Journal of Controlled Release 255 (2017) 258–269

Contents lists available at ScienceDirect

Journal of Controlled Release

journal homepage: www.elsevier.com/locate/jconrel

Assembly of polymer micelles through the sol-gel transition for effective cancer therapy

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ARTICLE INFO

Keywords:
Sol-gel transition
Apoptosis
Photodynamic therapy
Prodrug
Pluronic nanoparticles

ABSTRACT

Photo-induced apoptosis-targeted chemotherapy (PIATC) was designed and characterized to propose a new protocol for improved chemotherapy. Intratumoral injection was selected as the mode of administration of the anticancer drug, doxorubicin (DOX). To extend the retention time of DOX at the tumor parenchyma, *in-situ* gel formation was induced through the sol-gel transition of the Pluronic NPs containing a prodrug of DOX or a photosensitizer. The prodrug (DEVD-S-DOX) was designed to be inactive with a peptide moiety (Aspartic acid-Glutamic acid-Valine-Aspartic acid: DEVD) linked to DOX and to be cleaved into free DOX by caspase-3 expressed with apoptosis. For reactive oxygen species (ROS)-mediated apoptosis, photo-irradiation with methylene blue (MB, photosensitizer) was utilized. The sol-gel transition of the Pluronic NPs containing reactive species, DEVD-S-DOX or MB, was examined by measuring the cloud point and the gel strength in response to temperature change. ROS-mediated apoptosis was observed by measuring the ROS and membrane integrity with induced apoptosis. The *in vivo* antitumor efficacy of PIATC was measured with a cardiotoxicity assay in tumor-bearing mice.

1. Introduction

Three modalities, namely, sol-gel transition, apoptosis-targeted chemotherapy, and photodynamic therapy (PDT), were combined to accomplish an efficient chemotherapy. An *in-situ* forming gel system was characterized based on the assembly of nanoparticles (NPs) in the aqueous medium through the sol-gel transition. An activatable prodrug (DEVD-S-DOX) that contained doxorubicin (DOX) linked to a peptide moiety (DEVD: Aspartic acid-Glutamic acid-Valine-Aspartic acid) was prepared as a model chemotherapeutic drug for apoptosis-targeted chemotherapy. We then utilized light energy from the PDT to induce apoptosis as shown in Fig. 1.

The main issues in intravenous injections for chemotherapy are as follows: low drug accumulation at the tumor target sites [1], rapid clearance from the tumor through blood circulation [2], the non-specific toxicity of selective chemotherapeutics [3], and repeated administration for the maintenance of therapeutic activity [4]. To overcome these difficulties, the therapeutic concentration of the anticancer drug was maintained by several approaches from drug-loaded NPs to an injectable *in-situ* forming hydrogel [5–7].

With fuller understating of their micellization and sol-gel transition phenomena, Pluronics have been recognized as a nanoscale building block in biomedical applications [8–10]. Besides the phase behaviors from the micellization to the gelation, Pluronics were also considered as biological response modifiers below the critical micelle concentration (CMC). Because of these interesting features, various attempts have been made to demonstrate efficient nanomedicine for chemotherapy [11–13].

PDT is a light-based cancer therapy and considered as effective for curative and palliative treatment [14–16]. The treatment modality of PDT is promising to eradicate small areas of benign or malignant tumors by killing the tumor cells through apoptosis and necrosis as well

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http://dx.doi.org/10.1016/j.jconrel.2017.04.039
Received 24 January 2017; Received in revised form 2 April 2017; Accepted 26 April 2017
Available online 26 April 2017

