Hydrogel based drug carriers for controlled release of hydrophobic drugs and proteins

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Chapter 7

Summary and perspectives

Summary

Hydrogel materials are widely used in a diverse range of applications, especially for drug delivery, biosensing, and tissue engineering, due to their excellent biocompatibility and capability as a container for fragile bioactive molecules. Their high water content and three dimensional polymer network resemble a similiarity to natural tissue, which can increase its accommodability for more molecules and decrease its irritation to the biological surroundings. Recently, hydrogels that can be formed *in situ* under physiological conditions have been paid much attention as potential drug carriers. This is because *in situ* hydrogel formation allows for preparation of complex shapes and applications with minimal surgical wounds; furthermore, bioactive macromolecules such as peptides or proteins can be encapsulated simply by mixing with the polymer solutions before gelation.

In this thesis, *in situ* forming hydrogels based on biocompatible polymers for the controlled release of hydrophobic drug and proteins have been prepared and characterized. In order to load hydrophobic drug to the hydrophilic hydrogel matrix, β-cyclodextrin and human serum albumin were introduced to the hydrogel network respectively and acted as the primary accommodation for those hydrophobic molecules, and these hydrogels can be formed simply by mixing two solutions *via* Michael addition. Furthermore, supramolecular crosslinked and covalently crosslinked light sensitive hydrogels were prepared whose potential applications for light controlled protein release system have been shown.

Chapter 1 gives a general introduction about hydrogels and their applications, which emphasize *in situ* forming hydrogels and their applications for drug delivery. Furthermore, the aim and outline of this thesis are also presented.

In **Chapter 2**, a rapid *in situ* hydrogel forming system composed of thiol functionalized β-cyclodextrin and maleimide functionalized dextran has been prepared and characterized with rheology measurements and scanning electron microscopy, the *in vitro* release profile of the hydrophobic drug all-*trans* retinoic acid was studied.

Chapter 3 reports on hydrogel based drug carriers which have been developed from biocompatible materials, cyclodextrin, dextran and poly(ethylene glycol) and their application in zebrafish embryos. Maleimide modified dextrans (Dex-mal) were

functionalized with cyclodextrins and crosslinked to form a hydrogel using either per-6-thio-β-cyclodextrin (PSCD) or a combination of mono-6-thio-β-cyclodextrin (MSCD) and di-thiolated poly(ethylene glycol) (DSPEG). Using all-*trans* retinoic acid (RA) as a model hydrophobic drug, a sustained release from these cyclodextrin modified hydrogels was observed *in vitro* without an initial burst. This is because the cyclodextrin moiety in these hydrogels acts as a binding site for the RA. Furthermore, the nanosized hydrogel particles were injected into early stage zebrafish embryos in order to test *in vivo* release of RA and biocompatibility. We found the gel particles prepared from Dex-mal, MSCD and DSPEG were suitable for use in zebrafish embryos and it showed the release of RA in the embryos occurs in a controlled manner.

In **Chapter 4**, an *in situ* forming, covalently crosslinked hydrogel system composed of human serum albumin and maleimide functionalized dextran was prepared without any chemical modification on the protein. The obtained hydrogel was characterized with rheology measurements and scanning electron microscopy, and tested as a drug carrier using diclofenac, ibuprofen and ketoprofen as model drugs.

Chapter 5 focuses on using the inclusion complex of *trans* azobenzene and cyclodextrin as a photo-switchable crosslinker to construct a dextran based photo-responsive supramolecular hydrogel system which has the potential application as a light controlled protein release system.

Chapter 6 deals with a photodegradable, covalently crosslinked hydrogel system which has been constructed from the biocompatible polymers dextran and poly(ethylene glycol) using the acrylate-thiol Michael addition as the crosslinking method. Light sensitivity of the hydrogel was introduced by using a non-toxic photolabile *o*-nitrobenzyl moiety. Hydrogels were prepared under physiological conditions without the need of any additional reagents by mixing solutions of *o*-nitrobenzyl–modified dextran bearing acrylates and dithiolated poly(ethylene glycol). The degradation of the hydrogels due to UV irradiation was investigated with scanning electron microscopy, infrared and UV-vis spectroscopy. Using green fluorescent protein (GFP) as a model protein, light triggered protein release from the obtained gel matrices were investigated in different forms. Furthermore, photodegradation of the hydrogel *via* two photon excitation was also examined using focused pulsed near infrared (NIR) laser beam as a light source.

Perspectives

Hydrogel-based drug delivery systems are of interest due to their attractive characteristics, which can lead to targeting delivery, extension of circulation time, and reduction of toxicity and side effects. Particularly, hydrogels that can be formed *in situ* under physiological conditions have recently been paid much attention as promising drug carriers. Many hydrogels have been developed that can maintain drug levels within the narrow concentration window by controlled release, which is essential to avoid toxicity due to overdose or ineffectiveness from underdosing. Furthermore, hydrogel based controlled drug delivery systems can avoid frequent or continuous administrations which is beneficial to patients' convenience and comfort. However, current hydrogels as drug delivery tools are limited by their hydrophilic nature, which prevents their use as drug delivery systems for hydrophobic drugs because of a rapid drug release in the initial phase. Furthermore, because of the hydrophobic interactions, large drug aggregates may be formed during the drug loading process, which can result in a high local concentration and cause side effects or even toxicity. To achieve a hydrogel based controlled delivery systems for hydrophobic drugs, we have introduced β-cyclodextrin or human serum albumin to the hydrogel matrix as a primary accommodation site for those hydrophobic molecules and *in vitro* controlled release of model hydrophobic drugs were observed. Although positive preliminary *in vivo* results have been obtained by using zebrafish embryos as the animal models, further systematic *in vivo* studies have to be performed by using different mammalian animals before the clinical trials.

In the past few years, a number of therapeutic proteins have been developed against a broad range of diseases such as cancers, autoimmune diseases and metabolic disorders. However, most of these effective therapeutic proteins are prevented from clinical use by fundamental technical hurdles particularly with regard to delivery. Hydrogels are an ideal candidate for protein delivery, as they contain large amounts of water in the polymer network in a way similar to body tissues. Thus it allows to retain the proteins in the protective 3-D network in their active forms and prevents them from denaturation during administrations. Sustained delivery of proteins using hydrogel systems have been reported, in which the loaded proteins are released from the hydrogel matrix in a time dependent manner. However, a triggerable delivery system for proteins which enables an

on-demand controlled release profile might enhance its therapeutic effectiveness and reduce systemic toxicity. Therefore, we have developed both noncovalently and covalently crosslinked UV light responsive hydrogels for the purpose of releasing the entrapped proteins in a UV light controlled manner. By using green fluorescent protein as a model protein, light controlled protein release was shown *in vitro*. However, due to the reason that UV light can be absorbed by skin and tissue, biological related applications of our hydrogels are limited. Future work will concentrate on developing hydrogels that can respond to lights with great promise for clinical applications such as near-infrared light.

Recent advances in nanotechnology have spurred developments in hydrogel based drug delivery systems, nanosized hydrogels (nanogels) have attracted considerable attention, which offers the possibilities of intracellular delivery and administration by intravenous injection. Furthermore, nanogel particles can maintain the properties of their hydrogel counterparts but with a largely increased surface area and controlled sizes or shapes, which can contribute to improving efficiency of drug delivery to tumors. Macroscopic hydrogels are often employed as a model for proof-of-concept studies, which are easy to prepare and characterize. The promising macroscopic hydrogels can be reformulated into nano form with different techniques and contribute to the drug delivery field.