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# Peritoneal adhesion prevention by a biodegradable hyaluronic acid-based hydrogel formed in situ through a cascade enzyme reaction initiated by contact with body fluid on tissue surfaces

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ABSTRACT

Postsurgical peritoneal adhesion is a serious surgical complication. In situ hydrogel formation on the surface of tissues, which will develop adhesions, is a recent feasible approach to prevent peritoneal adhesion. Here, we report on-tissue surface formation of a hyaluronic acid-based hydrogel by administration of a pre-hydrogel aqueous solution. The hydrogelation was initiated by contact with body fluid containing glucose on tissue surfaces. During the hydrogelation, a hyaluronic acid derivative possessing phenolic hydroxyl moieties (HA-Ph) was cross-linked by a cascade reaction of glucose oxidase (GOx) and horseradish peroxidase (HRP). About 5 s of hydrogelation was accomplished using a solution containing 1.5% (w/v) HA-Ph, 5 U/mL HRP, and 2.5 U/mL GOx in 1 mg/mL glucose that is equivalent to the normal blood glucose concentration. The hydrogel was degradable by hyaluronidase and much softer than rat peritoneal sidewalls. We confirmed the efficiency of the hydrogel to prevent post-operative peritoneal adhesions by applying the solution containing HA-Ph, GOx, and HRP to animals with bowel abrasion-abdominal sidewall defects. A significant reduction in the development of peritoneal adhesions was found compared with animals applied with phosphate-buffered saline or saline containing HA-Ph alone.

**Statement of Significance**

Postsurgical peritoneal adhesion is a serious surgical complication. In this paper, we report a novel system for preventing it through an on-tissue surface formation of a biodegradable and biocompatible hyaluronic acid-based hydrogel by administration of a pre-hydrogel aqueous solution. The in situ hydrogelation is mediated by a cascade enzyme reaction of glucose oxidase (GOx) and horseradish peroxidase (HRP) initiated by contacting with body fluid containing glucose. The efficiency of the system was confirmed by applying the system to animals with bowel abrasion-abdominal sidewall defects.



## 1. Introduction

Peritoneal adhesions are pathological bonds that usually occur between the abdominal wall, omentum, and loops of the bowel. They are mostly induced by surgery in the peritoneal cavity and frequently cause multiple serious complications such as chronic pelvic pain, small intestinal obstructions, and difficulties in re-operations [1,2]. In the past three decades, various approaches have been examined to prevent these adhesions, including pharmacological and barrier-based approaches [1–3]. However, even now, peritoneal adhesions remain as a serious surgical complication. Recently, barrier-based approaches have attracted significant attention to prevent peritoneal adhesions. Polymer solutions [4,5], preformed solid films and membranes [6,7], and hydrogels [8–16] have been investigated for these approaches. Polymer solutions are useful to cover injured surfaces even with a complex geometry. However, persistent covering is greatly limited by the short residence time of the solution on the administrated site. For preformed solid films and membranes, it is difficult to cover injured surfaces with a complex geometry and extensive survey surface. A recent promising approach employs in situ cross-linkable hydrogels that can form hydrogels when applied in situ [8–14]. This strategy has advantages over approaches using polymer solutions and preformed solid films. The administered pre-hydrogel solution can cover injured surfaces

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with a complex geometry. Then, a hydrogel forms on the surface, resulting in a physical barrier to prevent direct contact with surfaces for a certain period of time. Injectable hydrogel systems can be classified into two types. One involves initiation of hydrogelation by mixing multiple cross-linkable precursor solutions immediately before application [8–11]. The other type initiates hydrogelation on the surface of the administered surface [12–14]. This system is simpler than the former type. One of these systems employing thermosensitive hydrogel has attracted attention because there is no need for additional factors such as ultraviolet light irradiation. The hydrogelation is induced by body temperature [14]. However, the relatively long time necessary for hydrogelation may be impractical. Therefore, there is still a need to develop an in situ gellable system that is triggered by the body.

Here, we describe a system employing a biodegradable hyaluronic acid (HA)-based hydrogel for peritoneal adhesion prevention, in which hydrogelation is initiated by contact with body fluids. The hydrogelation is based on co-enzymatic cross-linking mediated by a cascade reaction of glucose oxidase (GOx) and horseradish peroxidase (HRP) (Fig. 1a). HA and its derivatives have been previously used for adhesion prevention, including in situ gellable hydrogel systems because of their high biocompatibility and biodegradability [9–11]. In this study, we used the HA derivative possessing phenolic hydroxyl moieties (HA-Ph) developed by Kurisawa et al. [17]. The feasibility of an HA-Ph hydrogel formed by HRP-catalyzed reaction has been investigated for various biomedical applications such as drug delivery and tissue engineering [18–20]. Hydrogelation of a HA-Ph aqueous solution containing HRP can be initiated by mixing with a solution containing $H_2O_2$, an electron donor of the HRP-catalyzed oxidative reaction, resulting in cross-linking between Ph moieties. GOx catalyzes the oxidation of glucose to $H_2O_2$ and glucono-δ-lactone. Therefore, a solution containing glucose and GOx can be used as an alternative to a $H_2O_2$ solution. Glucose is a common component of body fluids. Thus, hydrogels can be formed by contacting an aqueous solution containing polymer with Ph moieties, HRP, and GOx with body fluids [21] (Fig. 1b). We examined the feasibility of a hydrogelation system consisting of a HA-Ph aqueous solution containing HRP and GOx, in which hydrogelation is initiated by contact with body fluids for peritoneal adhesion prevention.