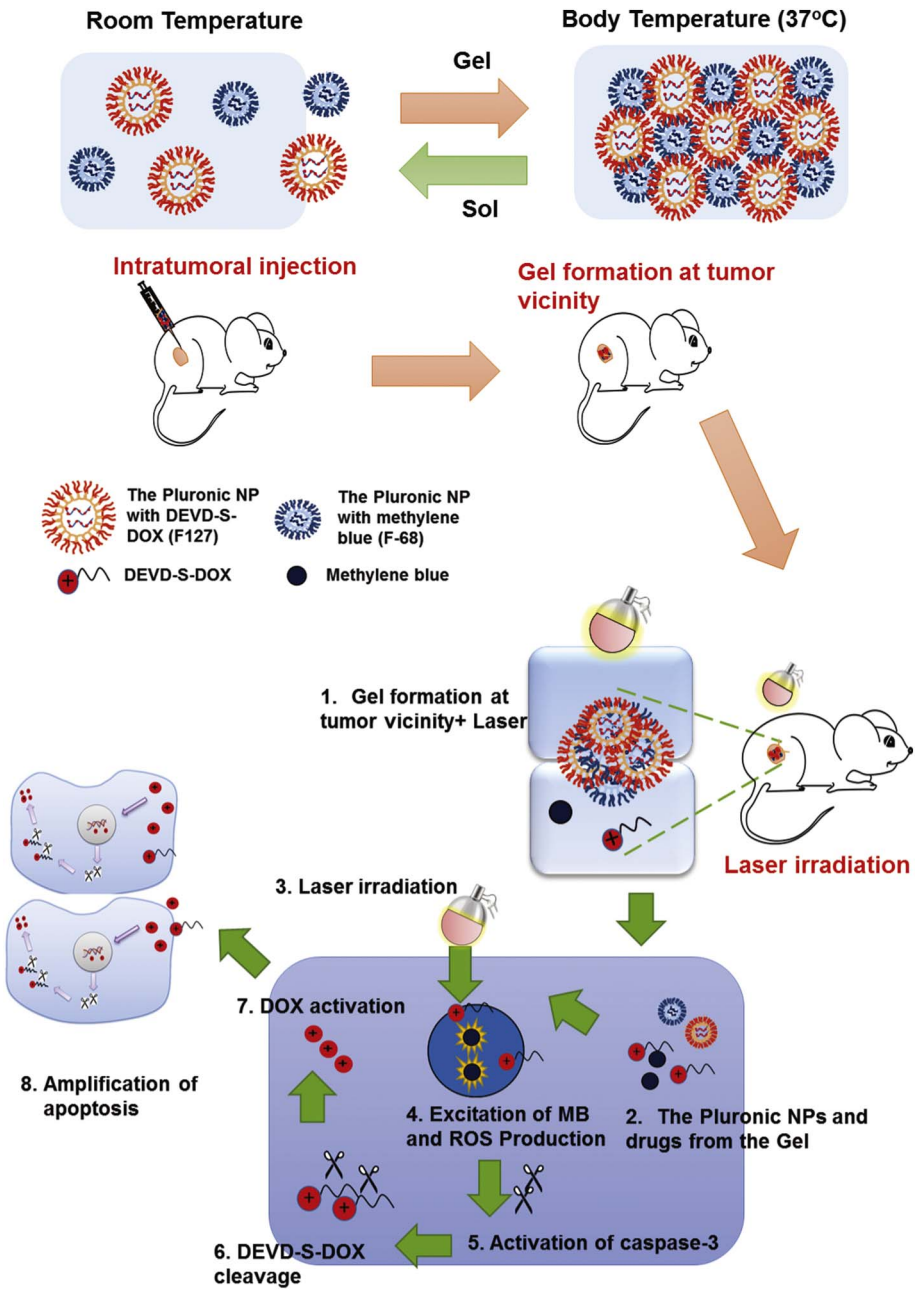

Fig. 1. The schematic description of photo-induced apoptosis-targeted chemotherapy in tumor parenchyma.

as by antitumor vasculature effects [17–20]. Work is in progress to improve the PDT efficiency by the use of different photosensitizers with certain properties such as the following: strength of light absorption by a substance in the wavelength range 600–750 nm per molar concentration, a high quantum yield of singlet oxygen, and selective affinity for tumor tissue [21–23]. From this perspective, methylene blue (MB), a thiazinium dye, has been renowned for generating singlet oxygen [22], destruction of nucleic acid in a nuclease like manner [23], low toxicity [24–26], and *in vivo* staining of tumors [27,28].

Our previous reports demonstrated various types of induced apoptosis targeted chemotherapy using a caspase-3 specific activatable prodrug DEVD-S-DOX [29,30]. To induce apoptosis at the tumor site for the expression of a caspase-3, we employed radiation and free DOX. Here, we propose photo-induced apoptosis-targeted chemotherapy (PIATC) through the sol-gel transition in an aqueous mixture of two functional nanoparticle systems as shown schematically in Fig. 1.

Two types of Pluronic NPs were prepared with Pluronics F-68 and F-127. Pluronics F-68 and F-127 exhibited gelation in the aqueous media at 58 °C and 17 °C, respectively, when they formed 25 wt% aqueous solutions [31]. When the surface of NPs is predominantly covered by Pluronics, the gelation behavior of the aqueous solution containing NPs is expected to be similar to that of Pluronics. With the appropriate combination of the NPs composed of Pluronics F-127 or F-68, the gel formation in the aqueous solution containing the NPs should be demonstrable at body temperature as displayed in Fig. 1.

We evaluated the sol-gel transition with the assembly of NPs containing DEVD-S-DOX or MB to confirm the stability of the *in-situ* gel at the tumor tissue. To verify the concept of PIATC, the cellular uptake behaviors and therapeutic efficacy of *in-situ* gel were observed as a function of time. And the photo-initiated apoptosis was observed and evaluated using ROS detection and a membrane integrity test. Finally, the antitumor efficacy and cardiotoxicity were evaluated in tumor-bearing mice.