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Title: Sustained, low-dose intraperitoneal cisplatin improves treatment outcome in ovarian cancer mouse models

Authors: Hongye Ye^{a,b,†}, Laura M. Tanenbaum^{a,b,†}, Young Jeong Na^c, Aikaterini Mantzavinou^{a,b}, Giulia Fulci^c, Marcela G. del Carmen^d, Michael J. Birrer^{c,e,f}, and Michael J. Cima^{b,g,*}.

Affiliations:

^a Harvard-MIT Division of Health Sciences and Technology, Massachusetts Institute of Technology, Cambridge, MA, USA.

^b David H. Koch Institute for Integrative Cancer Research, Massachusetts Institute of Technology, Cambridge, MA, USA.

^c Center for Cancer Research, Massachusetts General Hospital, Boston, MA, USA.

^d Department of Obstetrics and Gynecology, Massachusetts General Hospital, Boston, MA, USA.

^e Division of Hematology/Oncology, Massachusetts General Hospital, Boston, MA, USA.

^f Department of Medicine, Massachusetts General Hospital, Boston, MA, USA.

^g Department of Materials Science, Massachusetts Institute of Technology, Cambridge, MA, USA.

[†] These authors contributed equally to this work.

* Corresponding author.

Corresponding Author Information:

Michael J. Cima
Koch Institute for Integrative Cancer Research
Massachusetts Institute of Technology
500 Main Street
Building 76 Room 653
Cambridge, MA 02139
mjcima@mit.edu
Tel: (617) 253-6877
Fax: (617) 258-6936

Abstract: Intraperitoneal (IP) chemotherapy for ovarian cancer treatment prolongs overall survival by 16 months compared to intravenous chemotherapy but is not widely practiced due to catheter-related complications and complexity of administration. An implantable, nonresorbable IP microdevice was used to release chemotherapeutic agent at a constant rate of approximately 1.3 µg/hour *in vitro* and 1.0 µg/hour *in vivo*. Studies conducted in two orthotopic murine models bearing human xenografts (SKOV3 and UCI101) demonstrate that continuous dosing reduces tumor burden to the same extent as weekly IP bolus drug injections. Treatment-induced toxicity was quantified via body weight loss and complete blood count. The microdevice resulted in significantly less toxicity than IP bolus injections, despite administration of higher cumulative doses (total area under the concentration-time curve of 3,049 ng-day/mL with the microdevice vs. 2,118 ng-day/mL with IP bolus injections). This preclinical study supports the concept that reduced toxicity with similar efficacy outcomes can be achieved by continuous dosing in ovarian cancer patients currently treated with IP therapy.

Keywords: drug delivery; intraperitoneal; continuous; cisplatin; ovarian cancer

Chemical compounds: cisplatin - PubChem CID 441203

1. Introduction

Ovarian cancer is the fifth leading cause of cancer-related deaths in women and has the highest case-fatality rate of all gynecologic cancers [1]. It is estimated that there were 21,980 cases of ovarian cancer resulting in 14,270 deaths in 2014 [1]. The current standard treatment for advanced stage ovarian cancer includes cytoreduction surgery to leave no macroscopic residual disease followed by intravenous (IV) or intraperitoneal (IP) chemotherapy with a platinum-based agent [2-4]. Three-quarters of women treated for advanced stage ovarian cancer develop disease recurrence within two years after therapy. Multiple randomized phase III trials comparing IV and IP therapy have demonstrated a survival advantage with IP therapy [5-7]. The most recent Gynecologic Oncology Group (GOG) clinical trial 172 found that IP cisplatin treatment was able to prolong overall survival from 49.7 months with IV therapy alone to 65.5 months with IV/IP therapy [7]. A recently published retrospective analysis of GOG clinical trials 114 and 172 with an overall median patient follow-up of 10.7 years also concluded that IV/IP chemotherapy was associated with a 23% decreased risk of death compared to IV therapy [8].