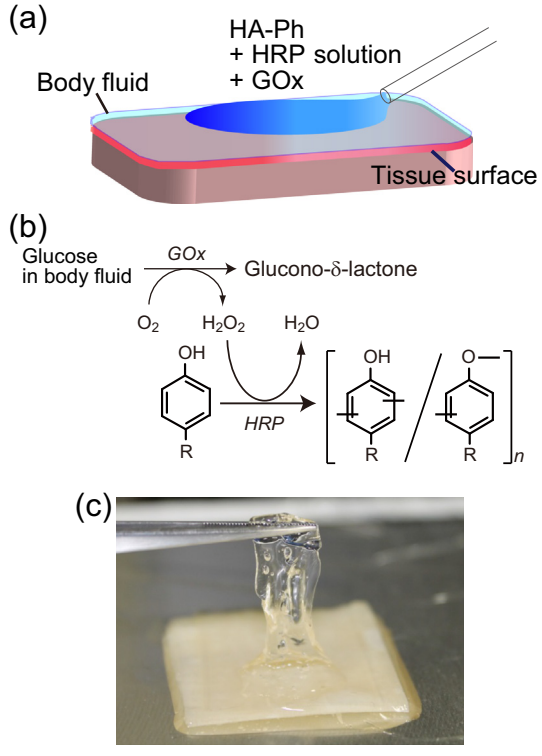


**Fig. 1.** (a) Schematic of covering an abdominal tissue surface with a HA-Ph hydrogel by pouring a gellable solution containing HA-Ph, HRP, and GOx onto the tissue surface. (b) Scheme for HA-Ph hydrogel formation via GOx- and HRP-catalyzed reactions by consuming glucose in body fluid. (c) Image of a pinched HA-Ph hydrogel formed by pouring a solution containing HA-Ph, HRP and GOx onto a dialysis membrane moistened with FBS.

## 2. Materials and methods

### 2.1. Materials

Sodium hyaluronate (MW:*ca.* 1000 kDa) and GOx from *Aspergillus niger* (287 U/mg) were purchased from JNC (Tokyo, Japan) and Serva Electrophoresis GmbH (Heidelberg, Germany), respectively. Hyaluronidase from ovine testes (1100 U/mg), HRP (190 U/mg), *N*-hydroxy sulfosuccinimide (NHS), catalase (10,000 U/mg), and D-glucose were purchased from Wako Pure Chemical Industries (Osaka, Japan). Tyramine hydrochloride and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) were purchased from Tokyo Chemical Industry (Tokyo, Japan) and Peptide Institute (Osaka, Japan), respectively. HA-Ph ($1.75 \times 10^{-4}$ mol-Ph/g-HA) was synthesized by combining HA and tyramine via carbodiimide-mediated condensation of the carboxyl groups of HA and amino groups of tyramine using EDC and NHS according to a previous report [17].

### 2.2. Gelation time

The time necessary for hydrogelation of the HA-Ph solution through GOx and HRP-catalyzed reactions was evaluated by a previously reported method [22]. Briefly, 180 μL phosphate-buffered saline (PBS, pH 7.4) containing HA-Ph, HRP, and GOx was added to a well of a 48-well plate and stirred with a magnetic bar. Subsequently, 20 μL PBS containing glucose was added to the well with stirring. Swelling of the solution surface and hindrance of the magnetic stirring were considered to indicate hydrogelation. One of the concentrations of HA-Ph, HRP, and GOx were altered to determine the effect of these parameters from the following condition: 1.5% (w/v) HA-Ph, 5 U/mL HRP, and 2.5 U/mL GOx. The concentration of glucose in each well was fixed at 1 mg/mL that is equivalent to the normal blood glucose concentration. All solutions before mixing and the 48-well plate were kept at 37 °C. The mean and standard deviations of triplicates were calculated.

### 2.3. Biodegradability

HA-Ph, HRP, and GOx were dissolved in PBS, and 1 mL of the solution was added to a cylindrical vessel (15 mm in diameter) with a dialysis membrane at the bottom. The vessel was then soaked in 12 mL PBS containing 1 mg/mL glucose for 12 h at 37 °C. After rising with PBS, the resultant hydrogel disk was soaked in PBS at 37 °C for 2 h. The hydrogel disks were then soaked in 13 mL PBS containing 50 U/mL hyaluronidase at 37 °C. Changes in the hydrogel weight were measured to evaluate degradation. The degree of degradation was calculated using the following formula: degradation (%) = $\{(W_0 - W_t)/W_0\} \times 100$; where $W_0$ and $W_t$ are the weights of hydrogel disks before and after soaking in PBS containing hyaluronidase, respectively. The mean and the standard deviations of triplicates were calculated.

### 2.4. Mechanical properties

Compression-repulsion stress profiles of HA-Ph hydrogel disks prepared under the conditions described in Section 2.3 were

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