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

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Chapter 1

General introduction

1. Hydrogels.

Hydrogels are three-dimensional hydrated network formed by crosslinking polymers through either covalent bonds or noncovalent interactions. The high water content of hydrogels renders them biocompatible to living systems and their soft nature can minimize damages to the surrounding tissues. Due to these reasons, hydrogels have received significant attentions in recent years for biomedical applications, such as drug delivery and tissue engineering.

2. Natural and synthetic hydrogels.

Hydrogels can be prepared from polymers derived from nature or synthesis. Examples of natural polymers such as collagen, gelatin, fibrin, silk, agarose, hyaluronic acid, chitosan, dextran and alginate have been employed for hydrogel preparations. Hydrogels based on naturally derived polymers usually possess innate biocompatibility and biodegradability. However, these polymers from nature are often expensive with high batch-to-batch variations and possible chronic immunogenic responses. Furthermore, fine structural modifications of those natural polymers are often limited due to their complex structures and fragile nature. Hydrogels based on synthetic polymers such as poly(ethylene glycol) (PEG), Poly(lactic acid) (PLA), poly(2-hydroxypropyl methacrylamide) (pHPMAm), poly(vinyl alcohol) (PVA) and poly(hydroxyethyl methacrylate) (pHEMA) offer great versatility in controlling polymer chemical structure and architecture, which is essential to prepare hydrogels with tailorable network and mechanical strengths. By careful choice of the polymers, well designed and tailored hydrogel materials can be obtained to satisfy specific biomedical applications.

3. *In situ* foming hydrogels.

Hydrogels that can be formed *in situ* allow 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 situ* forming hydrogels can be prepared by either physically crosslinking methods including ionic interactions, hydrophobic interactions and

stereocomplexation or chemically crosslinking approaches such as photo crosslinking, Schiff-base formation and Michael addition. Generally, the chemically crosslinked hydrogels exhibit enhanced mechanical strength and better stability than the physically crosslinked hydrogels.

4. Applications of hydrogels.

Hydrogels have been widely studied as biomaterials in a diverse range of applications due to their structural similarities to the body tissues. For example, hydrogel based drug delivery systems are of great interest since they can be easily modified to tune their characteristics and can lead to targeted delivery, extension of circulation time, and reduction of toxicity and side effects. Drug molecules are often physically entrapped in the network and are released from the hydrogel matrix by diffusion, which can be adjusted by changing the mesh of the network and/or their affinity to the drug. Furthermore, hydrogels can be an excellent carrier not only for small molecules but also for fragile bioactive macromolecules such as proteins. As hydrogels contain large amounts of water in the polymer network, it allows for retaining the activity of proteins in the protective polymer network and prevents them from denaturation, which makes hydrogel an ideal material to store and release proteins.

In recent years, hydrogels have also been used as three dimensional extracellular matrix mimics to circumvent the limitations of traditional two dimensional cell culture conditions. Hydrogels such as matrigel and collagen, which are based on natural polymers, have been explored extensively for applications in three dimensional cell cultures. Although promising results have been observed from cells cultured with these natural gels, due to their complex, variable and ill-defined composition, precisely controlled cell culture can not be achieved. In contrast, synthetic hydrogels with well defined network and mechanical strength can provide a regular three dimensional platform for cell growth but lacking biological signals that can communicate with cells and control their behaviors. Thus synthetic hydrogels conjugated with different biological signal molecules are being investigated to replace those natural gels.

5. Aim of the study.

The aim of this study is to prepare *in situ* forming hydrogels based on biocompatible polymers for the controlled release of hydrophobic drug and proteins. In order to load hydrophobic drug to the hydrophilic hydrogel matrix, β-cyclodextrin and human serum albumin was introduced to the hydrogel network respectively and acted as the primary accommodation for those hydrophobic molecules within the hydrogel network. Furthermore, supramolecular crosslinked and covalently crosslinked light sensitive hydrogels were prepared whose potential application for light controlled protein release system has been shown.

6. Outline of the thesis.

In this thesis *in situ* forming hydrogels and their application as controlled release systems for hydrophobic drugs and proteins are described. 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 contruct 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.