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Thermosensitive poly-(D,L-lactide-co-glycolide)-*block*-poly(ethylene glycol)-*block*-poly-(D,L-lactide-co-glycolide) hydrogels for multi-drug delivery

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Abstract

A current treatment strategy for peritoneal ovarian cancer is a combination of peritoneal surgery and multi-drug-based chemotherapy that often involves intraperitoneal (IP) injection. A thermosensitive poly-(D,L-lactide-co-glycolide)-*block*-poly(ethylene glycol)-*block*-poly-(D,L-lactide-co-glycolide) (PLGA-*b*-PEG-*b*-PLGA) hydrogel platform (thermogels) enabled gel loading of poorly work-soluble paclitaxel (cytotoxic agent), 17-allylamino-17-demethoxygeldanamycin (17-AAG, heat shock protein inhibitor), and rapamycin (mammalian target of rapamycin protein inhibitor). PLGA-*b*-PEG-*b*-PLGA thermogels (15%) carrying paclitaxel, 17-AAG, and rapamycin (named Triogel) made a successful transition from a free-flowing solution below ambient temperature to a gel depot at body temperature. Triogel gradually released paclitaxel, 17-AAG, and rapamycin at an equal release rate in response to the physical gel erosion. In an ES-2-luc ovarian cancer xenograft model, a single IP injection of Triogel (60, 60, and 30 mg/kg of paclitaxel, 17-AAG, and rapamycin, respectively) significantly reduced tumor burden and prolonged survival of ES-2-luc-bearing nude mice without notable systemic toxicity relative to those delivered by poly(ethylene glycol)-*block*-poly(D,L-lactic acid) (PEG-*b*-PLA) micelles in solution *via* IP or intravenous (IV) injection route. These results show a great potential of a biodegradable thermogel platform carrying multi-drugs for IP chemotherapy in peritoneal ovarian cancer.

Keywords

PLGA-*b*-PEG-*b*-PLGA; Regal; paclitaxel; 17-AAG; rapamycin; multi-drug delivery; ovarian cancer

Introduction

Cancers commonly associated with the peritoneal cavity, such as colorectal, ovarian, and pancreatic cancers, account for approximately 110,000 new cases and 60,000 deaths estimated in 2013 in the United States [1]. Peritoneal malignancies often create barriers in the vicinity of peritoneal organs because a number of cancer cells block the abdominal

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lymphatic ducts, cause obstruction of lymphatic drainage, and as a result, lead to a decreased outflow of peritoneal fluid [2]. These unique anatomical and physiological properties of peritoneal cancers have drawn attention to potential benefits of locoregional chemotherapy. Lately, progress has been made in drug delivery strategies for intraperitoneal (IP) therapy of ovarian cancer [2-5]. The primary rationale of IP administration of drugs is to increase exposure of drugs to cancer cells within the peritoneal cavity while reducing systemic toxicity [4,5]. Several studies have shown that IP delivery of cisplatin and paclitaxel could result in 10 and 1000 times higher distribution, respectively, in peritoneal tumor tissues than systemic delivery [4]. IP administration of drugs is exposed primarily to the large surface of the membrane in the peritoneal cavity, absorbed through the portal circulation, and detected in the systemic blood stream after a significant lag time [6]. With this regard, ideal IP chemotherapy seems to improve both therapeutic efficacy and safety. Previously, we proved the unique potential of IP drug delivery of paclitaxel (cytotoxic agent), cyclopamine (hedgehog inhibitor), and gossypol (Bcl-2 inhibitor) enabled by poly(ethylene glycol)-*block*-poly(ϵ -caprolactone) (PEG-*b*-PCL) micelles for the locoregional treatment of metastatic ovarian cancers [3]. PEG-*b*-PCL micelles containing paclitaxel, cyclopamine, and gossypol satisfied requirements for a combination drug delivery system such as biocompatibility, multiple hydrophobic drug solubilization in water, and sustained release of payloads. A 3-drug combination of paclitaxel, cyclopamine, and gossypol delivered by PEG-*b*-PCL micelles was highly effective in metastatic ES-2-luc and SKOV-3-luc ovarian cancer-bearing xenograft models by eradicating peritoneal tumors and prolonging survival rate of xenograft models without notable toxicity.

