrary.wiley.com/doi/10.1002/adfm.201000410/abstract

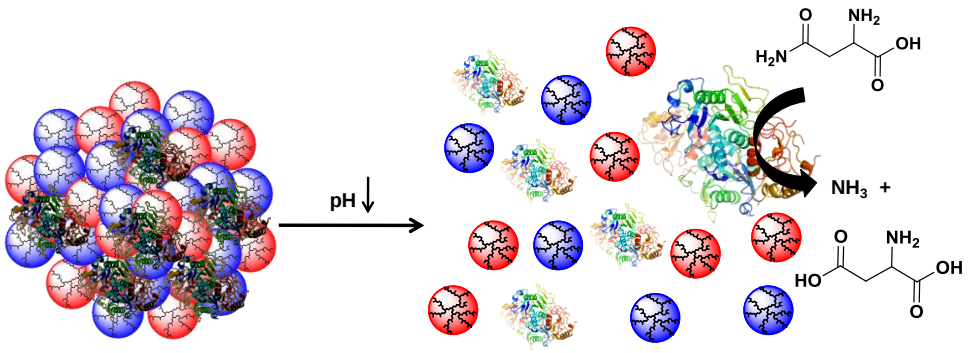
Author contributions

D. Steinhilber: Monomer synthesis, miniemulsion polymerisation, characterisation, degradation studies, fluorescence labelling, preparation of the manuscript.
A. L. Sisson: Supervison, preparation of the manuscript.
D. Mangoldt, P. Welker, K. Licha: Cellular studies.
R. Haag: Supervision, correction of the manuscript, scientific discussion of the data.

Abstract

In this manuscript biodegradable polyglycerol nanogels were prepared for the first time in inverse miniemulsion via an acid catalyzed ring-opening polyaddition of disulfide containing polyols and polyepoxides. Particle degradation under reductive intracellular conditions was studied by various analytical techniques. Gel permeation chromatography indicates that final degradation products have low molecular weights (≤ 5 kDa), thus ensuring renal clearance. In addition, studies in cell culture show that these nanoscale materials are highly biocompatible. Dye-labelled nanogels are visualized by optical microscopy techniques to readily internalize into cells by endocytotic mechanisms. This study highlights the great potential of these particles to function as sophisticated nanotransporters that deliver cargo to a certain tissue or cell target and then biodegrade into smaller fragments which would be cleared. In general this publication demonstrates the expertise of the applicant on the preparation and characterisation of a new class of biodegradable materials and their biomedical applications.

3.3 Surfactant free preparation of biodegradable dendritic polyglycerol nanogels by inverse nanoprecipitation for encapsulation and release of pharmaceutical enzymes



D. Steinhilber, M. Witting, X. Zhang, M. Staegemann, W. Friess, S. Küchler, F. Paulus, R. Haag
J. Control. Release **2013**, *169*, 289-295.
DOI: 10.1016/j.jconrel.2012.12.008
http://www.sciencedirect.com/science/article/pii/S016836591200836X

Author contributions

D. Steinhilber: Conceptual development and synthesis of acetal cleavable macromonomers, conceptual development of the novel inverse nanoprecipitation method to prepare nanogels, characterization, pH-controlled degradation studies, fluorescence labelling, enzyme encapsulation, preparation of the manuscript.
M. Witting: Enzyme release and activity, correction of the manuscript.
X. Zhang, M. Staegemann, F. Paulus: Macromonomer synthesis
W. Friess, S. Küchler: Selection of enzyme, correction of the manuscript.
R. Haag: Correction of the manuscript, supervision, scientific discussions of the data.

Abstract

In this manuscript the applicant developped the inverse nanoprecipitation method as a novel route towards biomacromolecule-laden nanogels. Enzymes were encapsulated with an efficacy of almost 100 % and after drug release, full enzyme activity and structural integrity were retained. Surfactant free nanoparticle templation was performed and in situ crosslinking of the precipitated nanoparticles resulted in size defined polyglycerol nanogels (100 - 1000 nm). Biodegradability was achieved by the introduction of benzacetal bonds into the net points of the nanogel. Interestingly, the polyglycerol nanogels quickly degraded into low molecular weight fragments at acidic pH values, which are present in inflamed and tumor tissues as well as intracellular organelles, and they remained stable at physiological pH values for a long time. This manuscript demonstrates the applicant's ability to independently develop a novel biomacromolecule-formulation technique, which has already been applied for other guests and gelation chemistries.

3.4 Hyperbranched polyglycerols on the nanometer and micrometer scale

D. Steinhilber, S. Seiffert, J. A. Heyman, F. Paulus, D. A. Weitz, R. Haag
Biomaterials **2011**, *32*, 1311-1316.
DOI: 10.1016/j.biomaterials.2010.10.010
http://www.sciencedirect.com/science/article/pii/S0142961210012986

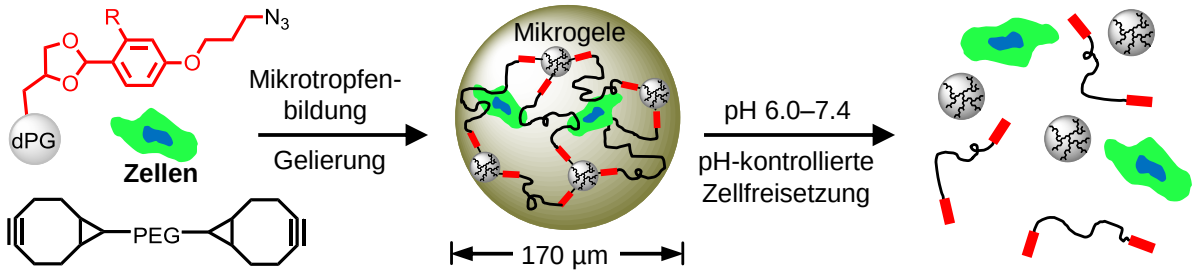
Author contributions

D. Steinhilber: Synthesis of macromonomers, development miniemulsion polymerization using commercial and cytocompatible surfactants, characterization, preparation of the manuscript.
S. Seiffert: Microfluidic templating, cell encapsulation, rheology, preparation of the manuscript.
J. A. Heyman: Cell culture.
F. Paulus: Polyglycerol synthesis.
D. A. Weitz, R. Haag: Correction of the manuscript, supervision, scientific discussions of the data.

Abstract

In this manuscript polyglycerol gel particles were prepared for the first time on the nano- and microscale. The use of a free-radical polymerization of dPGDecaacrylate in miniemulsion droplets, initiated by APS/TEMED, yields defined nanogels with a mean hydrodynamic diameter of 32 nm. By extension of this method to the use of micrometer-sized droplets, as obtained through microfluidic emulsification, we are able to form monodisperse hPG microgels with uniform diameters of several tens or hundreds of micrometers. These microgels formed at polymerization conditions that can be used for the encapsulation of yeast cells. For the encapsulation of more sensitive mammalian cells, however, more cytocompatible cross-linking reactions are needed.

3.5 A microgel construction kit for the bioorthogonal encapsulation and pH-controlled release of living cells



D. Steinhilber, T. Rossow, S. Wedepohl, F. Paulus, S. Seiffert, R. Haag
Angew. Chem. Int. Ed. **2013**, *52*, 13538-13543.
DOI: 10.1002/anie.201308005
http://onlinelibrary.wiley.com/doi/10.1002/anie.201308005/abstract

Author contributions

D. Steinhilber: Synthesis of azide functionalized macromonomers with varying hydrolysis kinetics of azide linkers, synthesis of electrophilic cyclooctynes on multigram scale, synthesis of homobifunctional cyclooctyne-crosslinkers, gelation by strain promoted azide-alkyne click chemistry, microfluidic templation and cell encapsulation, pH- controlled cell release and degradation, preparation of the manuscript.
T. Rossow: microfluidic templation and cell encapsulation, pH- controlled cell release and degradation, preparation of the manuscript.
S. Wedepohl: Donation of NIH-3T3 cells, correction of manuscript.
F. Paulus: Polyglycerol synthesis.
S. Seiffert: Correction of the manuscript.
R. Haag: Supervision, correction of the manuscript, scientific discussions of the data.

Abstract

This publication describes a fundamental new concept for the bioorthogonal encapsulation and the pH- controlled release of mamalian cells from bioinert microgels. In contrast to conventionally used enzymatic cell release mechanism, this novel approach provides high control over release kinetics because acidic hydrolysis shows no diffusion limitation due to the small size of the proton catalyst. The encapsulated cells could be encapsulated and cultured inside the microgels with full retention of their viability over several weeks. Interestingly, the on demand pH-mediated microgel degradation that was precisely controlled by different substituted benzacetals as pH-cleavable crosslinker had no detrimental effect on the encapsulated and released cells. As a result, the microgel particles can be used for temporary cell encapsulation, allowing the cells to be studied and manipulated during the encapsulation and then be isolated and harvested on demand by decomposition of the microgel scaffolds. The methodologies developed are well suited for applications in cell therapy and tissue engineering.

