Thermosensitive injectable doxorubicin hydrogels for localized cancer therapy

Hua-Jing Jhan, Ming-Thau Sheu, and Hsiu-O Ho
School of Pharmacy, College of Pharmacy, Taipei Medical University, 250 Wu-Hsing Street, Taipei 110, Taiwan (ROC)
E-mail: d301098002@tmu.edu.tw

ABSTRACT
In this study, doxorubicin (Dox) was loaded into a thermosensitive injectable hydrogel that was prepared using a physical mixing method. The optimal formulation, DHF-15, was composed of 1 mg/mL of Dox, 0.1% hyaluronic acid, 0.1 M magnesium chloride, and 0.15 mg/mL of Pluronic F127. Rheology test results indicated that DHF-15 is an injectable flowing solution that forms a nonflowing gel at body temperature. The structure and surface morphology of the hydrogel were characterized using scanning electron microscopy. Intratumorally administering the DHF-15 hydrogel resulted in efficient growth inhibition of C26 colon cancer cells in a Balb/c mouse model. The tumor inhibition rate of the DHF-15 hydrogel was 84.51%, whereas that of the free Dox solution was 80.65%. The developed in situ injectable Dox hydrogel was a potential drug delivery system that can increase the efficacy of cancer chemotherapy.

INTRODUCTION
Injectable hydrogel formulation has received attention because it offers several advantages such as local treatment, reduction in drug circulation, and alleviation of side effects. In addition, hydrogels can control drug release by changing the gel structure in response to environmental stimuli, such as the temperature, pH, ionic strength, and electric field. Pluronic F127 is a block copolymer of poly(ethylene oxide-b-propylene oxide-b-ethylene oxide) (PEO-PPO-PEO, Poloxamer 407, PF127), which is a nontoxic polymer that is an ideal candidate for thermosensitive delivery and has been applied in localized drug delivery such as intraperitoneal, subcutaneous, and intramuscular injections [1]. Hyaluronic acid (HA), a naturally ionic polysaccharide, is composed of repeating disaccharide units of D-glucuronic acid and N-acetyl-D-glucosamine. HA is biocompatible and biodegradable, and is extensively used in commercial applications such as pharmaceutical, medical, and cosmetic products. The Food and Drug Administration approved HA to be adequately safe for medical applications. Because CD44 is the HA receptor of cancer cells and HA-mediated motility is overexpressed in cancer cells, they can be used as a specific target in cancer treatment [2].

Doxorubicin (Dox) has been widely used in the clinical treatment of various cancers, successfully producing regression in acute leukemia, lymphomas, soft-tissue and osteogenic sarcomas, pediatric malignancies, and adult solid tumors, particularly breast and lung carcinomas. The cardiotoxicity of Dox limits its clinical use in treating cancer.

In this study, we developed a novel in situ injectable hydrogel based on HA, MgCl₂, and Pluronic F127 (PF127) to encapsulate Dox. The hydrogel can be delivered intratumorally to treat cancer. The viscoelastic properties and gelation temperature were investigated by performing a rheological analysis, and the structure of the hydrogel was characterized using scanning electron microscopy (SEM). Moreover, the in vivo antitumor efficacy of the Dox/HA hydrogel tested in the tumor xenograft mouse model is reported.

EXPERIMENTAL METHODS
Preparation of the Dox-loaded thermosensitive hydrogel
The thermosensitive Dox/HA hydrogel was prepared using a physical mixing method as follows. An HA solution, MgCl₂ solution, and PF127 solution were combined in a tube and placed in an ice bath for 30 min. The mixture was then mechanically vortexed at 2000 (1/min) by using an IKA MS1 mini shaker (IKA, Germany), and a Dox solution was added slowly [3].