In the past few years, a number of polymer-based hydrogels have been shown great potential in the biomedical field and locoregional chemotherapy. One of the most popular polymer-based hydrogels is thermosensitive poly-(D,L-lactide-co-glycolide)-*block*-poly(ethylene glycol)-*block*-poly-(D,L-lactide-co-glycolide) (PLGA_{1,500}-*b*-PEG_{1,000}-*b*-PLGA_{1,500}) triblock copolymer hydrogel (ReGel) due to its reversible sol-gel transition as a function of temperature, ability to increase the solubility of hydrophobic compounds, extended release of payloads, biodegradability, excellent safety profile, and clinical potentials in the biomedical field [7,8].

The formation of thermosensitive hydrogels takes places *via* physical association of hydrophobic PLGA segments: At low temperature, majority of triblock copolymers tend to form individual loops joining two hydrophobic PLGA segments together to the center of each loop and the association of several loops occurs sharing the hydrophobic PLGA center (micelle formation) [9,10]. A few linear triblock copolymers that do not participate in the loop formation provide bridges among micelles. At this stage, the hydrogen bonding between hydrophilic PEG segments of triblock copolymers and water molecules dominates and as a result, the water phase takes on a sol-like suspension. As temperature elevates, the hydrophobic interaction among hydrophobic PLGA segments increases, micelles are aggregated into micelle-networks, and water loses flowability, eventually inducing a sol-to-gel transition. At even higher temperature, due to the overly strengthened hydrophobic interaction, precipitation of micelle-networks occurs by separating the water phase from the precipitation phase [9,10]. PLGA-*b*-PEG-*b*-PLGA triblock copolymer thermogels can entrap

hydrophobic compounds in the hydrophobic regions of a hydrogel matrix as well as hydrophilic compounds near the PEG segments bridging multiple micelles. The main release mechanism of hydrophilic compounds is diffusion from hydrogels prior to the physical gel degradation or erosion whereas major driving force of release for hydrophobic compounds is the physical erosion of a hydrogel matrix [9]. In particular, as a release of hydrophobic compounds is highly dependent on the hydrogel matrix degradation, which is a sustained process, an extended release of hydrophobic compounds is anticipated. Several studies have proven that PLGA-*b*-PEG-*b*-PLGA thermogels could be used as a matrix material exhibiting a successful sol-gel transition upon an increase in temperature to provide sustained release profiles of drugs, such as dexamethasone acetate, doxorubicin, paclitaxel, and docetaxel, and ultimately improve therapeutic/pharmacokinetic efficacies of payloads [7,8,11,12]. For example, a series of preclinical and clinical studies of OncoGel, a biodegradable Regal (PLGA-*b*-PEG-*b*-PLGA) depot formulation of paclitaxel, increased the water solubility of paclitaxel by three orders of magnitude, enabled a continuous release of paclitaxel directly to the solid tumor and surrounding tissues for 6 weeks for locoregional chemotherapy, resulted in improved survival of a subcutaneous breast tumor xenograft model (MDA-MB-231) compared to intravenous (IV) or IP administration of Taxol (paclitaxel formulation dissolved in ethanol/Cremophor), and provided no treatment-limiting toxicities in several clinical trials [8].

The aforementioned properties of PLGA-*b*-PEG-*b*-PLGA thermogels are ideal not only for locoregional chemotherapy but also for a barrier device *via* peritoneal surgery to prevent postsurgical intra-abdominal adhesions. In clinics, almost all patients develop adhesions after transperitoneal surgery with various degrees and the consequences of peritoneal adhesions can be severe pain, infertility, and lethal bowel obstruction [13]. After peritoneal surgery, surgical injury and surgically traumatized peritoneal tissues increase vascular permeability mediated by histamine and form fibrin matrix. Under the ischemic condition present in surgical trauma, the activity of fibrinolysis is suppressed and as a result, fibrin bands are infiltrated with fibroblasts, further forming adhesions between intraperitoneal organs or omentum and wound [14]. Barrier devices, membranes and thin film of hydrogels, in general, can be placed directly onto the potential site of adhesions to prevent severe tissue adhesions and malfunctions of peritoneal organs. For example, Interceed (regenerated cellulose) and Seprafilm (hyaluronic acid-carboxymethylcellulose), which are non-toxic and biodegradable, have been used as post-gynecological surgery barrier devices in the US [15]. PLGA-*b*-PEG-*b*-PLGA triblock copolymer thermogels presumably have great potential in gynecology with the dual functionality, offering effective adjuvant IP chemotherapy and preventing tissue adhesion after peritoneal surgery.

