Design and application of dextran based cross-linked networks

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Chapter 7

Summary and Perspectives

As early as the 1950s, a variety of hydrogels have been designed, characterized and evaluated for biomedical applications. Commercial products based on hydrogels include contact lenses, wound dressings and hygiene products, due to their high water absorption, porosity and unique mechanical properties. In the last two decades, the focus has shifted to explore the potential of hydrogels in the fields of drug delivery and tissue engineering. As a result, the design of hydrogels evolved from relatively simple chemical or physical crosslinked networks to complex multicomponent systems with a high level of spatial and temporal control. The specific demand for multi-functional and well-controlled hydrogels resulted in the development of networks with novel synthetic methodologies and dynamic chemistry/assembly strategies.

In this thesis, new crosslinked hydrogels were designed and applications of these materials in the field of drug delivery were explored. These hydrogels mainly consist of the neutral polysaccharide dextran (Dex) due to its favorable properties like its biodegradable and biocompatible nature, relatively low cost, good water-solubility, and ease of chemical modification.

Inspired by natural systems, novel drug delivery platforms were established in which human serum albumin (HSA) acts simultaneously as an affinity-based drug carrier and crosslinker. **Chapter 2** focuses on the modification of HSA by thiolation, using it to crosslink a maleimide modified dextran (Dex-Mal) polymer, and the sustained delivery of low molecular drugs. To maintain the drug-binding capability of albumin, 2-iminothiolane was used to introduce thiols to albumin, which prevents denaturation by maintaining the surface charge distribution. A further extension of this work on affinity-based drug delivery hydrogels can be to use thiolated albumin in combination with other covalent polymers functionalized with Michael acceptors to gain access to hydrogel materials with a range of properties.

In **Chapter 3**, two approaches were explored to improve the stability and mechanical properties of the dextran-albumin hydrogels. In the first approach, the modification of dextran with vinyl sulfone through an ether linkage to reduce crosslinker hydrolysis was studied. The second approach involved the introduction of a third macromolecular precursor, poly(ethylene glycol) end-functionalized with thiol groups, to the dextran-albumin hydrogel system in order to tune the gelation kinetics and physical properties. The resulting Dex(VS)-sHSA-PEG hydrogels showed improved stability and increased mechanical stiffness making it more suitable for sustained drug delivery applications.

Besides the use of these gels as a drug delivery tool, other applications of these hydrogels were explored in **Chapter 4** and **5**. The uptake of a low molecular weight drug in a zebrafish embryo toxicity assay using the dextran based crosslinked drug carriers was studied in **Chapter 4**. The model drug valproate was solubilized using β-Cyclodextrins conjugated to a dextran-PEG network.

These Dex-CD/PEG carriers (without valproate) were taken up by 4-days post fertilization old (4-dpf) zebrafish embryos into the gastrointestinal tract. Next, 4-dpf zebrafish embryos were exposed to the model drug valproate, which was encapsulated in the drug carrier. As a result, the zebrafish embryos became more sensitive toward valproate strongly suggesting that the Dex-CD/PEG drug carriers are indeed capable of improving its uptake efficiency in the zebrafish embryo toxicity assay. Our results indicate that the Dex-CD/PEG carriers may assist zebrafish embryo-based toxicity assays of any drug that is able to form an inclusion complex with β-cyclodextrin.

Giant unilamellar vesicles (GUV) are used to study membrane biophysics and membrane related processes in general. In **Chapter 5**, a new method to form GUVs using hydrogel film-assisted hydration of a lipid film was established. A thin layer of a hydrogel was formed by a Michael addition reaction between maleimide-modified dextran and dithiol terminated poly(ethylene glycol) on a thiolated glass slide. Covalently linking the hydrogel film to the glass slides ensures that the formed GUVs are not contaminated with polymer fragments. Upon hydration of the polymer film using a buffer of physiological ionic strength, GUVs were formed rapidly in a high yield. The yield and size distribution of the formed GUVs were systematically investigated by varying the hydrogel composition. In order to obtain GUVs with a narrow size distribution in high yield, it was essential that the hydrogel films had a continuous pore structure with a suitable size. Also, the interactions between the lipid components and hydrophilic polymers is an important factor for successful GUV formation. Compared to electroformation methods, this method requires no special equipment and is applicable to any lipid formulation in high ionic strength buffer. This hydrogel film-assisted hydration method generates GUVs within minutes in high yield making it an easy to use method.