IP therapy has not been widely accepted despite these results. The toxicity of IP therapy in the GOG trial 172 was substantial, with only 42% of patients able to complete all intended cycles [7, 10]. Early termination of IP therapy was primarily due to catheter-related complications [7, 9, 10]. The catheter implantation site is susceptible to infection and obstruction [9]. Clinical adoption of IP therapy is also limited by the technical difficulty of catheter implantation, higher associated costs compared to IV therapy, and expertise required for treating associated complications [7, 10]. The significant survival benefits of IP therapy should, however, outweigh the greater toxicity and lower patient quality of life [10]. A new treatment regimen that eliminates the need for a catheter, and is cost-efficient and simple to administer, will be favorable to both clinicians and patients and, ultimately, increase clinical adoption of IP therapy.

Improved efficacy of continuous IP dosing has been demonstrated previously in ovarian cancer models, but published work faces significant limitations in (i) the clinical relevance of the evaluated treatment periods and (ii) the translatability of the proposed delivery systems to humans. Work by Piquette-Miller and Allen has focused on eliminating drug-free periods between IP infusions of chemotherapy for ovarian cancer, and they have published results to support superior antitumor response of continuous delivery compared to intermittent dosing [11-15]. They used commercially available osmotic pumps and custom-made injectable gels as vehicles to deliver drug locally to the peritoneal cavity. Such vehicles are ill-suited for clinical translation because of the impractical volumes required to contain the therapeutic payload when scaled for human use. Injected gels, additionally, achieve poorly controlled release rates as the polymer degrades.

This paper seeks to address the urgent need for a better IP drug delivery system in ovarian cancer. The results presented here demonstrate the efficacy of sustained cisplatin delivery *in vivo* in ovarian cancer mouse models over a period of 6 weeks, using custom-made implantable microdevices. The 6-week period allowed administration of 6 cycles of bolus therapy for comparison to the current clinical standard of care. Systemic toxicity was evaluated via complete blood cell counts (CBC) in addition to total body weight, as leukopenia was the main side effect observed in GOG trial 172 with significantly higher incidence in the IP bolus arm of the study [7]. The results presented here demonstrate that continuous dosing significantly reduces the toxicity of IP bolus therapy in both platinum-resistant and platinum-sensitive xenograft ovarian cancer models.

2. Materials and Methods

2.1. Chemicals and Materials

Phosphate-buffered saline (PBS), diethyldithiocarbamate trihydrate (DDTC), nickel (II) chloride, high-performance liquid chromatography (HPLC)-grade water, HPLC-grade methanol and cisplatin powder were purchased from Sigma-Aldrich (St. Louis, MO, USA). Luciferin was obtained from Perkin-Elmer (Hopkinton, MA, USA). RPMI 1640 medium, McCoy's 5a medium, MTT assay kits and fetal bovine serum (FBS) were purchased from Life Technologies (Grand Island, NY, USA). Medical grade cisplatin solution for bolus injections was purchased from McKesson (San Francisco, CA, USA).

2.2. Cisplatin Quantification

The HPLC method was modified from the method published by V. Augey *et al.* [16]. The samples were diluted in PBS to a final volume of 450 μL before adding 50 μL of nickel chloride (0.1 mg/mL in PBS) and 100 μL of DDTC (0.1 g/mL in 0.1 M sodium chloride) solutions. The samples were then incubated at 37°C for 30 minutes before passing them through the HPLC column. An Agilent 1200 LC was used for cisplatin quantification. An ODS Hypersil 250 x 4.6 mm column (Thermo Scientific, Waltham, MA, USA) was heated to 30°C and the sample holder was cooled to 4°C prior to the run. A mobile phase of 75% methanol in water (HPLC-grade) was used at a flow rate of 1.4 mL/min. 100 μL of cisplatin-containing sample were injected into the column. The area under the concentration-time curve (AUC) of the cisplatin peak that appeared at 5.1 min on the 254 nm spectrum was normalized to the AUC of the internal standard peak at 6.0 min on the 250 nm spectrum. A linear calibration curve was obtained by plotting the ratio of the cisplatin AUC to the internal standard AUC against a concentration range of 0.1 to 20 $\mu\text{g/mL}$ ($r^2 = 0.9996$).