In this study, we observed that PLGA-*b*-PEG-*b*-PLGA triblock copolymer thermogels successfully carried paclitaxel, 17-AAG, and rapamycin in their gel matrix, gradually released drugs at the equal rate from the gel matrix, and showed the potential for IP chemotherapy in peritoneal ovarian cancer by inhibiting tumor growth of an IP metastatic ES-2-luc-bearing xenograft model.

Materials and methods

Preparation of Triolimus and thermosensitive hydrogels carrying drug(s)

PEG_{4,000}-*b*-PLA_{2,200} (Polymer Source, Dorval, Canada) micelles containing paclitaxel, 17-AAG, and rapamycin (Triolimus) (LC Laboratories, Woburn, MA) were prepared as previously described [16]. Briefly, 150 mg of PEG-*b*-PLA and 6, 6, and 3 mg of paclitaxel, 17-AAG, and rapamycin were dissolved in 2 mL of acetonitrile. Acetonitrile was then removed by reduced pressure using rotary evaporator at 60 °C. Thin film consisting of a mixture of polymer and three drugs was rehydrated with 1 mL of pre-warmed distilled water at 60 °C at the final concentrations of 6, 6, and 3 mg/mL of paclitaxel, 17-AAG, and rapamycin. The aqueous solution was centrifuged and passed through 0.22 µm regenerated cellulose (RC) filter to remove unincorporated drugs. The content of drugs incorporated in Triolimus was quantified using Reverse Phase HPLC (RP-HPLC) analysis with a Shimadzu Prominence HPLC system (Shimadzu, Japan). Samples (10 µL) were injected into Zobrax SB-C8 Rapid Resolution cartridge (4.6 × 75 mm, 3.5 µm, Agilent, Santa Clara, CA). The flow rate was 1.0 mL/min and column was kept at 40 °C. The separation of paclitaxel, 17-AAG, and rapamycin was carried out in an isocratic mode with mobile phase consisting of 55% acetonitrile and 45% water (containing 0.1% phosphoric acid and 1% of methanol). Paclitaxel, 17-AAG, and rapamycin were monitored at 227, 333 and 279 nm, respectively, and eluted at 2.8, 3.3, and 8.6 min, respectively.

Thermosensitive PLGA-*b*-PEG-*b*-PLGA hydrogels (Polysciotech, West Lafayette, IN) were prepared as follows: PLGA_{1,500}-*b*-PEG_{1,000}-*b*-PLGA_{1,500} triblock copolymer dissolved in 1 mL of cold water (4 °C) was mixed with 6, 6, and 3 mg of paclitaxel, 17-AAG, and rapamycin, individually or in combinations, aiming for 10% w/w loading (% drug(s)/polymer), in 1 mL of *tert*-butanol at 60 °C and lyophilized for 24 h. The lyophilized cake was then rehydrated with 1 mL of cold water at 4 °C and gently stirred for > 6 h in the cold room. Rehydrated solution was incubated in the cold room for 30 min and passed through 0.22 µm regenerated cellulose (RC) filter to remove unincorporated drugs. The hydrogel was diluted with cold acetonitrile in solution and the content of drugs incorporated in hydrogel was quantified by RP-HPLC.

In vitro drug release study for hydrogel

An aqueous solution of drug-loaded hydrogels (kept cold) was put into dialysis cassettes ($n=4$ /each time point) (MWCO 20,000, Thermo Fischer Scientific Inc., Rockford, IL) and cassettes were placed in 2 L of water at 37 °C with stirring. At various time points, 0, 0.5, 1, 3, 6, 9, 24, and 48 h, cassettes ($n=4$) were removed and kept at 4 °C to liquefy hydrogels. Solution was then transferred to the cold tube at 4 °C and supernatant was collected for the further quantification of residual drug contents in the hydrogel matrix. Assuming a drug release from hydrogels was rate-limiting, curve-fitting of % drug release was done based on a first-order association using GraphPad Prism version 5.00 for Mac OS X (San Diego, CA).