4 Conclusions and outlook

In this thesis a miniemulsion approach has been designed, which to the synthesis of polyglyerol nanogels of dimensions previously unobtainable, providing simple access to materials in the nanoscale. Interestingly, these nanoparticles had a narrow size distribution and can be readily functionalized with a wide range of groups by click chemistry. The prepared nanogels were highly biocompatible and are therefore very promising for future applications as delivery vehicles for drugs and dyes. Owing to the size of these polyglycerol nanogels they rapidly and harmlessly internalized into cells by an endocytotic pathway.

Additionally, the polyether miniemulsion chemistry has been expanded to biodegradable polyglycerol nanogels with promising transport properties for cellular delivery. A simple one step process was developed and conditions were tuned to vary particle size and nanogel composition. A variety of polyols and polyepoxides, which led to new functional materials, could be polymerized following our general procedure. These disulfide containing nanogels were degraded into small oligomeric subunits in reducing environments as shown by three different assays. Furthermore, disulfide containing polyglycerol nanogels were found to be the same biocompatibele as non-degradable polyglycerol based nanogels. These studies show that these materials are able to rapidly enter cells by endocytic mechanisms, thus entering the reductive intracellular environment.

The developed epoxide cross-linking chemistry, however, requires high reaction temperatures, which are not suitable for the encapsulation of living cells and proteins during gel formation. Additionally, block-co-polymer surfactants were necessary which are difficult to be prepared on large-scale. Therefore, a gelation procedure was established which can be performed at room temperature and which allows for the stabilization of miniemulsion droplets by commercial surfactants. The use of a free-radical polymerization of acrylate functionalized polyglycerol macromonomers in miniemulsion droplets, initiated by

APS/TEMED, yielded defined nanogels with a mean hydrodynamic diameter of 32 nm. By extension of this method to the use of micrometer-sized droplets, as obtained through microfluidic emulsification, monodisperse dPG microgels with uniform diameters of several tens or hundreds of micrometers were obtained. These microgels formed at polymerization conditions that can be used for the encapsulation of yeast cells.

The reaction conditions in form of free radicals and high mechanical energy input by ultrasonication were still too harsh for the encapsulation of enzymes into nanogels and living cells into microgels. Therefore, a novel inverse nanoprecipitation method was developed to obtain enzyme-laden nanogels. Guests were encapsulated with an efficacy of almost 100 % and after drug release, full enzyme activity and structural integrity were retained. Surfactant free nanoparticle templation was performed and in situ crosslinking by copper catalyzed azide-alkyne cycloaddition of the precipitated nanoparticles resulted in size defined polyglycerol nanogels (100 - 1000 nm). Biodegradability was achieved by the introduction of benzacetal bonds into the net points of the nanogel. Interestingly, the polyglycerol nanogels quickly degraded into low molecular weight fragments at acidic pH values, which are present in inflamed and tumor tissues as well as intracellular organelles, and they remained stable at physiological pH values for a long time.

This gelation chemistry, however, cannot be applied for the encapsulation of living cells because of the cytotoxicity of the copper catalyst. Therefore, a new macromonomer construction kit has been designed that allows the bioorthogonal encapsulation of living mammalian cells by strain promoted azide-alkyne click chemistry into microgels. Additionally the encapsulated cells were released upon demand for the first time under acidic conditions. The encapsulated cells could be cultured inside the microgels with full retention of their viability. Microgel degradation was precisely controlled by different substituted benzacetals as pH-cleavable linkers on the dendritic building block and had no detrimental

effect on the encapsulated and released cells. As a result, the microgel particles can be used for temporary cell encapsulation, allowing the cells to be studied and manipulated during the encapsulation and then isolated and harvested on demand by decomposition of the microgel scaffolds. Thus, our approach will advance the understanding of cellular survival in artificial 3D matrix environments. Additionally, our construction kit has potential for the stabilization and controlled release of many other therapeutic relevant biological systems such as proteins, genes, and even bacteria.

In future the nanoprecipitation procedure presented in this work could be further investigated. Application of the bioorthogonal gelation chemistry for the nanogel formation might enable the encapsulation of even more sensitive therapeutic enzymes such as epo and somatostatin. Furthermore, the nanoprecipitation technique is well suited for a process up-scaling. This might be interesting for applications that require big material quantities. Additionally, more pH cleavable acetal linkers with varying cleavage kinetics could be synthesized to get an even higher control over degradation rates.

5 Kurzzusammenfassung

In dieser Arbeit wurden erstmalig Polyglycerinnanogele durch Polyadditions-vernetzungsreaktionen zwischen Glycerin und Glycerin-basiertem Polyepoxid in Miniemulsionströpfchen hergestellt. Durch die Reaktion von nicht umgesetzten Epoxidgruppen mit Natriumazid konnten diese Nanogele durch Kupfer-katalysierte Azid-Alkin Zykloaddition mit Fluoreszenzfarbstoffen markiert werden um die Zellaufnahme der Nanogele zu verfolgen. Interessanterweise wurde festgestellt, dass Nanogele mit einem Durchmesser von 50 nm schnell und effizient in Tumorzellen mittels Endozytose aufgenommen wurden. Kleine Polyglycerindendrimere mit einem Durchmesser von etwa einem Nanometer wiesen nur eine geringe Zellaufnahme auf. Zusammenfassend konnte gezeigt werden, dass die Größe der Nanogele einen entscheidenden Einfluss auf die Zellaufnahme hat und somit ein wichtiger Parameter für die intrazelluläre Freisetzung von Wirkstoffen darstellt.

Desweiteren konnte dieser Ansatz zur Herstellung von bioabbaubaren Nanogelen verwendet werden, welche Disulfidbrücken im Nanogelnetzwerk aufwiesen. Mittels mehrerer Charakterisierungsmethoden konnte gezeigt werden, dass die Nanogele unter reduktiven Bedingungen, welche in intrazellulären Umgebungen vorliegen, in kleine oligomere Bausteine gespalten werden. Durch diesen Abbau können Wirkstoffe intrazellulär freigesetzt und die zytokompatiblen Abbauprodukte können letztendendes aufgrund ihrer geringen Größe durch die Nieren wieder ausgeschieden werden.

Obwohl diese Herstellungsmethode vielversprechende Ergebnisse lieferte, können aufgrund der hohen Reaktionstemperaturen keine therapeutischen Proteine in die Nanogele verkapselt werden. Diese neuartigen Therapeutika wären jedoch für zukünftige Therapieansätze sehr vielversprechend. Daher wurde die Polyadditionschemie durch die freie radikalische Vernetzung von Acrylat-funktionalisierten Polyglycerinen ersetzt, welche bei Raumtemperatur vernetzen. Hierdurch wurden erstmalig Polyglycerin-Nano- und Polyglycerin-Mikropartikel durch die Gelierung in Miniemulsions- und mikrofluidischen Tröpfchen hergestellt. Bemerkenswerterweise konnte die Gelierung der Mikrotröpfchen in Anwesenheit von Hefezellen durchgeführt werden, wodurch die Zellen in den Mikrogelen mit einer Zellüberlebensrate von 40% verkapselt wurden. Allerdings stellte sich die freie radikalische Vernetzungsreaktion als zu zelltoxisch heraus und die Anwendung von energiereicher Ultraschallbehandlung zur Miniemulgierung würde zu Proteindenaturierungen führen.

Demzufolge wurde in dieser Arbeit eine neuartige inverse Nanofällungsmethode entwickelt um enzymbeladene Nanogele unter milden Bedingungen herzustellen. Hierbei konnten die Gäste mit einer Beladungseffizienz von beinahe 100% verkapselt werden und nach pH-kontrollierter Freisetzung wurde eine vollständige Enzymaktivität festgestellt. Die Nanopartikelherstellung konnte ohne Verwendung von Tensiden durchgeführt werden, wodurch Proteindenaturierungen vermieden wurden. Interessanterweise bauten sich die Nanogele bei niederen pH-Werten, welche in intrazellulären Umgebungen vorliegen, schnell durch Hydrolyse ab. Da die Nanogele bei physiologischem pH-Wert von 7,4 über einen langen Zeitraum stabil blieben, sind diese Systeme bestens für die kontrollierte intrazelluläre Wirkstofffreisetzung geeignet. Die Azid-Alkin Vernetzungschemie ist allerdings für in-vivo-Anwendungen kritisch, da verwendete Kupferkatalysatoren toxische Wirkungen hervorrufen können.