The inductively-coupled plasma mass spectrometry (ICP-MS) cisplatin assay technique was used for samples with very low concentrations of cisplatin (less than 0.1 $\mu\text{g/mL}$). The ICP-MS protocol was provided by the Trace Metal Laboratory at the Harvard School of Public Health (Boston, MA, USA). The samples, along with a set of calibration samples, were then run on a PerkinElmer Elan 6100 DRC-II ICP-MS machine in the Harvard School of Public Health, Trace Metal Laboratory. A linear calibration in the range of 0.1 ng/mL to 10 $\mu\text{g/mL}$ was obtained ($r^2 = 1$).

2.3. Microdevice Design and Fabrication

The microdevices used in this study were fabricated by microPEP (East Providence, RI, USA) and consist of an injection-molded reservoir and cap (Fig. 1A). The microdevices are injection-molded into a cylindrical reservoir of approximately 3 mm in diameter, 3.5 mm in height, and 0.3 mm in thickness. The cap is a thin disc, 3 mm in diameter and 400 μm in thickness, with a 180- μm orifice in the center. The orifice diameter was selected to control release from the reservoir based on the known solubility of cisplatin and its approximate diffusivity in water. The rate-controlling mass transfer resistance was diffusion through the orifice, by design. The microdevices can be fabricated from any biocompatible moldable material. The microdevices used in this study are fabricated using poly-L-lactic acid (PLLA) with a molecular weight of 217 kDa from SurModics Inc (Eden Prairie, MN, USA). PLLA is known to degrade via bulk degradation where water is first absorbed, followed by swelling of the material before the material loses its integrity and disintegrates [17]. Visual observations upon microdevice explantation after the treatment period showed that there was no swelling of the material. All explanted microdevices had their caps removed in order to measure the amount of

drug remaining in the microdevices. All caps were intact. It was concluded that the PLLA microdevices do not lose mechanical integrity over the release period of 42 days *in vivo*.