Human ovarian cancer xenograft and drug treatment

Female 6-8 week-old athymic nude mice were obtained from Harlan Laboratories (Madison, WI). General anesthesia was induced with 1.5% isoflurane/oxygen and maintained with 1%

isoflurane/oxygen during the experiment. All animal experiments were approved by UW-Madison's Institutional Animal Care and Use Committee and conducted in accordance with institutional and NIH guidance. All animals were euthanized at the time of reaching a moribund condition by medical grade carbon dioxide with the flow rate of 10-30% of the euthanasia chamber volume per minute.

In the intraperitoneal retention study, aqueous Triolimus micelle solution (~ 200 μ L) or aqueous Triogel solution (kept cold, ~ 400 μ L) was injected into peritoneal cavity of normal nude mice at 60, 60, and 30 mg/kg of paclitaxel, 17-AAG, and rapamycin, respectively. Mice were sacrificed at 2, 8, 24, 48, and 120 h post injection of Triolimus or Triogel, and remnants were removed from the peritoneal cavity for the quantitative analyses. Collected remnants were dissolved in acetonitrile and the level of drugs in supernatant was analyzed by RP-HPLC.

For an anticancer efficacy study, ES-2-luc human ovarian cancer cells were transfected with luciferase-expressing plasmid pGL4.51 as previously reported and cultured in McCoy's 5a medium (ATCC, Manassas, VA) supplemented with 1% L-glutamine, 10% fetal bovine serum, and 1% penicillin/streptomycin.³ ES-2-luc cells (1×10^6 cells/animal) were injected into peritoneal cavity of anesthetized mice and 4 days after cell inoculation, drug treatment was initiated. ES-2-luc-bearing xenograft model was divided into 5 groups ($n=5$): Triolimus (IV), PEG-*b*-PLA micelles containing paclitaxel, 17-AAG, and rapamycin at 60, 60, and 30 mg/kg, respectively; Triolimus (IP); Triogel (IV), PLGA-*b*-PEG-*b*-PLGA thermogels containing paclitaxel, 17-AAG, and rapamycin at 60, 60, and 30 mg/kg, respectively; Empty PEG-*b*-PLA micelles (IV); Empty PLGA-*b*-PEG-*b*-PLGA thermogels (IP). Approximately 200 μ L of aqueous micelles and 400 μ L of cold aqueous thermogels were injected IV and IP into anesthetized mice, respectively. Body weights, general appearance, and mortality of animals were monitored up to 60 days post ES-2-luc cell inoculation.

Whole-body bioluminescence imaging

Whole-body bioluminescence images were obtained using Xenogen IVIS[®] 200 Series and Live Imaging[®] software was used for image acquisition/quantification of the total photon counts in the regions of interest (ROIs). Color-coded whole-body images of anesthetized mice were recorded on 0, 1, 2, 3, 7, 14, 21, and 28 days after the each formulation treatment. Throughout experiments, all images were acquired and collected using identical system settings: exposure time = 1s; binning = medium; f/stop 2). D-Luciferin (Caliper Life Science, Hopkinton, MA) at 113 mg/kg was injected IP into ES-2-luc-bearing xenograft model 5 min prior to whole-body imaging.

Statistical analysis

Data were represented as mean $\pm$ standard deviation (SD). Statistical analysis was conducted using one-way ANOVA at 5% significance level combined with Tukey's multiple comparison tests provided by GraphPad Prism. *, **, and *** signify $P < 0.05$, < 0.01 , and < 0.001 , respectively.

Results and discussions

Characterization of thermosensitive hydrogels carrying drug(s)