Deshalb wurde in dieser Arbeit ein Makromonomerbaukasten für die bioorthogonale Verkapselung und pH-gesteuerte Freisetzung von lebenden Zellen entwickelt. pH-Spaltbare zellbeladene Mikrogele mit exzellenten Langzeitüberlebensraten wurden durch Kombination bioorthogonaler spannungsvermittelter Azid-Alkin-Cycloaddition (SPAAC) und Tröpfchen-basierter Mikrofluidik hergestellt. Poly(ethylenglykol)dicyclooctin und dendritisches Poly(glycerinazid) dienten als bioinerte Mikrogelbausteine. Die Azid-Konjugation erfolgte mithilfe unterschiedlicher säurelabiler Benzacetallinker, wodurch eine präzise Steuerung der Abbaukinetik im interessanten pH-Bereich zwischen 4,5 und 7,4 ermöglicht wurde. Hierdurch konnte eine pH-gesteuerte Freisetzung der verkapselten Zellen auf Abruf erreicht werden, ohne ihre Überlebensrate oder Ausspreizung zu beeinträchtigen. Folglich können die Mikrogelpartikel für die temporäre Verkapselung von Zellen verwendet werden, wobei sich die Zellen während der Verkapselung studieren und manipulieren lassen. Anschließend können die Zellen durch Zersetzung des Mikrogelgerüstes isoliert und freigesetzt werden.

6 References

[1] N. Huebsch, D. J. Mooney, *Nature* **2009**, *462*, 426–432.

[2] J. E. Phillips, K. L. Burns, J. M. Le Doux, R. E. Guldberg, A. J. García, *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105*, 12170–12175.

[3] R. Gauvin, A. Khademhosseini, *ACS Nano* **2011**, *5*, 4258–4264.

[4] S. A. Newman, *Science* **2012**, *338*, 217–219.

[5] Y.-C. Toh, K. Blagovic, H. Yu, J. Voldman, *Integr. Biol.* **2011**, *3*, 1179–1187.

[6] D. Hanahan, R. A. Weinberg, *Cell* **2011**, *144*, 646–674.

[7] T.-M. Achilli, J. Meyer, J. R. Morgan, *Expert Opin. Biol. Ther.* **2012**, *12*, 1347–1360.

[8] N. Matsuda, T. Shimizu, M. Yamato, T. Okano, *Adv. Mater.* **2007**, *19*, 3089–3099.

[9] M. Matsusaki, K. Kadowaki, Y. Nakahara, M. Akashi, *Angew. Chem. Int. Ed. Engl.* **2007**, *46*, 4689–4692.

[10] H. Sekine, T. Shimizu, K. Sakaguchi, I. Dobashi, M. Wada, M. Yamato, E. Kobayashi, M. Umezu, T. Okano, *Nat. Commun.* **2013**, *4*, 1399.

[11] Y. Du, E. Lo, S. Ali, A. Khademhosseini, *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105*, 9522–9527.

[12] H. Qi, M. Ghodousi, Y. Du, C. Grun, H. Bae, P. Yin, A. Khademhosseini, *Nat. Commun.* **2013**, *4*, 2275.

[13] D. E. Discher, D. J. Mooney, P. W. Zandstra, *Science* **2009**, *324*, 1673–1677.

[14] B. Trappmann, J. E. Gautrot, J. T. Connelly, D. G. T. Strange, Y. Li, M. L. Oyen, M. A. Cohen Stuart, H. Boehm, B. Li, V. Vogel, et al., *Nat. Mater.* **2012**, *11*, 642–649.

[15] J. Swift, I. L. Ivanovska, A. Buxboim, T. Harada, P. C. D. P. Dingal, J. Pinter, J. D. Pajerowski, K. R. Spinler, J.-W. Shin, M. Tewari, et al., *Science* **2013**, *341*, 1240104.

[16] N. Huebsch, P. R. Arany, A. S. Mao, D. Shvartsman, O. A. Ali, S. A. Bencherif, J. Rivera-Feliciano, D. J. Mooney, *Nat. Mater.* **2010**, *9*, 518–526.

[17] T. M. Kauer, J.-L. Figueiredo, S. Hingtgen, K. Shah, *Nat. Neurosci.* **2012**, *15*, 197–204.

[18] A. M. Shapiro, J. R. Lakey, E. A. Ryan, G. S. Korbutt, E. Toth, G. L. Warnock, N. M. Kneteman, R. V Rajotte, *N. Engl. J. Med.* **2000**, *343*, 230–238.

[19] F. Lim, A. Sun, *Science* **1980**, *210*, 908–910.

[20] M. Qi, B. L. Strand, Y. Mørch, I. Lacík, Y. Wang, P. Salehi, B. Barbaro, A. Gangemi, J. Kuechle, T. Romagnoli, et al., *Artif. Cells. Blood Substit. Immobil. Biotechnol.* **2008**, *36*, 403–420.

[21] P. Soon, E. Feldmant, R. Nelson, R. Heintz, Q. Yao, Z. Yao, T. Zheng, N. Merideth, G. Skjak-braek, T. Espevik, et al., *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 5843–5847.

[22] Y. Sun, X. Ma, D. Zhou, I. Vacek, A. M. Sun, *J. Clin. Invest.* **1996**, *98*, 1417–1422.

[23] R. B. Elliott, L. Escobar, P. L. J. Tan, M. Muzina, S. Zwain, C. Buchanan, *Xenotransplantation* **2007**, *14*, 157–161.

[24] R. Calafiore, *Diabetes Care* **2006**, *29*, 137–138.

[25] B. E. Tuch, G. W. Keogh, L. J. Williams, W. Wu, J. L. Foster, V. Vaithilingam, R. Philips, *Diabetes Care* **2009**, *32*, 1887–1889.

[26] R. R. Caspi, *Nat. Rev. Immunol.* **2008**, *8*, 970–976.

[27] L. Steinman, J. T. Merrill, I. B. McInnes, M. Peakman, *Nat. Med.* **2012**, *18*, 59–65.

[28] D. V Goeddel, D. G. Kleid, F. Bolivar, H. L. Heyneker, D. G. Yansura, R. Crea, T. Hirose, A. Kraszewski, K. Itakura, a D. Riggs, *Proc. Natl. Acad. Sci. U. S. A.* **1979**, *76*, 106–110.

[29] B. Leader, Q. J. Baca, D. E. Golan, *Nat. Rev. Drug Discov.* **2008**, *7*, 21–39.

[30] L. a Holladay, D. Puett, *Proc. Natl. Acad. Sci. U. S. A.* **1976**, *73*, 1199–1202.

[31] M. C. Manning, D. K. Chou, B. M. Murphy, R. W. Payne, D. S. Katayama, *Pharm. Res.* **2010**, *27*, 544–575.

[32] W. Wang, *Int. J. Pharm.* **1999**, *185*, 129–188.

[33] A. S. De Groot, W. Martin, *Clin. Immunol.* **2009**, *131*, 189–201.

[34] L. Tang, A. M. Persky, G. Hochhaus, B. Meibohm, *J. Pharm. Sci.* **2004**, *93*, 2184–2204.

[35] W. Jiskoot, T. W. Randolph, D. B. Volkin, C. R. Middaugh, C. Schöneich, G. Winter, W. Friess, D. J. A. Crommelin, J. F. Carpenter, *J. Pharm. Sci.* **2012**, *101*, 946–954.

[36] R. Haag, F. Kratz, *Angew. Chem. Int. Ed. Engl.* **2006**, *45*, 1198–1215.

[37] K. Knop, R. Hoogenboom, D. Fischer, U. S. Schubert, *Angew. Chem. Int. Ed. Engl.* **2010**, *49*, 6288–6308.

[38] J. M. Harris, R. B. Chess, *Nat. Rev. Drug Discov.* **2003**, *2*, 214–221.

[39] R. K. Jain, *Cancer Res.* **1987**, 3039–3051.

[40] Y. Matsumura, H. Maeda, *Cancer Res.* **1986**, *46*, 6387–6392.

[41] N. J. Ganson, S. J. Kelly, E. Scarlett, J. S. Sundy, M. S. Hershfield, *Arthritis Res. Ther.* **2006**, *8*, R12.

[42] F. M. Veronese, G. Pasut, *Drug Discov. Today* **2005**, *10*, 1451–1458.

[43] S. Jevsevar, M. Kunstelj, V. G. Porekar, *Biotechnol. J.* **2010**, *5*, 113–128.