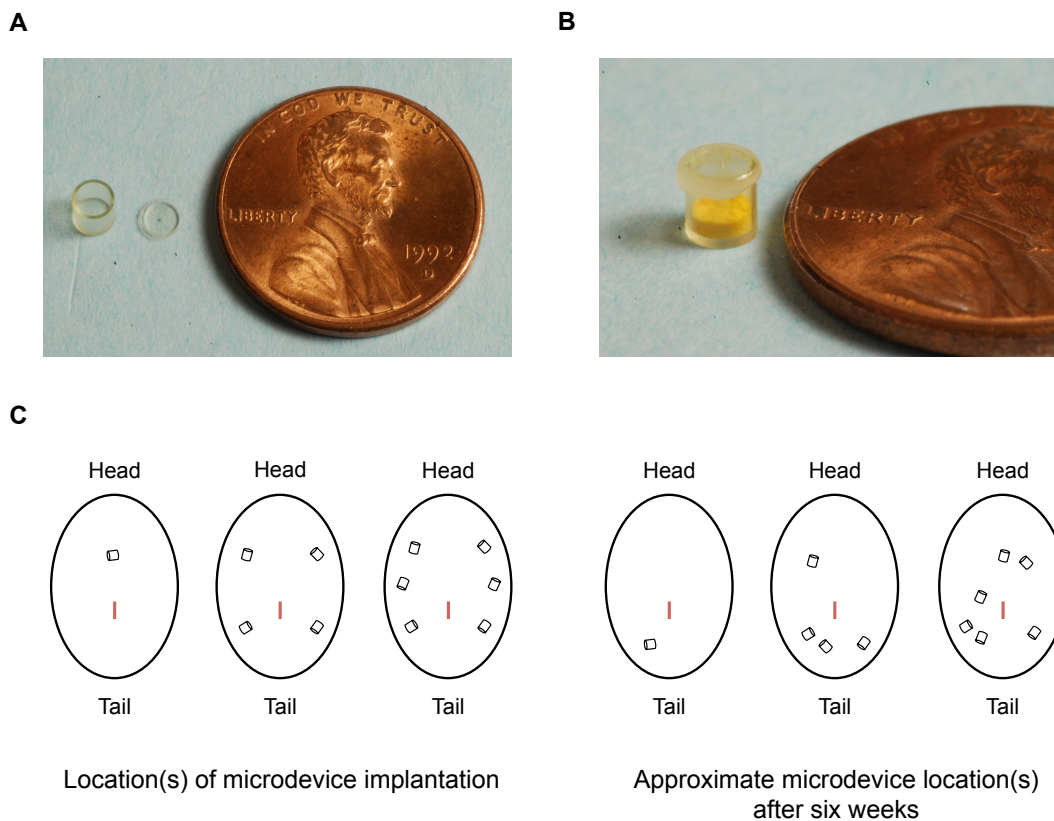


Fig. 1. Microdevice scale, preparation, and *in vivo* location at the beginning and end of treatment. (A) The microdevices consist of a cylindrical reservoir of approximately 3 mm in diameter, 3.5 mm in height, and 0.3 mm in thickness, and a cap of 3 mm in diameter and 400 μm in thickness. The cap contains a 180- μm orifice in the center to control the rate of drug release from the microdevice. A penny is included for scale. (B) The microdevices are filled with cisplatin powder and medical device epoxy is used to seal the cap-reservoir interface before implantation. (C) A schematic of the approximate microdevice location(s) in the peritoneal cavity is shown for 1 (left), 4 (middle), and 6 (right) microdevices following surgical implantation (left) and at the time of euthanasia (right). The black ellipse represents the mouse peritoneal cavity, and head and tail labels are included for orientation. The incision site is indicated with a red line. The microdevices were not fixed to the abdominal wall to minimize trauma to the surrounding tissues, so they migrated towards the pelvic region during the six-week treatment period.

2.4. Microdevice Preparation for *In Vitro* and *In Vivo* Studies

Each microdevice was loaded with 5 mg of cisplatin powder and the cap-reservoir interface was sealed using Loctite Hysol M-21HP Medical Device Epoxy Adhesive (McMaster-Carr, Elmhurst, IL, USA) (Fig. 1B). Cisplatin release was activated by placing the microdevices

in PBS solution under vacuum to replace the air in the microdevice with PBS and form a supersaturated solution within the reservoir.

2.5. *In Vitro Cytotoxicity*

The cisplatin-resistant cell lines SKOV3 (ATCC, Manassas, VA, USA) and OVCAR3 (ATCC) and the cisplatin-sensitive cell lines A2780 (Sigma-Aldrich) and UCI101 (Dr. G. Scott Rose, University of California, Irvine, CA, USA) were used. The OVCAR3 cell line is classified as cisplatin-resistant because it originates from a patient that exhibited platinum-resistance; however, these cells have demonstrated cisplatin sensitivity *in vitro* [18]. The cells were seeded in 96-well plates at a density of 10^4 cells/well and incubated in cell culture media with cisplatin concentrations of 0.1 $\mu\text{g/mL}$ to 10 $\mu\text{g/mL}$ over different time periods ranging from 2 hours to 7 days ($n = 16$ per concentration per time point). The UCI101 cell line has a doubling time of 24 hours, compared to the 48-hour doubling time of the other three cell lines, so the UCI101 study duration was halved to prevent cell overgrowth. The cisplatin-containing cell media was changed daily. An MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) was performed to quantify cell viability as a percentage of the control signal. The minimum efficacious AUC for each cell line was calculated by multiplying the lowest cisplatin concentration by the minimum number of days required to result in less than 5% cell viability. The range of efficacious AUC includes the minimum efficacious AUCs of all four ovarian cancer cell lines.

2.6. *In Vitro Microdevice Release*

The microdevices released drug into a PBS solution at 37°C, which was replaced by fresh PBS at various time points to maintain a constant sink condition around the microdevice. These PBS solutions were then assayed with HPLC to measure the mass of drug that had been released up until each time point. The cumulative mass of drug released was plotted versus time to obtain the *in vitro* release profile for the microdevice. *In vitro* release rate was estimated by a linear best-fit through the origin.