<table>
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<tr>
<th>Acronym</th>
<th>Dox (mg/mL)</th>
<th>HA (%)</th>
<th>MgCl₂ (M)</th>
<th>PF127 (mg/mL)</th>
<th>Temperature</th>
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<tr>
<td>DHF-5</td>
<td>1</td>
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<td>0.1</td>
<td>0</td>
<td>G</td>
</tr>
<tr>
<td>DHF-10</td>
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<td>0.1</td>
<td>0.05</td>
<td>G</td>
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<tr>
<td>DHF-15</td>
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<td>0.1</td>
<td>0.1</td>
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<td>DHF-20</td>
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RESULTS AND DISCUSSION
In this study, we developed a Dox/HA thermosensitive hydrogel as a novel injectable drug carrier that can potentially be applied in local cancer treatment. Dox was loaded into a thermosensitive injectable hydrogel that was prepared using a physical mixing method without adding a chemical linker. The optimal formulation, DHF-15, was composed of 1 mg/mL of Dox, 0.1% HA, 0.1 M MgCl₂, and 0.15 mg/mL of PF127. The in situ gelation of the DHF-15 formulation is shown in Figs. 1(A) and 1(B).

Figs. 1 (C) shows that G’ is higher than G” for a temperature above 36 °C, indicating that the DHF-15 hydrogel underwent a temperature-induced transition from a viscoelastic fluid to an elastic solid when the temperature was over 36 °C. The injection properties and in vivo gelation were studied by injecting the DHF-15 solution into the bodies of animals. Figs. 1 (D) and 1(E) show that a red hydrogel containing Dox rapidly formed in situ as a result of the temperature changes caused by the body conditions of the
mice. The results indicated that the DHF-15 solution is injectable at room temperature and forms a hydrogel within 5 min after being injected into the body.

![Image](https://via.placeholder.com/150)

Fig. 1. In vitro and in vivo sol-gel transition of the DHF-15 hydrogel: (A) a solution formed at room temperature (25 °C); (B) a gel formed at body temperature (37 °C); (C) Dynamic temperature ramp curves of the DHF-15 hydrogel; (D and E) Photographs of in situ Dox hydrogel formation 5 min after the subcutaneous injection of the DHF-15 solution into a male Balb/c mouse.

The hydrogels were frozen at -80 °C, and then lyophilized and sectioned for use in SEM analysis. Fig. 2 shows the SEM images of Dox hydrogels derived from various concentrations of PF-127 (0–0.2 mg/mL; w/v). All of the hydrogels exhibited a porous network comprising a wide distribution of interconnected pores of various sizes. Compared with the DHF-0 formulation, other formulations were less smooth and exhibited less porous structures and smaller pores (Fig. 2A); however, the pores of DHF-0 were larger than those of other formulations. By contrast, DHF-20 exhibited smaller pores and a more compact and rigid structure than DHF-0 did. A higher concentration of PF-127 produced a more compact microstructure. The results indicated that increasing the PF-127 content caused the release rate of Dox to decrease (data not shown).

![Image](https://via.placeholder.com/150)

Fig. 2. SEM images of Dox, HA, and MgCl2 mixed with various concentrations of PF127: (A) DHF-0; (B) DHF-5; (C) DHF-10; (D) DHF-15; (E) DHF-20.

The in vivo antitumor efficacy of the DHF-15 hydrogel formulation was evaluated in the C26 tumor-bearing mouse model. The mice were randomized into three groups when the tumor size was 100 mm³. Each group was intratumorally injected with either PBS, the DHF-15 hydrogel, or a free Dox solution. On Day 10, the tumors injected with the DHF-15 hydrogel and free Dox solution were 9.22% and 19.17% smaller than those injected with PBS (control group) (Fig. 3A).

In addition, we monitored the changes in body weight during the experiment, and the results indicated no systemic toxicity. Fig. 3B shows that neither toxicity-induced death nor severe body weight loss was observed in the mice after intratumoral injection with the DHF-15 hydrogel or the free Dox solution during the in vivo antitumor efficacy experiment.

![Image](https://via.placeholder.com/150)

Fig. 3. In vivo antitumor effect of the DHF-15 hydrogel. (A) Changes in tumor volume; (B) changes in the body weight of the mice.

CONCLUSION

The Dox-loaded DHF-15 hydrogel was prepared as an injectable formulation suitable for intratumorally delivering Dox into tumor cells. The preparation involved using a physical mix method, and no chemical linker agents were used. The DHF-15 hydrogel exhibited substantial antitumor activity and reduced toxicity in C26 tumors in vivo, indicating that the DHF-15 hydrogel is a potential and novel injectable formulation that can be used to deliver Dox in locally treating cancer. Our future studies will investigate the affinity of HA for CD44-positive tumor cells in order to enhance antitumor activity and reduce side effects in an animal model.

REFERENCES


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