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Design of high-performance anti-adhesion agent using injectable gel with an anti-oxidative stress function

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ABSTRACT

Postsurgical tissue adhesion formation caused by inflammation and oxidative stress is one of the serious issues because it induces severe clinical disorders. In this study, we designed redox injectable gel (RIG) which covalently possesses nitroxide radicals as a reactive oxygen species (ROS) scavenger for high performance anti-adhesion agent. The redox flower micelles exhibiting gelation under physiological conditions were prepared by a polyion complex (PIC) between polyamine-PEG-polyamine triblock copolymer possessing nitroxide radicals as a side chain of polyamine segments and poly(acrylic acid). RIG showed prolonged local retention in the abdominal cavity of the mice, which was monitored by *in vivo* imaging system (IVIS). Compared with a commercial anti-adhesion agent (Seprafilm®, Genzyme, Cambridge, MA), RIG dramatically inhibited the formation of tissue adhesions via a combination of physical separation and biological elimination of generated ROS in talc-induced adhesion model mice. Treatment with RIG suppressed inflammatory cytokines and neutrophil invasion, suppressing the increase in peritoneal membrane thickness. It is also emphasized that RIG suppressed the increase of white blood cells level, indicating that the present RIG treatment effectively prevents diffusion of local inflammation to entire body. These findings indicate that RIG has a great potential as a high performance anti-adhesion agent.

1. Introduction

Postsurgical adhesion is an inevitable consequence of most abdominal surgeries and causes serious complications [1,2]. Recent studies have reported that the incidence of adhesion after abdominal surgery is over 90% [3,4]. The consequences of adhesion formation includes chronic pelvic pain, ileus and infertility; these require readmission and reoperation, leading to low Quality of Life (QOL) for the patients [5]. Adhesion formation is strongly associated with the oxidative stress caused during surgical procedures [6]. When tissue is injured by mechanical damage, infection, or foreign body reactions during surgery, such factors induce the excess production of reactive oxygen species (ROS), which cause oxidative damage to cell membranes, proteins, and DNA [6–10]. Inflammation related to oxidative stress increases vascular permeability with an exudation of inflammatory mediators such as myeloperoxidase (MPO), tumor necrosis factor α (TNF-α) and interleukin 6 (IL-6) which in turn induce the increase in tissue thickness by causing coagulation or fibrin formation [6,11–13]. Through this acute inflammation cascade, injured sites interact with each other and form adhesions between tissues that are normally separated [12].

Numerous anti-adhesive agents have been studied for their

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effectiveness in preventing postsurgical adhesions. Currently, the most useful approach for reducing adhesions is the prevention of adhesions with physical barriers, using liquid or solid materials to isolate the traumatized tissue from the surrounding other tissues [14–16]. Although this approach has been clinically adopted, some limitations for its use remain. Such materials are difficult to handle during surgery and cannot completely cover the injured site. Thus, much attention has been focused on the use of injectable gel system that undergoes gelation under physiological conditions [17,18]. This system could effectively remove these limitations. However, these materials function only as a physical barrier and have no effect on the inflammation caused by tissue adhesions. Therefore, there is a need for an innovative anti-adhesion agent having both excellent physical barrier and anti-inflammatory functions. From these points, anti-adhesion agent containing an antioxidants such as 2,2,6,6-tetramethylpiperidine-N-oxyl (TEMPO) may be a good candidate for high-performance anti-adhesion agent. TEMPO has nitroxide radical and can effectively eliminate the excessively generated ROS which aggravates inflammation [19]. However, it was reported that low molecular weight (LMW) antioxidants cause serious adverse effect such as mitochondrial dysfunction due to uptake into normal cells by diffusion to entire body [20,21].

We have previously reported a core–shell type redox-nanoparticle (RNP) conjugating TEMPO which has nitroxide radical as free radical scavenger inside the core, works as a nano-medicine [22–24]. RNP has the following characteristics: (1) it prevents unintended diffusion of nitroxide radicals to the outside of the nanoparticle because it is covalently conjugated inside of the core; (2) it is not taken up by healthy cells due to the formation of nanoparticles [25,26]. Because of its excellent ROS-scavenging properties, RNP showed a good anti-oxidative function *in vitro* and therapeutic effects against various diseases including renal, cerebral and myocardial ischemia reperfusion injuries and peritoneal dialysis [27–32]. However, liquid type of RNP may not be suitable as an anti-adhesion agent because it is rapidly eliminated from the peritoneal cavity, reducing its ROS scavenging potential.