2.7. *Surgical and Euthanasia Procedures for In Vivo Experiments*

All listed procedures were in accordance with the Massachusetts Institute of Technology Committee on Animal Care and Massachusetts General Hospital Institutional Animal Care and Use Committee guidelines, as well as the NIH Guide for the Care and Use of Laboratory Animals. Animal surgeries to implant cisplatin-loaded microdevices were performed within a sterile field and all surgical equipment was autoclaved. Inhalation anesthesia was maintained throughout surgeries with isoflurane. Pre- and post-surgical analgesics were used as per recommendation by the animal care committees. The incision site was sterilized with iodine and isopropyl alcohol. A small abdominal incision of about 5mm was made through the skin and peritoneum to implant the microdevices. Microdevices were not fixed to the abdominal wall and were allowed to freely move within the peritoneal cavity, to minimize trauma to the surrounding tissues. The microdevices migrated towards the pelvic region during the course of treatment. A schematic is provided in Figure 1C to illustrate microdevice location(s) at the beginning and end of therapy. Mice were weighed daily to monitor overall health and euthanized upon weight loss greater than 20% or poor body condition. All animals were euthanized via carbon dioxide inhalation at a steadily increasing flow rate to minimize distress.

2.8. *In Vivo Microdevice Release and Pharmacokinetics*

Healthy, tumor-free female nu/nu nude mice of 25-30 g were divided into two groups: the microdevice group and the IP bolus injection (10 mg/kg) group. Microdevices were sterilized

with ethylene oxide prior to implantation. Animals were euthanized at various time points and peritoneal lavage and blood were harvested. Peritoneal lavage was obtained by injecting 1 mL of sterile saline into the peritoneal cavity and withdrawing the solution to collect the peritoneal fluid. Microdevice-bearing animals had their microdevices explanted after euthanasia. Lavage and serum samples were ultrafiltered to remove cisplatin bound to serum and peritoneal proteins (primarily albumin), in order to quantify the therapeutic, or unbound, cisplatin concentration. These samples were then assayed for cisplatin concentration using ICP-MS. The amount of drug remaining in each explanted microdevice at each time point was measured using HPLC. The mass of drug released by each microdevice was calculated by subtracting the mass of drug remaining from the mass originally loaded. The microdevice group time points were 2, 4, 7, 10, 14, 21, 35 and 42 days. The IP bolus injection group time points were 15 min, 30 min, 1 hour, 3 hours, 8 hours, 1 day and 7 days. The AUC for each group was calculated by the trapezoidal rule.

2.9. Orthotopic Tumor Models

An orthotopic tumor model with female nu/nu nude mice of 25-30 g (Charles River Laboratories, Wilmington, MA, USA) and SKOV3-Luc cells (Perkin Elmer, MA, USA) was used. SKOV3-Luc cells were trypsinized, centrifuged and re-suspended in sterile PBS. Each mouse was inoculated with 10^6 SKOV3-Luc cells on day 0, and the treatment (weekly 5 mg/kg IP bolus injection or microdevice implantation) began on day 14. The microdevices were sterilized with ethylene oxide prior to implantation. Animals were imaged twice weekly using the IVIS Spectrum Pre-Clinical In Vivo Imaging System from PerkinElmer. Animals receiving treatment were weighed daily throughout the course of the experiment.

An orthotopic tumor model with female BALB/c nude mice of 15-18 g (Charles River Laboratories) and UCI101 cells (Dr. G. Scott Rose) was used. UCI101 cells were trypsinized, centrifuged and re-suspended in sterile PBS. Each mouse was inoculated with 3×10^6 UCI101 cells on day 0, and treatment (weekly 4 mg/kg IP bolus injection, or 4 or 6 microdevices implanted per animal) began on day 21. Blood was sampled from the facial vein of all animals 48 hours after the 3rd and 6th IP bolus injection for CBC analysis with differential (Massachusetts General Hospital, Boston, MA, USA). Control animals were euthanized after the 3rd treatment cycle due to poor body condition, and 5 animals from each treatment arm were euthanized at the same time point for tumor burden comparison. All other animals were euthanized following the 6th treatment cycle.