[44] F. M. Veronese, *Biomaterials* **2001**, *22*, 405–417.

[45] J. P. Magnusson, A. O. Saeed, F. Fernández-Trillo, C. Alexander, *Polym. Chem.* **2011**, *2*, 48.

[46] W. R. Gombotz, D. K. Pettit, *Bioconjug. Chem.* **1995**, *6*, 332–351.

[47] H. Okada, *Adv. Drug Deliv. Rev.* **1997**, *28*, 43–70.

[48] J. L. Cleland, O. L. Johnson, S. Putney, A. J. . Jones, *Adv. Drug Deliv. Rev.* **1997**, *28*, 71–84.

[49] R. L. K. Fu, D. W. Pack, A. M. Klibanov, *Pharm. Res.* **n.d.**, *17*, 100–106.

[50] W. J. M. van de Weert, W. E. Hennink, *Pharm. Res.* **2000**, *17*, 1159–1167.

[51] A. G. A. Lucke, J. Kiermaier, *Pharm. Res.* **2002**, *19*, 175–181.

[52] J. Khandare, M. Calderón, N. M. Dagia, R. Haag, *Chem. Soc. Rev.* **2012**, *41*, 2824–2848.

[53] C. R. Alving, *J. Immunol. Methods* **1991**, *140*, 1–13.

[54] T. Vermonden, R. Censi, W. E. Hennink, *Chem. Rev.* **2012**, *112*, 2853–2888.

[55] T. Nochi, Y. Yuki, H. Takahashi, S. Sawada, M. Mejima, T. Kohda, N. Harada, I. G. Kong, A. Sato, N. Kataoka, et al., *Nat. Mater.* **2010**, *9*, 572–578.

[56] K. Akiyoshi, S. Kobayashi, S. Shichibe, D. Mix, M. Baudys, S. Wan Kim, J. Sunamoto, *J. Control. Release* **1998**, *54*, 313–320.

[57] Y. J. Kwon, E. James, N. Shastri, J. M. J. Frechet, *Proc. Natl. Acad. Sci.* **2005**, *102*, 18264–18268.

[58] N. Murthy, M. Xu, S. Schuck, J. Kunisawa, N. Shastri, J. M. J. Fréchet, *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100*, 4995–5000.

[59] Y. J. Kwon, S. M. Standley, S. L. Goh, J. M. J. Fréchet, *J. Control. Release* **2005**, *105*, 199–212.

[60] T. Nochi, Y. Yuki, H. Takahashi, S. Sawada, M. Mejima, T. Kohda, N. Harada, I. G. Kong, A. Sato, N. Kataoka, et al., *Nat. Mater.* **2010**, *9*, 572–578.

[61] R. Censi, P. Di Martino, T. Vermonden, W. E. Hennink, *J. Control. Release* **2012**, *161*, 680–692.

[62] T. Vermonden, R. Censi, W. E. Hennink, *Chem. Rev.* **2012**, *112*, 2853–2888.

[63] R. Langer, D. A. Tirrell, *Nature* **2004**, *428*, 487–492.

[64] R. D. A. Alshamkhani, *J. Bioact. Compat. Polym* **1995**, *10*, 4–13.

[65] T. Boontheekul, H.-J. Kong, D. J. Mooney, *Biomaterials* **2005**, *26*, 2455–2465.

[66] A. Murua, A. Portero, G. Orive, R. M. Hernández, M. de Castro, J. L. Pedraz, *J. Control. Release* **2008**, *132*, 76–83.

[67] G. Orive, R. Maria Hernández, A. Rodríguez Gascón, R. Calafiore, T. M. Swi Chang, P. de Vos, G. Hortelano, D. Hunkeler, I. Lacík, J. Luis Pedraz, *Trends Biotechnol.* **2004**, *22*, 87–92.

[68] S. Sugiura, T. Oda, Y. Izumida, Y. Aoyagi, M. Satake, A. Ochiai, N. Ohkohchi, M. Nakajima, *Biomaterials* **2005**, *26*, 3327–3331.

[69] E. J. Oh, K. Park, K. S. Kim, J. Kim, J.-A. Yang, J.-H. Kong, M. Y. Lee, A. S. Hoffman, S. K. Hahn, *J. Control. Release* **2010**, *141*, 2–12.

[70] J. A. Burdick, G. D. Prestwich, *Adv. Mater.* **2011**, *23*, H41–H56.

[71] B. D. Fairbanks, S. P. Singh, C. N. Bowman, K. S. Anseth, *Macromolecules* **2011**, *44*, 2444–2450.

[72] M. P. Lutolf, J. A. Hubbell, *Biomacromolecules* **2003**, *4*, 713–722.

[73] K. M. Schultz, A. D. Baldwin, K. L. Kiick, E. M. Furst, *Macromolecules* **2009**, *42*, 5310–5316.

[74] A. Metters, J. Hubbell, *Biomacromolecules* **n.d.**, *6*, 290–301.

[75] A. E. Rydholm, S. K. Reddy, K. S. Anseth, C. N. Bowman, *Polymer (Guildf).* **2007**, *48*, 4589–4600.

[76] D. S. W. Benoit, M. P. Schwartz, A. R. Durney, K. S. Anseth, *Nat. Mater.* **2008**, *7*, 816–823.

[77] A. A. Aimetti, A. J. Machen, K. S. Anseth, *Biomaterials* **2009**, *30*, 6048–6054.

[78] G. Pasut, F. M. Veronese, *Adv. Drug Deliv. Rev.* **2009**, *61*, 1177–1188.

[79] M.-H. Alves, B. E. B. Jensen, A. A. A. Smith, A. N. Zelikin, *Macromol. Biosci.* **2011**, *11*, 1293–1313.

[80] M. I. Baker, S. P. Walsh, Z. Schwartz, B. D. Boyan, *J. Biomed. Mater. Res. B. Appl. Biomater.* **2012**, *100*, 1451–1457.

[81] M. Calderón, M. A. Quadir, S. K. Sharma, R. Haag, *Adv. Mater.* **2010**, *22*, 190–218.

[82] D. Wilms, S.-E. Stiriba, H. Frey, *Acc. Chem. Res.* **2010**, *43*, 129–141.

[83] M. Weinhart, I. Grunwald, M. Wyszogrodzka, L. Gaetjen, A. Hartwig, R. Haag, *Chem. Asian J.* **2010**, *5*, 1992–2000.

[84] C. Siegers, M. Biesalski, R. Haag, *Chemistry* **2004**, *10*, 2831–2838.

[85] F. Paulus, M. E. R. Weiss, D. Steinhilber, A. N. Nikitin, C. Schütte, R. Haag, *Macromolecules* **2013**, *46*, 8458–8466.

[86] M. E. R. Weiss, F. Paulus, D. Steinhilber, A. N. Nikitin, R. Haag, C. Schütte, *Macromol. Theory Simulations* **2012**, *21*, 470–481.

[87] M. Calderón, P. Welker, K. Licha, I. Fichtner, R. Graeser, R. Haag, F. Kratz, *J. Control. Release* **2011**, *151*, 295–301.

[88] S.-E. Stiriba, H. Kautz, H. Frey, *J. Am. Chem. Soc.* **2002**, *124*, 9698–9699.

[89] R. Haag, A. Sunder, J.-F. Stumbé, *J. Am. Chem. Soc.* **2000**, *122*, 2954–2955.

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

[91] R. Chapanian, I. Constantinescu, N. A. A. Rossi, N. Medvedev, D. E. Brooks, M. D. Scott, J. N. Kizhakkedathu, *Biomaterials* **2012**, *33*, 7871–7883.

[92] R. K. Kainthan, D. E. Brooks, *Biomaterials* **2007**, *28*, 4779–4787.

[93] R. K. Kainthan, S. R. Hester, E. Levin, D. V Devine, D. E. Brooks, *Biomaterials* **2007**, *28*, 4581–4590.

[94] R. K. Kainthan, E. B. Muliawan, S. G. Hatzikiriakos, D. E. Brooks, *Macromolecules* **2006**, *39*, 7708–7717.

[95] N. E. Fedorovich, M. H. Oudshoorn, D. van Geemen, W. E. Hennink, J. Alblas, W. J. A. Dhert, *Biomaterials* **2009**, *30*, 344–353.

[96] M. H. M. Oudshoorn, R. Rissmann, J. A. Bouwstra, W. E. Hennink, *Biomaterials* **2006**, *27*, 5471–5479.

[97] M. H. M. Oudshoorn, R. Penterman, R. Rissmann, J. A. Bouwstra, D. J. Broer, W. E. Hennink, *Langmuir* **2007**, *23*, 11819–11825.