In **Chapter 6**, the heterodimeric coiled-coil forming peptides **E** and **K**, were conjugated to dextran in order to direct its self-assembly through non-covalent interactions. The peptides were conjugated either on their C- or N-terminus to dextran, resulting in four multivalent peptide-dextran conjugates. It was observed that the peptide conformation was altered after conjugation to dextran. Formation of large aggregates between dextran-peptide **E** and dextran-peptide **K** conjugates was revealed by dynamic light scattering measurements. However no specific coiled-coil interaction could be observed in fluorescence resonance energy transfer studies. This might be due to homomeric (**K**/**K**) coiled-coil formation in dextran-peptide **K** preventing heteromeric coiled-coil (**E**/**K**) formation. Furthermore, coiled-coil formation may be hampered by steric effects as a result of polymer-peptide conjugation.

In summary, this thesis describes the development of dextran-based hydrogels which can be tuned within a wide range of physicochemical properties for a given application. Moreover, this thesis also explores emerging applications of hydrogels in fields of biophysics and toxicology.

Regarding the use of hydrogels for albumin drug delivery, the binding pockets of albumin were utilized to construct hydrogels for spatial and temporal control over the release of therapeutic agents. Their sustained release was proven with several different drugs in our studies, but further optimization is required. Drug release from these hydrogels involves two stages: the release of the drug from the binding cavities, and subsequent drug diffusion through the insoluble polymer network. Computational simulations of the release process may provide a deeper insight into the release mechanism. From these studies, correlation between drug-albumin binding coefficients and release kinetics from the hydrogel can be established and thus aid in the further design optimization of these materials. Co-delivery of both hydrophobic and hydrophilic therapeutic agents from the various parts of the hydrogel may also be an interesting direction for further investigation.

Regarding the emerging applications of hydrogels described in this thesis, the crosslinked dextran-PEG hydrogel films provide a facile method to prepare GUVs with and without cargo, while the crosslinked Dex-CD/PEG carriers improve the uptake efficiency of low molecular weight compounds like valproate in a zebrafish embryo toxicity assay. It is expected that this method is suited to enhance the uptake of any compound that is able to form host-guest complex with β-cyclodextrin. Besides establishing these new approaches, a fundamental aspect of polymer science is explored in this thesis: orthogonal crosslinking methods in polymer networks and examination of their corresponding physiochemical properties. By careful design of the hydrogel building blocks, defects and inhomogeneities of the polymeric network could be reduced thereby resulting in better performance with respect to reproducibility and application.

Regarding the development of new biomolecule-dextran derivatives, the conjugation between the dextran polymer and biomolecules such as peptides, proteins and nucleic acids seems to be straightforward. However, it becomes apparent that the structure, conformation and functionality of the resulting multivalent biomolecule-dextran conjugates may be more complex than initially expected. Therefore it is necessary in the future to focus on the detailed interactions of biomolecules grafted to dextran backbones as the local environment may interfere with the biomolecule function. The distribution of the polymer grafts on the polymeric backbone and the distance between outer ends of polymer grafts as well as the polymeric backbone may also be important to be able to evaluate these interactions. However designing experiments that could reveal these parameters is not trivial. Nevertheless, a thorough understanding of the newly developed biomolecule-dextran derivatives can assist in their future design.

Tremendous progress was achieved over the years in the design of hydrogels as evidenced by the vast body of literature. Recent developments include the design of multi-component hydrogel systems to meet the needs of increasingly complex scaffolds for a wide range of biomedical applications. To this end, novel hydrogel systems are also designed for facilitating simple and

controllable manipulations such as *in situ* forming and 3D printed hydrogels. By continuing investigation in this research field, new frontiers and lasting contributions to polymer and biomedical sciences are within reach.