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（Summary）

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（要　旨）

Hydrogels are physically/chemically crosslinked natural/synthetic polymers widely used as biomaterials for drug delivery and tissue engineering. In particular, injectable hydrogel systems have drawn a lot of attention because of the elimination of surgical implantation. Although several *in situ* crosslinking strategies have been developed for the formation of injectable hydrogels, the crosslink density and gelation rate of most existing systems cannot be independently tuned. Crosslink density is an important parameter because it affects the rate of hydrogel degradation, the rate of drug release, and the viability, proliferation and differentiation of encapsulated cells. The gelation rate of an injectable hydrogel system should be tunable based on the application requirement.

In this thesis, injectable hydrogel systems composed of polymer-phenol conjugates were developed for various biomedical applications. First, a hydrogel system composed of hyaluronic acid-tyramine (HA-Tyr) conjugates was designed. The HA-Tyr conjugates were crosslinked by the oxidative coupling of tyramine moieties in the presence of horseradish peroxidase (HRP) and hydrogen peroxide (H_2O_2). It was shown that HRP predominantly controlled the gelation rate of the hydrogel, while H_2O_2 controlled the crosslink density. The HA-Tyr hydrogel system was applied for the delivery of interferon-α2a (IFN-α2a) and trastuzumab, which are therapeutic proteins used in cancer treatment. The aim was to reduce the injection frequency of the proteins *via* sustained release from hydrogels, while not compromising the therapeutic outcome. Two methods were developed to achieve sustained release of the proteins from the hydrogels. First, the crosslink density of hydrogel was varied to control the release rate of proteins. Second, electrostatic interactions between the proteins and the hydrogel were employed to immobilize the proteins in the hydrogel, and the release was regulated by hydrogel degradation. Using mice xenograft models of human cancers, it was demonstrated that the sustained release of proteins from hydrogels more effectively inhibited tumor growth than bolus injections.

In addition, a dextran-tyramine (Dex-Tyr) hydrogel, which was formed by HRP-mediated crosslinking reaction and contained poly(ethylene glycol) (PEG) microdomains, was developed for the sustained delivery of PEGylated IFN-α2a (PEG-IFN-α2a). It was shown that the release rate could be tuned by the PEG domain size, which in turn was controlled by the gelation rate. Remarkably, a one-time administration of PEG-IFN-α2a-loaded hydrogel provided sustained delivery of the protein for 3 months, and prevented liver injury as effectively as weekly administration of the protein in a humanized mice model of hepatitis C.

Besides protein delivery, the enzymatically crosslinked HA-Tyr hydrogel system was also used for tissue engineering applications. The goal of tissue engineering is to construct tissues from a combination of cells and scaffold materials. One of the challenges associated with tissue engineering is the insufficient vascularization of the tissue construct, which compromises the survival of embedded cells after transplantation. To address this challenge, two methods of vascularizing HA-Tyr hydrogels were developed. First, an interpenetrating polymer network (IPN) hydrogel composed of fibrin and HA-Tyr was formed. Compared to fibrin gels, the IPN hydrogels demonstrated improved structural stability against cell-induced contraction and degradation by proteases, while maintaining cell proliferation and capillary network formation. Second, an Arg-Gly-Asp (RGD) peptide containing phenol moieties (Ph_2-PEG-RGD) was designed for *in situ* conjugation into the HA-Tyr network during HRP-mediated crosslinking reaction. Conjugation of the peptide significantly improved cell adhesion, migration and capillary network formation in the hydrogel. *In vivo* study confirmed that functional vasculature could be formed in the peptide-conjugated hydrogels within two weeks post-injection. In addition, the HA-Tyr hydrogel system was investigated as a 3D culture system for the propagation of human embryonic stem cells, which are the most promising cell source for tissue engineering.

Lastly, a bioactive hydrogel composed of HA-(-)-epigallocatechin-3-gallate (HA-EGCG) conjugates was developed as an injectable and degradation-resistant system. EGCG, a green tea catechin, is a polyphenol known for its various health benefits. Therefore the conjugation of EGCG to HA would endow the resulting conjugates with the bioactivities of EGCG. Indeed, HA-EGCG conjugates exhibited radical scavenging, anti-proliferative and enzyme-inhibiting properties, which are absent or present at low levels in native HA. It is known that under aerobic conditions, EGCG autoxidizes and produces H_2O_2 as the by-product, which could serve as the oxidant in the HRP-mediated coupling of phenols. Thus the addition of HRP alone induced the gelation of a solution HA-EGCG conjugates within a few minutes through the coupling of EGCG moieties. Moreover, HA-EGCG hydrogels degraded slower *in vivo* compared with HA-Tyr hydrogels, which was attributed to the enzyme-inhibiting properties of EGCG. Furthermore, it was demonstrated that hydrogels could be formed through EGCG coupling even in the absence of HRP. The HRP-free gelation is a promising alternative approach to form injectable hydrogels composed of polymer-phenol conjugates, as it circumvented the safety concern associated with HRP due to its plant origin. In summary, enzymatically crosslinked hydrogels composed of polymer-phenol conjugates are promising injectable system for drug delivery and tissue engineering applications.