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# Preparation of gelatin hydrogels incorporating low-molecular-weight heparin for anti-fibrotic therapy

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ABSTRACT

The objective of this study is to design biodegradable hydrogels for the controlled release of low-molecular-weight heparin (LMWH) and evaluate the biological activity. Gelatin was cationized by chemically introducing ethylene diamine into the carboxyl groups in different conditions to obtain cationized gelatins. The cationized gelatin was mixed with the LMWH in aqueous solution to form the complex. Gelatin, together with the complex of LMWH and cationized gelatin, was dehydrothermally cross-linked for different time periods to prepare the gelatin hydrogel-incorporating complex. The hydrogel-incorporating complex was neither degraded in phosphate-buffered saline solution (PBS) at 37 °C nor did it release the LMWH complex. When placed in PBS containing collagenase, the hydrogel was enzymatically degraded to release the LMWH complex. The time profile of hydrogel degradation and the LMWH release depended on the condition of hydrogel cross-linking. The longer the cross-linking time period, the slower the hydrogel degradation and the subsequent LMWH release. The half-life period of LMWH release was in good correspondence with that of hydrogel degradation. It is possible that the LMWH was released as the result of hydrogel degradation. When applied to the mouse model of abdominal membrane fibrosis, the hydrogel system of LMWH release showed a promising anti-fibrotic effect.



## 1. Introduction

The therapeutic effects of many drugs with different physicochemical and biological properties have been investigated. However, drugs often show side-effects. The use of different drug delivery systems (DDS) has been explored as a practical technology that can modify the biodistribution and the consequent therapeutic effects of drugs. Among the DDS technologies, a variety of materials for the controlled release of drugs have been investigated [1–9]. However, after drug release, the release carrier material sometimes remains, even if these materials are biodegradable. The remaining materials often cause inflammatory reactions and therapeutically unacceptable responses. Therefore, in practice it is necessary to develop a carrier material for drug release systems which does not induce inflammatory reactions. It is well recognized that, compared with hydrophobic polymer materials, hydrophilic materials such as hydrogels show fewer inflammation responses [10,11].

Gelatin is a biodegradable material and has been extensively used for food, drug ingredients, and medical purposes. The biosafety of gelatin has been proven through its long practical applications. Gelatin has various side chains which can be chemically modified with ease. Dehydrothermal or chemical treatment enables gelatin to intermolecularly cross-link to obtain the hydrogel. Gelatin hydrogel can be enzymatically degraded and the degradability can be changed by altering the cross-linking condition. The time period of hydrogel degradation ranged from a few days to several months [12]. We have demonstrated that gelatin hydrogels could release plasmid DNA and proteins with biological activity and enhance their biological activities [13–23].

Heparin is a negatively charged glycosaminoglycan, which is composed of repeated disaccharide units of alternating glucosamine and glucuronic residues heterogeneously modified by carboxyl groups and N- or O-linked sulfate. It has been clinically used as an anticoagulant agent. In addition, other biological effects have been reported. For example, heparin enables cells to stimulate the production of hepatocyte growth factor (HGF) [24]. The anti-fibrotic effect of heparin is experimentally confirmed with a mouse model of $CCl_4$-induced hepatitis and unilateral ureteral obstruction (UUO) kidney fibrosis [25,26]. Heparin has a side-effect of bleeding acceleration [27]. It is reported that compared with normal heparin, low-molecular-weight heparin (LMWH) has the nature to induce less bleeding [28]. The potential to induce the HGF production is similar to that of normal heparin [29]. Based on these findings, the LMWH was chosen as an anti-fibrotic drug in this study.

The objective of this study is to design a gelatin hydrogel system for the controlled release of LMWH. Cationized gelatin was prepared to form the water-soluble complex of LMWH. The complex was mixed with gelatin, followed by dehydrothermal

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