2.10. Statistical Analysis

A one-way ANOVA test with a Tukey-Kramer multiple comparisons test (InStat GraphPad Software, San Diego, CA, USA) was used to compare the means of the following data sets: normalized bioluminescence intensity (BLI), tumor mass, percentage weight loss, and CBC. The reported *p* values describe comparisons among the control, weekly IP bolus injection, and microdevice groups. Reported results are expressed as mean \pm standard error of the mean.

3. Results

3.1. Design and Testing of the Microdevice for Drug Delivery

An implantable device was conceived to replace the current technology for IP chemotherapy delivery in ovarian cancer (Supplementary Fig. S1). The device will continuously release the drug payload for the complete course of therapy via passive diffusion. The optimal continuous dose that maximizes tumor kill and minimizes toxicity will be scaled up from experiments in ovarian cancer mouse models using a standard conversion equation [19]. A microdevice for mice was designed to continuously deliver drug over a prolonged period through

a circular orifice on its cap (Fig. 1A, B). Feasibility of surgical implantation in the peritoneal cavity limits the size of a peritoneal implant. The microdevice design maximizes drug load per unit volume, to better recapitulate this aspect of the human device. Loading the microdevice with drug in powder form – as opposed to drug in solution – minimizes the required size of the drug reservoir and prolongs the stability of drugs that degrade quickly in solution. The microdevice is diffusion-controlled, and the area of the orifice is directly proportional to the rate of release of agents as predicted by Fick's first law:

$$\dot{m} = - \frac{ADC_s}{\Delta x} \quad (1)$$

where \dot{m} is the mass diffusion rate (mass per unit time), A is the area of the release orifice, D is the diffusion coefficient of the drug in the diffusion medium, C_s is the solubility of the drug, and Δx is the diffusion length, assumed to be the depth of the orifice. The human device will similarly be designed for diffusion-controlled release. It can be made of a semipermeable material for drug dissolution by aqueous peritoneal fluid, and lined with orifices for release of the drug solution to the peritoneal cavity according to Fick's first law (Supplementary Fig. S1) (Eq. 1). The microdevice is designed to contain an excess of drug. This maintains a saturated drug solution within the microdevice, achieving a constant diffusion gradient and continuous, zero-order release.

3.2. *In Vitro* Cytotoxicity

The platinum sensitivity of various ovarian cancer cell lines was first quantified under the expected conditions, in order to employ an IP drug delivery device. The cytotoxicity curves (Fig. 2) demonstrate that these cell lines are sensitive to an overall AUC range of 500 ng-day/mL to 2500 ng-day/mL. This is consistent with what is reported in the literature [20].