[98] E. M. Sletten, C. R. Bertozzi, *Acc. Chem. Res.* **2011**, *44*, 666–676.

[99] J. A. Prescher, C. R. Bertozzi, *Nat. Chem. Biol.* **2005**, *1*, 13–21.

[100] E. M. Sletten, C. R. Bertozzi, *Angew. Chem. Int. Ed. Engl.* **2009**, *48*, 6974–6998.

[101] A. S. Sawhney, C. P. Pathak, J. A. Hubbell, *Macromolecules* **1993**, *26*, 581–587.

[102] J. L. Ifkovits, J. A. Burdick, *Tissue Eng.* **2007**, *13*, 2369–2385.

[103] J. A. Burdick, A. J. Peterson, K. S. Anseth, *Biomaterials* **2001**, *22*, 1779–1786.

[104] C.-C. Lin, A. T. Metters, *Pharm. Res.* **2006**, *23*, 614–22.

[105] A. Valdebenito, P. Espinoza, E. A. Lissi, M. V. Encinas, *Polymer* **2010**, *51*, 2503–2507.

[106] N. E. Fedorovich, M. H. Oudshoorn, D. van Geemen, W. E. Hennink, J. Alblas, W. J. A. Dhert, *Biomaterials* **2009**, *30*, 344–353.

[107] A. M. Kloxin, M. W. Tibbitt, A. M. Kasko, J. A. Fairbairn, K. S. Anseth, *Adv. Mater.* **2010**, *22*, 61–66.

[108] A. M. Kloxin, A. M. Kasko, C. N. Salinas, K. S. Anseth, *Science* **2009**, *324*, 59–63.

[109] D. R. Griffin, A. M. Kasko, *J. Am. Chem. Soc.* **2012**, *134*, 13103–13107.

[110] H. Shin, B. D. Olsen, A. Khademhosseini, *Biomaterials* **2012**, *33*, 3143–3152.

[111] P. Panda, S. Ali, E. Lo, B. G. Chung, T. A. Hatton, A. Khademhosseini, P. S. Doyle, *Lab Chip* **2008**, *8*, 1056–1061.

[112] S. R. Shin, S. M. Jung, M. Zalabany, K. Kim, P. Zorlutuna, S. B. Kim, M. Nikkhah, M. Khabiry, M. Azize, J. Kong, et al., *ACS Nano* **2013**, *7*, 2369–2380.

[113] L. Pescosolido, S. Miatto, C. Di Meo, C. Cencetti, T. Coviello, F. Alhaique, P. Matricardi, *Eur. Biophys. J.* **2010**, *39*, 903–909.

[114] R. Censi, T. Vermonden, M. J. van Steenbergen, H. Deschout, K. Braeckmans, S. C. De Smedt, C. F. van Nostrum, P. di Martino, W. E. Hennink, *J. Control. Release* **2009**, *140*, 230–236.

[115] M. A. Azagarsamy, D. L. Alge, S. J. Radhakrishnan, M. W. Tibbitt, K. S. Anseth, *Biomacromolecules* **2012**, *13*, 2219–2224.

[116] X.-M. Jiang, *J. Biol. Chem.* **1999**, *274*, 2416–2423.

[117] S. Aubry, F. Burlina, E. Dupont, D. Delaroche, A. Joliot, S. Lavielle, G. Chassaing, S. Sagan, *FASEB J.* **2009**, *23*, 2956–67.

[118] B. Vernon, N. Tirelli, T. Bächi, D. Haldimann, J. A. Hubbell, *J. Biomed. Mater. Res. A* **2003**, *64*, 447–456.

[119] D. L. Elbert, A. B. Pratt, M. P. Lutolf, S. Halstenberg, J. A. Hubbell, *J. Control. Release* **2001**, *76*, 11–25.

[120] M. P. Lutolf, J. A. Hubbell, *Biomacromolecules* **2003**, *4*, 713–722.

[121] S. C. Rizzi, J. A. Hubbell, *Biomacromolecules* **2005**, *6*, 1226–1238.

[122] S. K. Hahn, J. K. Park, T. Tomimatsu, T. Shimoboji, *Int. J. Biol. Macromol.* **2007**, *40*, 374–380.

[123] S. K. Hahn, E. J. Oh, H. Miyamoto, T. Shimobouji, *Int. J. Pharm.* **2006**, *322*, 44–51.

[124] E. A. Phelps, N. O. Enemchukwu, V. F. Fiore, J. C. Sy, N. Murthy, T. A. Sulchek, T. H. Barker, A. J. García, *Adv. Mater.* **2012**, *24*, 64–70.

[125] Y. Lei, T. Segura, *Biomaterials* **2009**, *30*, 254–265.

[126] G. A. Hudalla, T. S. Eng, W. L. Murphy, *Biomacromolecules* **2008**, *9*, 842–849.

[127] J. M. H. Staudinger, *Hel. Chim. Acta* **1919**, *2*, 635.

[128] E. Saxon, *Science* **2000**, *287*, 2007–2010.

[129] J. A. Prescher, D. H. Dube, C. R. Bertozzi, *Nature* **2004**, *430*, 873–877.

[130] R. Kleineweischede, C. P. R. Hackenberger, *Angew. Chem. Int. Ed. Engl.* **2008**, *47*, 5984–5988.

[131] Y.-X. Chen, G. Triola, H. Waldmann, *Acc. Chem. Res.* **2011**, *44*, 762–773.

[132] H. C. Hang, J. P. Wilson, G. Charron, *Acc. Chem. Res.* **2011**, *44*, 699–708.

[133] C. I. Schilling, N. Jung, M. Biskup, U. Schepers, S. Bräse, *Chem. Soc. Rev.* **2011**, *40*, 4840–4871.

[134] S. S. van Berkel, M. B. van Eldijk, J. C. M. van Hest, *Angew. Chem. Int. Ed. Engl.* **2011**, *50*, 8806–8827.

[135] K. M. Gattás-Asfura, C. L. Stabler, *Biomacromolecules* **2009**, *10*, 3122–3129.

[136] K. K. Hall, K. M. Gattás-Asfura, C. L. Stabler, *Acta Biomater.* **2011**, *7*, 614–624.

[137] K. M. Gattás-Asfura, C. A. Fraker, C. L. Stabler, *J. Biomed. Mater. Res. A* **2011**, *99*, 47–57.

[138] R. Huisgen, *Angew. Chemie* **1963**, *75*, 604–637.

[139] C. W. Tornøe, C. Christensen, M. Meldal, *J. Org. Chem.* **2002**, *67*, 3057–3064.

[140] V. V Rostovtsev, L. G. Green, V. V Fokin, K. B. Sharpless, *Angew. Chem. Int. Ed. Engl.* **2002**, *41*, 2596–9.

[141] H. C. Kolb, M. G. Finn, K. B. Sharpless, *Angew. Chem. Int. Ed. Engl.* **2001**, *40*, 2004–2021.

[142] M. G. Finn, V. V Fokin, *Chem. Soc. Rev.* **2010**, *39*, 1231–1232.

[143] D. C. Kennedy, C. S. McKay, M. C. B. Legault, D. C. Danielson, J. A. Blake, A. F. Pegoraro, A. Stolow, Z. Mester, J. P. Pezacki, *J. Am. Chem. Soc.* **2011**, *133*, 17993–18001.

[144] G. J. Brewer, *Clin. Neurophysiol.* **2010**, *121*, 459–460.

[145] J. Gierlich, G. A. Burley, P. M. E. Gramlich, D. M. Hammond, T. Carell, *Org. Lett.* **2006**, *8*, 3639–3642.

[146] E. Lallana, E. Fernandez-Megia, R. Riguera, *J. Am. Chem. Soc.* **2009**, *131*, 5748–5750.

[147] C. J. Duxbury, D. Cummins, A. Heise, *J. Polym. Sci. Part A Polym. Chem.* **2009**, *47*, 3795–3802.

[148] C. Barner-Kowollik, F. E. Du Prez, P. Espeel, C. J. Hawker, T. Junkers, H. Schlaad, W. Van Camp, *Angew. Chem. Int. Ed. Engl.* **2011**, *50*, 60–62.

[149] K. Kempe, A. Krieg, C. R. Becer, U. S. Schubert, *Chem. Soc. Rev.* **2012**, *41*, 176–191.

[150] V. Crescenzi, L. Cornelio, C. Di Meo, S. Nardecchia, R. Lamanna, *Biomacromolecules* **2007**, *8*, 1844–1850.