In this work, biodegradable and thermogelling PLGA_{1,500}-*b*-PEG_{1,000}-*b*-PLGA_{1,500} triblock copolymers permitted incorporation of highly hydrophobic drugs, paclitaxel (cytotoxic agent), 17-allylamino-17-demethoxygeldanamycin (17-AAG, heat shock protein 90 inhibitor), and rapamycin (mammalian target of rapamycin protein inhibitor), using a simple lyophilization method. PLGA-*b*-PEG-*b*-PLGA thermogels containing paclitaxel, 17-AAG, and rapamycin, named Triogel, was a free flowing solution below ambient temperature but formed a hydrogel depot at higher room temperature (Figure 1). As temperature further increased above 50 °C, thermogels shrank in volume, expelled water, leading to a phase separation between water and a mixture of polymer and drugs, and as a result, precipitated drugs. Table 1 presents the level of paclitaxel, 17-AAG, and rapamycin incorporated in solution in water (mg/mL) at 4 °C and visual conditions of hydrogels containing 1-, 2-, and 3-drugs at 37 °C. Paclitaxel and 17-AAG were successfully incorporated in thermogels in water at *ca.* 6 mg/mL and *ca.* 5-6 mg/mL, respectively, individually and in 2- and 3-drug combinations. Interestingly, thermogels lost a gel-like integrity at 37 °C when loaded with rapamycin alone whereas rapamycin was successfully incorporated in thermogels at *ca.* 3 mg/mL in 2-drug and 3-drug combinations with paclitaxel and rapamycin, *eg.* paclitaxel/rapamycin, rapamycin/17-AAG, and paclitaxel/rapamycin/17-AAG. This is the first report successfully incorporating 3 highly hydrophobic drugs in the platform of thermosensitive hydrogels for the IP multi-drug delivery in oncology.

In vitro drug release profiles

In vitro drug release patterns (Figure 2a) from Triogel at 37 °C presented that all three drugs were released in an identical monophasic pattern and individual curves were fit in a first-order association model with the goodness of fit (R^2) of 0.9763 for paclitaxel, 0.8911 for 17-AAG, and 0.9733 for rapamycin. Drug release curves for Triogel reached a plateau at 46% for paclitaxel, 46% for 17-AAG, and 44% for rapamycin within 48 h with a statistically equal release rate: rate constant (k , h^{-1}) of paclitaxel, 17-AAG, and rapamycin was 0.0577, 0.0770, and 0.0900, respectively. Release patterns of singly-loaded paclitaxel ($R^2 = 0.9868$, $k = 0.0672 h^{-1}$) and singly-loaded 17-AAG ($R^2 = 0.9341$, $k = 0.0671 h^{-1}$) at 37 °C were also identical, reaching a plateau at 60% for paclitaxel and 61% for 17-AAG over 48 h (Figure 2b). Not surprisingly, rapamycin-incorporated thermogels in a free-flowing solution at 37 °C showed a rapid release of rapamycin along with the immediate precipitation of rapamycin in dialysis cassettes, releasing 50% of rapamycin within 0.5 h whereas rapamycin in combinations with paclitaxel or 17-AAG, successfully formed thermogels, presented slow release kinetics (Figure 2b and 2c). It is because the major release mechanism for hydrophobic compounds successfully incorporated in thermogels is the physical erosion of the hydrogel matrix and the physical gel erosion takes place at slow pace at 37 °C. Previously, we obtained 3 distinctive release profiles of paclitaxel ($R^2 = 0.984$, $k = 0.075 h^{-1}$), 17-AAG ($R^2 = 0.996$, $k = 0.275 h^{-1}$), and rapamycin ($R^2 = 0.986$, $k = 0.050 h^{-1}$) from PEG-*b*-PLA micelles in solution (named Triolimus) [16]. As the main release mechanism of drugs from polymeric micelles in solution is diffusion, the release profile of drugs partially

relies on hydrophobicity of each drug components, resulting in 3 distinctive release profiles from polymeric micelles in the aqueous medium.

***In situ* gel formation and degradation**

In situ gel formation and degradation of Triogel at 60, 60, 30 mg/kg of paclitaxel, 17-AAG, and rapamycin, respectively, were determined in healthy nude mice shown in Figure 3a. Triogel was kept cold in solution prior to IP injection into nude mice. Visible gel depots (purple-in-color from 17-AAG) were found in peritoneum of animals at 2 h post IP injection, occupying gaps between surfaces of internal organs in peritoneum. At 8 h post IP injection of Triogel, purple-colored gel depots were found in the deeper peritoneum. At 24, 48, and 120 h post IP injection of Triogel, visible gel depots turned into white-colored gels, presumably due to the release of the majority of drugs. Collected gel depots from the peritoneum kept remnants, approximately 16% of paclitaxel, 6% of 17-AAG, and 8% of rapamycin, at 8 h post IP injection of Triogel and 1% of paclitaxel alone was detected at 48 h. In an identical setting of experiment, PEG-*b*-PLA micelles containing paclitaxel, 17-AAG, and rapamycin (Triolimus) in solution at 60, 60, and 30 mg/kg, respectively, rapidly disappeared within 2 h post IP injection (Figure 3b).