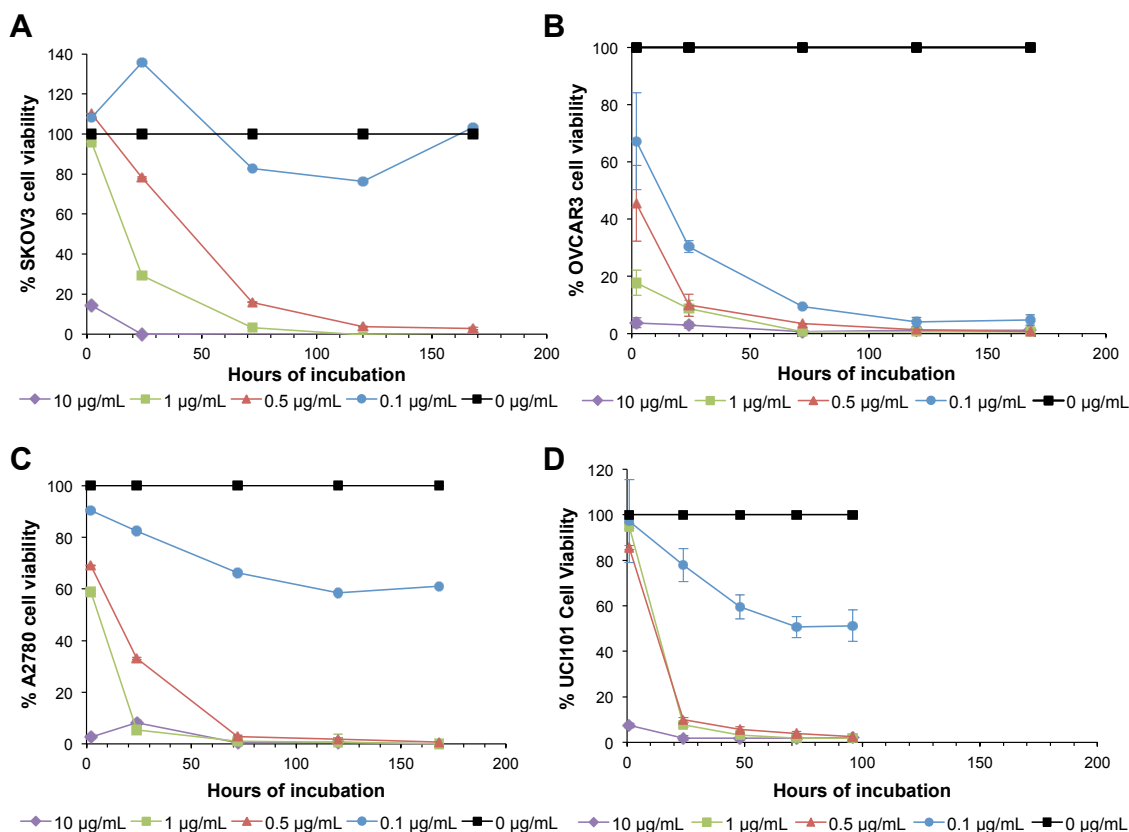


Fig. 2. *In vitro* cytotoxicity assay with continuous cisplatin exposure. The viability of four human ovarian cancer cell lines after treatment with various cisplatin concentrations was examined over 4 to 7 days: (A) SKOV3, (B) OVCAR3, (C) A2780, and (D) UCI101. The UCI101 cell line has a doubling time of 24 hours, compared to the 48-hour doubling time of the other three cell lines, so the UCI101 study duration was halved to prevent cell overgrowth. The three plots demonstrate the percentage viability of the cancer cells in comparison to the untreated controls after various durations of cisplatin treatment. A minimum cisplatin concentration of 0.1 µg/mL - 0.5 µg/mL maintained over 3 to 5 days was required to eliminate all types of ovarian cancer cells.

3.3. *In Vitro* and *In Vivo* Release of Cisplatin Using the IP Microdevice

Figure 3A shows that the microdevice is able to release cisplatin at 37°C *in vitro* in a linear and reproducible fashion, without an initial burst ($r^2 = 0.996$, $n = 3$). The average release rate is 1.3 µg/hour. The microdevice was implanted surgically in the peritoneal cavity of mice to determine the release profile of cisplatin *in vivo*. The microdevice is able to release cisplatin continuously throughout the period of 42 days *in vivo* with a linear profile (Fig. 3B, $r^2 = 0.965$, $n = 3$). No degradation products were observed in these samples, indicating that drug stability and device functionality were preserved throughout the treatment period. The *in vivo* release rate of 1.0 µg/hour, or 172.2 µg/wk, is similar to the *in vitro* release rate of 1.3 µg/hour. Approximately 20% of the initial 5 mg microdevice payload was released from each microdevice *in vivo*.