[151] S. Q. Liu, P. L. R. Ee, C. Y. Ke, J. L. Hedrick, Y. Y. Yang, *Biomaterials* **2009**, *30*, 1453–1461.

[152] A. K. G. Wittig, **1961**, *94*, 3260.

[153] N. J. Agard, J. M. Baskin, J. A. Prescher, A. Lo, C. R. Bertozzi, *ACS Chem. Biol.* **2006**, *1*, 644–648.

[154] J. M. Baskin, J. A. Prescher, S. T. Laughlin, N. J. Agard, P. V Chang, I. A. Miller, A. Lo, J. A. Codelli, C. R. Bertozzi, *Proc. Natl. Acad. Sci. U. S. A.* **2007**, *104*, 16793–16797.

[155] S. T. Laughlin, J. M. Baskin, S. L. Amacher, C. R. Bertozzi, *Science* **2008**, *320*, 664–667.

[156] E. Lallana, E. Fernandez-Megia, R. Riguera, *J. Am. Chem. Soc.* **2009**, *131*, 5748–5750.

[157] J. Dommerholt, S. Schmidt, R. Temming, L. J. A. Hendriks, F. P. J. T. Rutjes, J. C. M. van Hest, D. J. Lefeber, P. Friedl, F. L. van Delft, *Angew. Chem. Int. Ed. Engl.* **2010**, *49*, 9422–9425.

[158] M. F. Debets, S. S. van Berkel, J. Dommerholt, A. T. J. Dirks, F. P. J. T. Rutjes, F. L. van Delft, *Acc. Chem. Res.* **2011**, *44*, 805–815.

[159] R. van Geel, G. J. M. Pruijn, F. L. van Delft, W. C. Boelens, *Bioconjug. Chem.* **2012**, *23*, 392–398.

[160] C. A. DeForest, K. S. Anseth, *Nat. Chem.* **2011**, *3*, 925–931.

[161] J. Xu, T. M. Filion, F. Prifti, J. Song, *Chem. Asian J.* **2011**, *6*, 2730–2737.

[162] C. A. DeForest, B. D. Polizzotti, K. S. Anseth, *Nat. Mater.* **2009**, *8*, 659–664.

[163] R. A. Carboni, R. V. Lindsey, *J. Am. Chem. Soc.* **1959**, *81*, 4342–4346.

[164] G. Clavier, P. Audebert, *Chem. Rev.* **2010**, *110*, 3299–314.

[165] J. Balcar, G. Chrisam, F. X. Huber, J. Sauer, *Tetrahedron Lett.* **1983**, *24*, 1481–1484.

[166] J. Sauer, D. K. Heldmann, J. Hetzenegger, J. Krauthan, H. Sichert, J. Schuster, *European J. Org. Chem.* **1998**, *1998*, 2885–2896.

[167] F. Thalhammer, U. Wallfahrer, J. Sauer, *Tetrahedron Lett.* **1990**, *31*, 6851–6854.

[168] N. K. Devaraj, G. M. Thurber, E. J. Keliher, B. Marinelli, R. Weissleder, *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109*, 4762–4767.

[169] N. K. Devaraj, R. Weissleder, S. A. Hilderbrand, *Bioconjug. Chem.* **2008**, *19*, 2297–2299.

[170] J. L. Seitchik, J. C. Peeler, M. T. Taylor, M. L. Blackman, T. W. Rhoads, R. B. Cooley, C. Refakis, J. M. Fox, R. A. Mehl, *J. Am. Chem. Soc.* **2012**, *134*, 2898–2901.

[171] Y. Xia, G. M. Whitesides, *Angew. Chemie Int. Ed.* **1998**, *37*, 550–575.

[172] J. K. Oh, D. J. Siegwart, H. Lee, G. Sherwood, L. Peteanu, J. O. Hollinger, K. Kataoka, K. Matyjaszewski, *J. Am. Chem. Soc.* **2007**, *129*, 5939–5945.

[173] A. L. Sisson, I. Papp, K. Landfester, R. Haag, *Macromolecules* **2009**, *42*, 556–559.

[174] J. K. Oh, R. Drumright, D. J. Siegwart, K. Matyjaszewski, *Prog. Polym. Sci.* **2008**, *33*, 448–477.

[175] K. Landfester, A. Musyanovych, *Adv. Polym. Sci.* **2010**, *234*, 39–63.

[176] K. McAllister, P. Sazani, M. Adam, M. J. Cho, M. Rubinstein, R. J. Samulski, J. M. DeSimone, *J. Am. Chem. Soc.* **2002**, *124*, 15198–15207.

[177] J.-Z. Du, T.-M. Sun, W.-J. Song, J. Wu, J. Wang, *Angew. Chem. Int. Ed. Engl.* **2010**, *49*, 3621–3626.

[178] M. Antonietti, *Angew. Chemie Int. Ed. English* **1988**, *27*, 1743–1747.

[179] M. Antonietti, K. Landfester, *Chemphyschem* **2001**, *2*, 207–210.

[180] M. Antonietti, E. Wenz, L. Bronstein, M. Seregina, *Adv. Mater.* **1995**, *7*, 1000–1005.

[181] M. Antonietti, *Prog. Polym. Sci.* **2002**, *27*, 689–757.

[182] W. Z. Ostwald, *Phys. Chem.* **1901**, *37*, 385.

[183] J. Ugelstad, M. S. El-Aasser, J. W. Vanderhoff, *J. Polym. Sci. Polym. Lett. Ed.* **1973**, *11*, 503–513.

[184] K. Landfester, *Adv. Mater.* **2001**, *13*, 765–768.

[185] K. Landfester, N. Bechthold, S. Förster, M. Antonietti, *Macromol. Rapid Commun.* **1999**, *20*, 81–84.

[186] K. Landfester, M. Willert, M. Antonietti, *Macromolecules* **2000**, *33*, 2370–2376.

[187] K. Landfester, N. Bechthold, F. Tiarks, M. Antonietti, *Macromolecules* **1999**, *32*, 5222–5228.

[188] K. Landfester, *Annu. Rev. Mater. Res.* **2006**, *36*, 231–279.

[189] D. J. Siegwart, A. Srinivasan, S. A. Bencherif, A. Karunanidhi, J. K. Oh, S. Vaidya, R. Jin, J. O. Hollinger, K. Matyjaszewski, *Biomacromolecules* **2009**, *10*, 2300–2309.

[190] J. H. Schulman, W. Stoeckenius, L. M. Prince, *J. Phys. Chem.* **1959**, *63*, 1677–1680.

[191] M. Kahlweit, R. Strey, G. Busse, *J. Phys. Chem.* **1991**, *95*, 5344–5352.

[192] C. C. Co, P. Cotts, S. Burauer, R. de Vries, E. W. Kaler, *Macromolecules* **2001**, *34*, 3245–3254.

[193] H.-P. Hentze, E. W. Kaler, *Curr. Opin. Colloid Interface Sci.* **2003**, *8*, 164–178.

[194] J. P. Rolland, B. W. Maynor, L. E. Euliss, A. E. Exner, G. M. Denison, J. M. DeSimone, *J. Am. Chem. Soc.* **2005**, *127*, 10096–10100.

[195] K. Chen, T. J. Merkel, A. Pandya, M. E. Napier, J. C. Luft, W. Daniel, S. Sheiko, J. M. DeSimone, *Biomacromolecules* **2012**, *13*, 2748–2759.

[196] J. Xu, J. Wang, J. C. Luft, S. Tian, G. Owens, A. A. Pandya, P. Berglund, P. Pohlhaus, B. W. Maynor, J. Smith, et al., *J. Am. Chem. Soc.* **2012**, *134*, 8774–8777.

[197] M. H. M. Oudshoorn, R. Penterman, R. Rissmann, J. A. Bouwstra, D. J. Broer, W. E. Hennink, *Langmuir* **2007**, *23*, 11819–11825.

[198] M. C. M. van Oers, F. P. J. T. Rutjes, J. C. M. van Hest, *J. Am. Chem. Soc.* **2013**, *135*, 16308–16311.

[199] C. J. Rijcken, C. J. Snel, R. M. Schiffelers, C. F. van Nostrum, W. E. Hennink, *Biomaterials* **2007**, *28*, 5581–5593.

[200] Y.-L. Li, L. Zhu, Z. Liu, R. Cheng, F. Meng, J.-H. Cui, S.-J. Ji, Z. Zhong, *Angew. Chem. Int. Ed. Engl.* **2009**, *48*, 9914–9918.

[201] J.-H. Ryu, R. T. Chacko, S. Jiwpanich, S. Bickerton, R. P. Babu, S. Thayumanavan, *J. Am. Chem. Soc.* **2010**, *132*, 17227–17235.