***In vitro* cytotoxicity**

In vitro cytotoxicity of paclitaxel, 17-AAG, and rapamycin, individually and in combinations was assessed in ES-2-luc human ovarian cancer cells and IC₅₀ values of drug(s) dissolved in a mixture of DMSO and medium were summarized in Table 2. Individual treatment of rapamycin (IC₅₀: 2×10^{11} nM) or 17-AAG (IC₅₀: 934 nM) did not induce significant cytotoxic effect in ES-2-luc cells whereas a 2-drug combination of 17-AAG/rapamycin (2:1 w/w ratio) treated ES-2-luc cells with much lower IC₅₀ value of 343 nM, indicating synergistic cell-killing effect in ES-2-luc cells. Paclitaxel alone and combinations of paclitaxel/rapamycin (1:1 molar ratio) and paclitaxel/17-AAG/rapamycin (2:2:1 w/w/w ratio) resulted in comparably low IC₅₀ values at 125, 112, and 168 nM, respectively in ES-2-luc cells.

Anticancer efficacy of paclitaxel, 17-AAG, and rapamycin in thermogel depot vs. in solution after IP or IV injections

Anticancer efficacies of Triogel and Triolimus at 60, 60, and 30 mg/kg of paclitaxel, 17-AAG, and rapamycin, respectively, were assessed in IP metastatic ES-2-luc human ovarian cancer-bearing nude mice (Figure 4). ES-2-luc tumor progression was observed longitudinally in groups of 5 mice by monitoring bioluminescence signals in the peritoneum and calculating % bioluminescence intensity relative to the initial bioluminescence signal (%BLI). At day 4 post IP inoculation of ES-2-luc cells, strong regional bioluminescence signals were detected in the peritoneum, indicating that IP metastatic ES-2-luc human ovarian cancer was formed. An ES-2-luc xenograft model was treated with a combination of paclitaxel, 17-AAG, and rapamycin at 4 days post cell inoculation *via* either IP or IV route. In an IV empty PEG-*b*-PLA vehicle control, bioluminescence from ES-2-luc cancer cells and tissues rapidly increased, reaching 1123% of %BLI at day 14 post treatment. Substantial amount of ascites (body fluid in peritoneum) rapidly formed in animals showing notably

increased radii of abdomen and strong bioluminescence signals in peritoneum at days 21 and 28 post treatment. In an IP empty PLGA-*b*-PEG-*b*-PLGA thermogel control, there was also a rapid increase in bioluminescence signals in the peritoneum, reaching 2695% of %BLI at day 21 post treatment and abdomen of animals was notably expanded. Tumor burden killed 100% of an ES-luc ovarian cancer-bearing xenograft model for IV and IP controls within 23 and 28 days post vehicle injection. A single IV or IP injection of Triolimus was effective in delaying tumor growth for 3 days, but %BLI rapidly increased up to 412% and 460%, respectively, after 7 days post IV or IP treatment and reached 2080% and 1925%, respectively, after 21 days post treatment. Hundred percent ES-luc ovarian cancer-bearing nude mice treated with IV and IP Triolimus died of cancer within 30 and 28 days post treatments. Surprisingly, a single IP injection of Triogel was highly effective in reducing tumor growth and ascites formation; almost no bioluminescence signals were detected in animals at days 7 (3% of %BLI) and 14 (6% of %BLI) post treatment, possibly eradicating large metastases and ascites fluid, but bioluminescence signals appeared near the kidney and fallopian tube in whole-body images at day 21 (32% BLI) post treatment. Tumor regression upon the treatment of IP Triogel was approximately 70-fold and 80-fold superior than tumor regressions by Triolimus treatments (IP and IV) and controls (IP and IV), respectively (Figure 4b). Twenty percent ES-luc ovarian cancer-bearing nude mice treated with an IP Triogel survived for 60 days post treatment (Figure 4c). This interesting result demonstrates that right selections of a type of formulation (solution *vs.* thermogels) and an administration route (IV *vs.* IP) can make a significant difference in treatment outcome in oncology.