In order to develop a high performance anti-adhesion agent, we have focused on the injectable gel system with an anti-oxidative stress function. Recently stimuli-responsive hydrogel based on ABA-type triblock copolymer have been widely studied [33,34]. The ABA-type triblock copolymer which possesses a hydrophilic B-segment as a center and a hydrophobic A-segment on both sides forms core–shell type of nanoparticle at room temperature. This type of core–shell polymer micelle with loop chain is named as “flower micelle” [33,34]. The flower micelle is transformed into a hydrogel via cross-linking structure between micelles in response to a stimulus such as temperature and pH. In this study, we designed a redox injectable gel system based on our previously reported original material [35]. First, we synthesized cationic poly [4-(2,2,6,6-tetramethylpiperidine-N-oxyl) aminomethylstyrene]-b-poly(ethylene glycol)-b-poly[4-(2,2,6,6-tetramethylpiperidine-N-oxyl) aminomethylstyrene] (PMNT-PEG-PMNT) tri-block copolymer which covalently conjugate TEMPO moieties as ROS scavenger into the side chains via amino linkage. When conjugated to a polymer, TEMPO specifically exhibited an anti-oxidative function against inflammation without internalization into healthy cells [25,26]. Cationic tri-block polymer and anionic poly(acrylic acid) (PAAc) forms flower-like polyion complex (PIC) micelles via electrostatic interactions. Because PIC micelles used as redox-injectable gel (RIG) forms an irreversible gel matrix in response to temperature under physiological ionic strength, they are anticipated to be retained at the injection site and prevent tissue adhesions both physically and biologically. At low ionic strength, PIC micelles do not transform into gel, but once the environmental ionic strength increased, it begins to form a gel, making it suitable for both open surgery and minimally invasive surgical procedures, i.e., endoscopic, catheterized, and robotic procedures (Fig. 1). In this study, peritoneal adhesion model mice were prepared by intraperitoneal injection of talc [36,37]. RIG was administered to the peritoneal cavity of the mice to confirm its effectiveness in preventing tissue adhesions. RIG effectively prevented peritoneal adhesions and significantly suppressed inflammation due to its sustained ROS scavenging activity *in vivo*. In comparison, adhesion model mice treated with a commercial anti-adhesion agent (Seprafilm®, Genzyme, Cambridge, MA, USA) suffered from tissue adhesions even after treatment. These findings indicate that RIG has great potential for high performance anti-adhesion agent.

2. Materials and methods

2.1. Materials

4-Amino-2,2,6,6-tetramethylpiperidine-N-oxyl (4-amino-TEMPO) (Aldrich Chemical Co. Inc., Milwaukee, WI, U.S.A), poly(-acrylic acid) (PAAc) (Mn = 5,000), dimethyl sulfoxide (DMSO), diethyl ether, N,N-dimethylformamide (DMF), ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC), 5-aminofluorescein, and talc (Wako Pure Chemical Industries, Ltd., Osaka, Japan) were used without further purification. Commercial PEG possessing sulfanyl groups at both ends (SH-PEG-SH) (Mn = 10,000) (NOF CORPORATION Co., Ltd., Tokyo, Japan) was used without further purification. 2,2′-Azobisisobutyronitrile (AIBN; Kanto Chemical Co., Inc., Tokyo, Japan) was purified by recrystallization from methanol. Chloromethylstyrene (CMS) was kindly provided by Seimi Chemical Co., Ltd. (Kanagawa, Japan) and purified on a silica gel column by washing with alkali to remove nitrophenol and other inhibitors, followed by vacuum distillation under a nitrogen gas atmosphere. Seprafilm was purchased from Kaken Pharmaceutical Co., Ltd. (Tokyo, Japan).

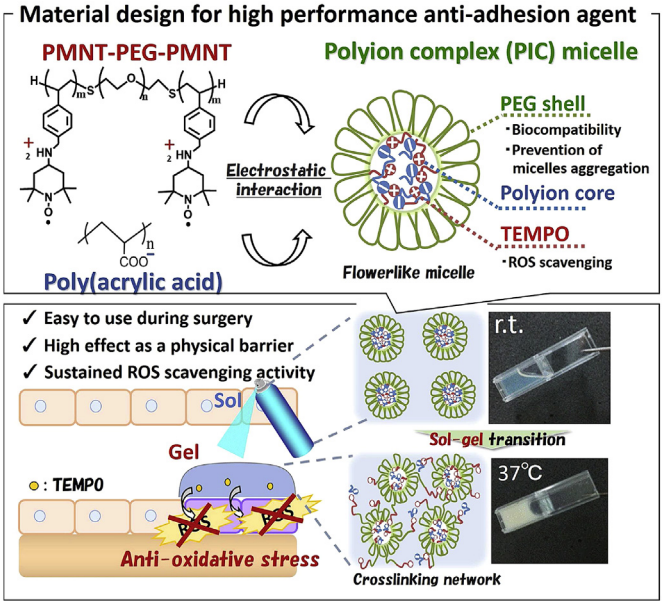

Fig. 1. Schematic illustration of redox injectable gel (RIG) for use as a high performance anti-adhesion agent. Polyion complex micelles were formed via electrostatic interaction between cationic poly[4-(2,2,6,6-tetramethylpiperidine-N-oxyl)amino-methylstyrene]-b-poly(ethylene glycol)-b-poly [4-(2,2,6,6-tetramethylpiperidine-N-oxyl)aminomethylstyrene] (PMNT-PEG-PMNT) triblock copolymer conjugating TEMPO moiety and anionic poly(acrylic acid) (PAAc). After administration, the redox flower micelle starts to form gel (RIG) as can be seen in the figure.