[202] N. V Nukolova, H. S. Oberoi, S. M. Cohen, A. V Kabanov, T. K. Bronich, *Biomaterials* **2011**, *32*, 5417–5426.

[203] S. Herlambang, M. Kumagai, T. Nomoto, S. Horie, S. Fukushima, M. Oba, K. Miyazaki, Y. Morimoto, N. Nishiyama, K. Kataoka, *J. Control. Release* **2011**, *155*, 449–457.

[204] X. Shuai, T. Merdan, A. K. Schaper, F. Xi, T. Kissel, *Bioconjug. Chem.* **2004**, *15*, 441–448.

[205] C. Cheng, K. Qi, D. S. Germack, E. Khoshdel, K. L. Wooley, *Adv. Mater.* **2007**, *19*, 2830–2835.

[206] Y. Li, K. Xiao, J. Luo, W. Xiao, J. S. Lee, A. M. Gonik, J. Kato, T. A. Dong, K. S. Lam, *Biomaterials* **2011**, *32*, 6633–6645.

[207] S. Liu, J. V. M. Weaver, Y. Tang, N. C. Billingham, S. P. Armes, K. Tribe, *Macromolecules* **2002**, *35*, 6121–6131.

[208] K. B. Thurmond, T. Kowalewski, K. L. Wooley, *J. Am. Chem. Soc.* **1996**, *118*, 7239–7240.

[209] V. K. LaMer, R. H. Dinegar, *J. Am. Chem. Soc.* **1950**, *72*, 4847–4854.

[210] G. François, J. L. Katz, *Chemphyschem* **2005**, *6*, 209–216.

[211] C. Zhang, V. J. Pansare, R. K. Prud'homme, R. D. Priestley, *Soft Matter* **2012**, *8*, 86–93.

[212] S. Schubert, J. T. Delaney, Jr, U. S. Schubert, *Soft Matter* **2011**, *7*, 1581–1588.

[213] I. Y. Perevyazko, J. T. Delaney, A. Vollrath, G. M. Pavlov, S. Schubert, U. S. Schubert, *Soft Matter* **2011**, *7*, 5030–5035.

[214] E. M. Sussman, M. B. Clarke, V. P. Shastri, *Langmuir* **2007**, *23*, 12275–12279.

[215] S. Seiffert, *Angew. Chem. Int. Ed. Engl.* **2013**, *52*, 11462–11468.

[216] D. Velasco, E. Tumarkin, E. Kumacheva, *Small* **2012**, *8*, 1633–1642.

[217] B. G. Chung, K.-H. Lee, A. Khademhosseini, S.-H. Lee, *Lab Chip* **2012**, *12*, 45–59.

[218] S. Seiffert, D. a. Weitz, *Polymer (Guildf).* **2010**, *51*, 5883–5889.

[219] J. C. McDonald, D. C. Duffy, J. R. Anderson, D. T. Chiu, H. Wu, O. J. Schueller, G. M. Whitesides, *Electrophoresis* **2000**, *21*, 27–40.

[220] R. Shah, H. Shum, A. Rowat, D. Lee, J. Agresti, A. Utada, L. Chu, J. Kim, A. Fernandeznieves, C. Martinez, et al., *Mater. Today* **2008**, *11*, 18–27.

[221] W.-H. Tan, S. Takeuchi, *Adv. Mater.* **2007**, *19*, 2696–2701.

[222] A. Kumachev, J. Greener, E. Tumarkin, E. Eiser, P. W. Zandstra, E. Kumacheva, *Biomaterials* **2011**, *32*, 1477–1483.

[223] P. Panda, S. Ali, E. Lo, B. G. Chung, T. A. Hatton, A. Khademhosseini, P. S. Doyle, *Lab Chip* **2008**, *8*, 1056–1061.

[224] E. Gil, S. Hudson, *Prog. Polym. Sci.* **2004**, *29*, 1173–1222.

[225] J. Hu, G. Zhang, S. Liu, *Chem. Soc. Rev.* **2012**, *41*, 5933–5949.

[226] A. Kikuchi, T. Okano, *Adv. Drug Deliv. Rev.* **2002**, *54*, 53–77.

[227] E. C. Novosel, C. Kleinhans, P. J. Kluger, *Adv. Drug Deliv. Rev.* **2011**, *63*, 300–311.

[228] W. Chen, F. Meng, J. Feijen, Z. Zhong, *J. Control. Release* **2013**, *172*, e115.

[229] P. Li, Z. Luo, P. Liu, N. Gao, L. Cai, Y. Ma, *J. Control. Release* **2013**, *172*, e119–120.

[230] D. Maciel, P. Figueira, S. Xiao, D. Hu, X. Shi, J. Rodrigues, H. Tomás, Y. Li, *Biomacromolecules* **2013**, *14*, 3140–3146.

[231] W. Chen, M. Zheng, F. Meng, R. Cheng, C. Deng, J. Feijen, Z. Zhong, *Biomacromolecules* **2013**, *14*, 1214–1222.

[232] S. Singh, F. Topuz, K. Hahn, K. Albrecht, J. Groll, *Angew. Chem. Int. Ed. Engl.* **2013**, *52*, 3000–3003.

[233] D. C. González-Toro, J.-H. Ryu, R. T. Chacko, J. Zhuang, S. Thayumanavan, *J. Am. Chem. Soc.* **2012**, *134*, 6964–6967.

[234] Y.-Y. Yuan, J.-Z. Du, W.-J. Song, F. Wang, X.-Z. Yang, M.-H. Xiong, J. Wang, *J. Mater. Chem.* **2012**, *22*, 9322.

[235] M. C. Parrott, J. C. Luft, J. D. Byrne, J. H. Fain, M. E. Napier, J. M. Desimone, *J. Am. Chem. Soc.* **2010**, *132*, 17928–17932.

[236] K. S. Anseth, A. T. Metters, S. J. Bryant, P. J. Martens, J. H. Elisseeff, C. N. Bowman, *J. Control. Release* **2002**, *78*, 199–209.

[237] E. Fleige, M. A. Quadir, R. Haag, *Adv. Drug Deliv. Rev.* **2012**, *64*, 866–884.

[238] J. Patterson, J. A. Hubbell, *Biomaterials* **2010**, *31*, 7836–7845.

[239] A. M. Kloxin, A. M. Kasko, C. N. Salinas, K. S. Anseth, *Science* **2009**, *324*, 59–63.

[240] N. Fomina, C. McFearin, M. Sermsakdi, O. Edigin, A. Almutairi, *J. Am. Chem. Soc.* **2010**, *132*, 9540–9542.

[241] F. Q. Schafer, G. R. Buettner, *Free Radic. Biol. Med.* **2001**, *30*, 1191–1212.

[242] G. Saito, J. A. Swanson, K.-D. Lee, *Adv. Drug Deliv. Rev.* **2003**, *55*, 199–215.

[243] J. Yang, H. Chen, I. R. Vlahov, J.-X. Cheng, P. S. Low, *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103*, 13872–13877.

[244] S. Khetan, M. Guvendiren, W. R. Legant, D. M. Cohen, C. S. Chen, J. A. Burdick, *Nat. Mater.* **2013**, *12*, 458–465.

[245] G. P. Raeber, M. P. Lutolf, J. A. Hubbell, *Biophys. J.* **2005**, *89*, 1374–1388.

[246] L. M. Weber, C. G. Lopez, K. S. Anseth, *J. Biomed. Mater. Res. A* **2009**, *90*, 720–729.

[247] C. Siegers, M. Biesalski, R. Haag, *Chemistry* **2004**, *10*, 2831–2838.

7 Publications, patent applications and conference contributions

Publications with peer review process

[1] A. L. Sisson, **D. Steinhilber**, T. Rossow, P. Welker, K. Licha, R. Haag
"Biocompatible functionalized polyglycerol microgels with cell penetrating properties"
Angew. Chem. **2009**, *121*, 7676-7681. *Angew. Chem. Int. Ed. Engl.* **2009**, *48*, 7540-7545.

[2] **D. Steinhilber,** A. L Sisson, D Mangoldt, P. Welker, K. Licha, R. Haag
"Synthesis, reductive cleavage, and cellular interaction studies of biodegradable, polyglycerol nanogels"
Adv. Funct. Mater. **2010**, *20*, 4133-4138.

[3] **D. Steinhilber**, S.Seiffert, J. A. Heyman, F. Paulus, D. A. Weitz, R. Haag
"Hyperbranched polyglycerols on the nanometer and micrometer scale"
Biomaterials **2011**, *32*, 1311-1316.