Conclusions

In conclusion, we successfully incorporated paclitaxel, 17-AAG, and rapamycin in biocompatible and biodegradable PLGA-*b*-PEG-*b*-PLGA thermogels that enabled three highly hydrophobic drug components soluble in water. Triogel (thermosensitive PLGA-*b*-PEG-*b*-PLGA hydrogels carrying paclitaxel, 17-AAG, and rapamycin) made a successful sol-gel transition upon the temperature changes, extended release of payloads *in vitro* and *in vivo*, and induced significant anticancer efficacy in ES-2-luc peritoneal ovarian cancer bearing nude mice without systemic toxicity.

In the future, biomedical potentials of thin film of Triogel as adjuvant IP chemotherapy after peritoneal surgery for killing residual tumor tissues and cells and as a barrier device for preventing postsurgical tissue adhesions will be assessed in a peritoneal disease-bearing rat model in surgical oncology.

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Figure 1. Schematic illustration of a sol-gel transition of thermosensitive PLGA-*b*-PEG-*b*-PLGA hydrogels carrying paclitaxel, 17-AAG, and rapamycin.

Figure 2. Release kinetics of drugs for thermosensitive PLGA-*b*-PEG-*b*-PLGA hydrogels carrying paclitaxel (PTX), 17-AAG, and rapamycin (RAPA) in a 3-drug combination (a), individually (b), and in 2-drug combinations (c).

Figure 3.

In situ thermogel formation of Tiogel at 60, 60, and 30 mg/kg of paclitaxel (PTX), 17-AAG, and rapamycin (RAPA) in the peritoneum of healthy nude mice (a), detection of Triolimus at 60, 60, and 30 mg/kg of PTX, 17-AAG, and RAPA in the peritoneum of healthy nude mice (b), and the level of residual drugs from Triogel in the peritoneum of healthy nude mice (c).

Figure 4. Noninvasive bioluminescence imaging and treatment assessment for an ES-2-luc xenograft model. Results are presented in whole-body bioluminescence images (a), quantitative bioluminescence intensity (BLI) in regions of interest (ROIs). * $P < 0.05$ (IP Triogel vs. IP control, IV Triolimus, or IP Triolimus on day 21) (b), and Kaplan-Meier analysis for survival. * $P < 0.05$ (IV control vs. IP Triolimus, IP control vs. IV Triolimus), ** $P < 0.01$

(IV control *vs.* IV Triolimus), *** $P < 0.001$ (IV control, IP control, IP Triolimus, or IV Triolimus *vs.* IP Triogel) (c).

Characterizations of PLGA-*b*-PEG-*b*-PLGA thermogels in 1-drug, 2-drug, and 3-drug combinations of paclitaxel (PTX), 17-AAG, and rapamycin (RAPA).

Table 1

Drug(s)	PTX	17-AAG	RAPA	PTX/17-AAG	PTX/RAPA	RAPA/17-AAG	PTX/17-AAG/RAPA
Polymer (mg/mL)	60	60	30 or 60	120	90	90	150
Initial drug(s) added (mg/mL)	6	6	3	6/6	6/3	3/6	6/6/3
Final drugs (mg/mL)	5.8 ± 0.5	5.3 ± 0.3	1.6 ± 0.2	5.6 ± 0.4/5.5 ± 0.3	6.0 ± 0.5/3.0 ± 0.2	2.5 ± 0.2/4.8 ± 0.4	5.7 ± 0.4/5.7 ± 0.4/3.1 ± 0.2
Physical condition	Gel	Gel	Solution	Gel	Gel	Gel	Gel

IC₅₀ values of paclitaxel (PTX), 17-AAG, and rapamycin (RAPA) in 1-drug, 2-drug, and 3-drug combinations in ES-2-luc human ovarian cancer cells.

Table 2

Drug(s)	PTX	17-AAG	RAPA	PTX/17-AAG	PTX/RAPA	RAPA/17-AAG	PTX/17-AAG/RAPA
IC ₅₀ (nM)	124.5 ± 25.2	933.6 ± 1.8	> 2.2×10 ¹¹	518.8 ± 14.4	112.0 ± 11.3	342.9 ± 74.3	168.1 ± 37.2