[4] J. Cuggino, C. I. Alvarez, M. Strumia, P. Welker, K. Licha, **D. Steinhilber,** R. C. Mutihac, M. Calderón
"Thermosensitive nanogels based on dendritic polyglycerol and N-isopropylacrylamide for biomedical applications"
Soft Mater **2011**, *7*, 11259-11266.

[5] H. Zhou, **D. Steinhilber,** H. Schlaad, A. L. Sisson, R. Haag
"*Glycerol based polyether-nanogels with tunable properties via acid-catalyzed epoxide-opening in miniemulsion"*
React. Funct. Polym. **2011**, *71*, 356-361.

[6] **D. Steinhilber**, R. Haag, A. L. Sisson
"Multivalent biodegradable polyglycerol hydrogels
Int. J. Artif. Organs **2011**, *34*, 118-122.

[7] **D. Steinhilber**, F. Paulus, A. T. Zill, S. C. Zimmerman, R. Haag
"*Calix[8]arene functionalized polyglycerol nanogels for encapsulation and stabilization of fluorescent dyes"*
MRS Proceedings 2012, 1403, doi: 10.1557/opl.2012.419, Published online by Cambridge University Press

[8] M. E. R. Weiss, F. Paulus, **D. Steinhilber**, A.Nikitin, R. Haag, C. Schütte
"*Estimating kinetic parameters for the spontaneous polymerization of glycidol at elevated temperatures"*
Macromolecular Theory and Simulations **2012**, *21*, 470-481.

[9] Asadian, M., Souza, A., **Steinhilber, D.**, Cuggino, J., Calderón M.
"Functional nanogels for biomedical applications"
Current Medicinal Chemistry **2012**, *19*, 5029-5043.

[10] **D. Steinhilber,** M. Witting, X. Zhang, M. Steagemann, W. Friess, S. Küchler, R. Haag
"*Surfactant free preparation of biodegradable dendritic polyglycerol nanogels by inverse nanoprecipiation for encapsulation and release of pharmaceutical enzymes"*
J. Control. Release **2013**, *169*, 289-295.

[11] F. Paulus, M. E. R. Weiss, **D. Steinhilber,** A. Nikitin, C.Schütte, R. Haag
"*Anionic ring-opening polymerization simulations for hyperbranched polyglycerols with defined molecular weights"*
Macromolecules **2013**, *46*, 8458-8466.

[12] X. Zhang, K. Achazi, D. Steinhilber, F. Kratz, J. Dernedde, R. Haag
"A facile approach towards dual-responsive prodrug nanogels based on dendritic polyglycerols with minimal leaching"
J. Control. Release **2013**, *174*, 209-216.

[13] **D. Steinhilber**, T. Rossow, S. Wedepohl, F. Paulus, S. Seiffert, R. Haag
"A microgel construction kit for the bioorthogonal encapsulation and pH-controlled release of living cells"
Angew. Chem. **2013**, *52*, 13538-13543.

[14] F. Paulus, R. Schulze, **D. Steinhilber,** M. Zieringer, I. Steinke, P. Welker, K. Licha, S. Wedepohl, J. Dernedde, R. Haag
"The effect of polyglycerol branching on inflammatory processes"
Macromol. Bio. Sci. in press, DOI: 10.1002/mabi.201300420.

Publications without peer review process

[16] I. Papp, A. L. Sisson, **D.**,**Steinhilber**, J. Dernedde, S. Enders, R. Haag
"Multivalent dendritic architectures"
Macromol. Bio. Sci. **2009**, *9*, F83-F84.

[17] **D. Steinhilber**, A. L. Sisson, P. Welker, K. Licha, R. Haag
"Biodegradable polyglycerol nanogels with excellent cell penetrating properties"
PMSE Preprints **2010**, *103*, 467-469.

[18] **D. Steinhilber**, I. Papp, F. Paulus, T. Rossow, S. Seiffert, R. Haag,
"Multifunctional dendritic polyglycerol nano- and microgels by miniemulsion, nanoprecipitation and microfluidic techniques"
Macromol. Chem. Phys. **2013**, *214*, F97-F98.

Patents

[19] R. Haag, A. L. Sisson, **D. Steinhilber,** H. Zhou, europäische Patentanmeldung **2009**, EP 08158867.5, Internationale Patentanmeldung **2009**, PCT / EP 2009 057924, *Method for producing a nanoparticle, nanoparticle, nanoparticle system and its use.*

[20] **D. Steinhilber**, R. Haag, W. Friess, S. Küchler, *deutsche Patentanmeldung* **2012**, DE 10 2012 108 345.2, *internationale PCT-Anmeldung* eingereicht. *Verfahren zur Herstellung eines Polyglycerin-Nanogels zur Verkapselung und Freisetzung biologisch aktiver Substanzen / Verfahren zur Herstellung von bioabbaubaren Polyglycerin Nanogelen durch Nanofällung zur Stabilisierung und kontrollierten Freisetzung von Therapeutischen Enzymen.*

Conference contributions

Oral presentations

[1] **D. Steinhilber**, A. L. Sisson, D. Mangoldt, P. Welker, K. Licha, R. Haag
"Biocompatible functionalized polyglycerol nanogels with cell penetrating properties"
8 th International Symposium on Polymer Therapeutics, Valencia, **Spain**, 2010

[2] **D. Steinhilber,** M. Staegemann, R. Haag
„Surfactant free preparation of pH labile polygylcerol nanogels by nanoprecipitation"
Materials Research Society Meeting, Boston, **USA**, 2011

[3] **D. Steinhilber**, F. Paulus, S Seiffert, R. Haag
„Biodegradable polyglycerol gel particles on various length scales"
Makromolekulares Kolloquium, Freiburg, **Germany**, 2012

[4] **D. Steinhilber,** R. Haag
"Protein- and cell-compatible polyglycerol nano- and microgels"
12 th International Conference on Polymers and Advanced Technologies, Berlin, Germany, 2013

Posterpresentations

[1] **D. Steinhilber**, A. L. Sisson, R. Haag
"Synthesis and degradation of disulfide containing dendritic polyglycerol microgels"
6 th International Dendrimer Symposium, Stockholm, **Sweden**, 2009

[2] **D. Steinhilber**, A. L. Sisson, P. Welker, K. Licha, R. Haag
"Polyglycerol microgels with excellent cell penetrating properties"
Tag der Chemie, Potsdam, **Germany**, 2009

[3] **D. Steinhilber**, A. L. Sisson, D. Mangoldt, P. Welker, K. Licha, R. Haag
"Biocompatible functionalized polyglycerol nanogels with cell penetrating properties"
8 th International Symposium on Polymer Therapeutics, Valencia, **Spain** 2010

[4] **D. Steinhilber**, A. L. Sisson, D. Mangoldt, P. Welker, K. Licha, R. Haag
"Biodegradable polyglycerol nanogels"
Tag der Chemie, Berlin, **Germany**, 2010

[5] **D. Steinhilber**, A. L. Sisson, D. Mangoldt, P. Welker, K. Licha, R. Haag
"Biodegradable polyglycerol nanogels for biomedical applications"
240 th American Chemical Society National Meeting, Boston, **USA**, 2010

[6] **D. Steinhilber**, M. Staegemann, R. Haag
"Surfactant free synthesis of pH labile polyglycerol nanogels by nanoprecipitation"
Tag der Chemie, Berlin, **Germany**, 2011

[7] **D. Steinhilber**, M. Staegemann, , M. Richter, R. Klitzing, R. Haag
"Surfactant free synthesis of pH labile polyglycerol nanogels by nanoprecipitation"
9 th Nanomedicin and Drug Delivery Symposium, Salt Lake City, **USA**, 2011

[8] **D. Steinhilber,** F. Paulus, S. Seiffert, R. Haag
"Biodegradable polyglycerol gel particles on various length scales"
Makromolekulares Kolloquium, Freiburg, **Germany**, 2012

[9] **D. Steinhilber,** T. Rossow, M. Richter, R. Klitzing, R. Haag
"Multifunctional polyglycerol micro- and nanogels for the stabilisation and release of enzymes and living cells"
2 nd International Conference on Biomaterials Science, Tsukuba, **Japan**, 2013
Poster Award and Travel Award

[10] D. Steinhilber, T. Rossow, S. Wedepohl, F. Paulus, S. Seiffert, R. Haag
"A microgel construction kit for the bioorthogonal encapsulation and pH-controlled release of living cells"
12 th International Conference on Polymers for Advanced Technologies, Berlin, **Germany,** 2013
First Poster Award

8 Curriculum vitae

For reasons of data protection, the curriculum vitae is not included in the online version