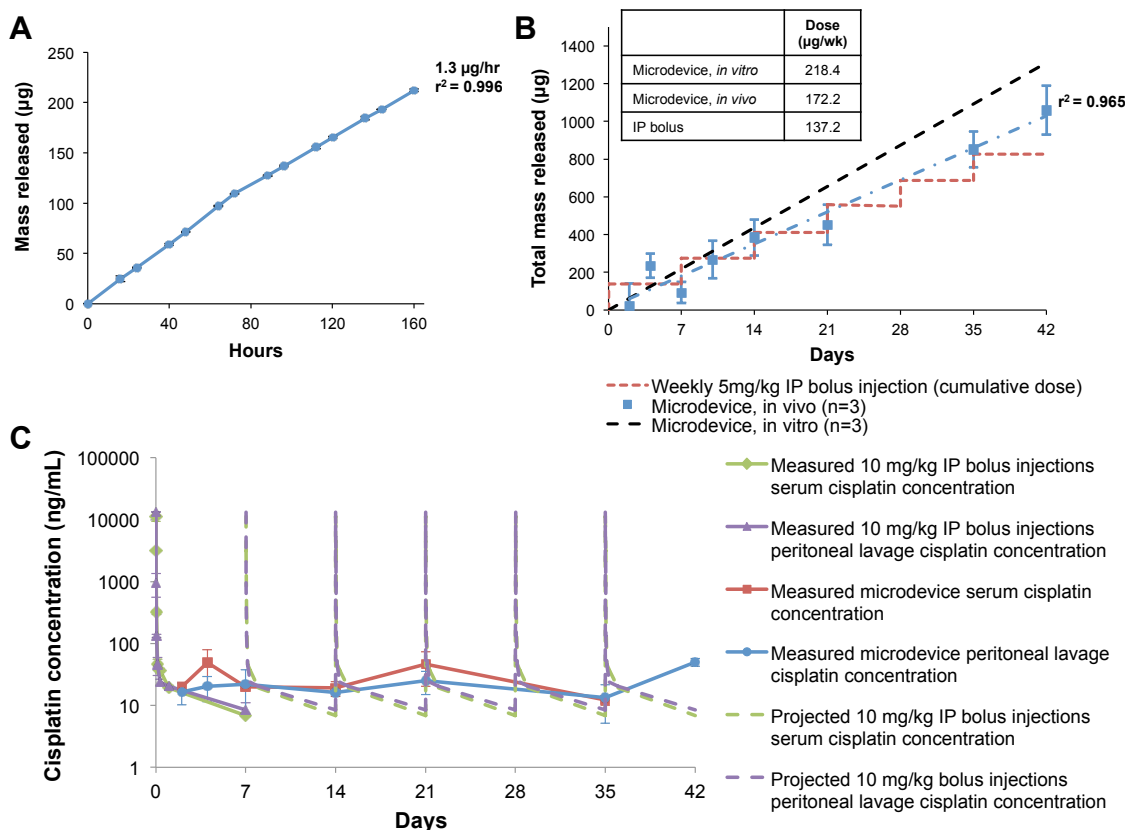


Fig. 3. Characterization of the microdevice *in vitro* and *in vivo* in comparison to bolus injections. (A) The *in vitro* release profile of the microdevice at 37°C in PBS is linear with a release rate of 1.3 $\mu\text{g/hr}$ ($n = 3$, $r^2 = 0.996$). (B) The microdevice was able to release cisplatin consistently *in vivo* throughout the period of 42 days with a linear profile ($n = 3$, $r^2 = 0.965$). The *in vivo* microdevice release rate of approximately 1.0 $\mu\text{g/hr}$ (172.2 $\mu\text{g/wk}$) was very similar to its *in vitro* release rate of 1.3 $\mu\text{g/hr}$ shown in (A). The microdevice also has a similar release profile as cumulative IP bolus injections at a dose of 5 mg/kg (represented here as a step function) over the same period of time. (C) Cisplatin concentration in the serum and peritoneal lavage samples over 42 days ($n = 3$). The IP bolus injections resulted in spikes of cisplatin in the serum and lavage while the microdevice demonstrated continuous release over the entire 42 days, maintaining a relatively constant concentration of 20 ng/mL in the serum and peritoneal cavity. The y-axis shown is on a logarithmic scale.

3.4. Pharmacokinetic Comparison of an IP Bolus Injection versus the IP Microdevice

A pharmacokinetic study was conducted to quantify the *in vivo* drug concentration in the serum and peritoneal cavity after either an IP bolus injection of 10 mg/kg or the IP implantation of a microdevice (Fig. 3C). An IP injection of cisplatin in mice caused a sharp spike in the serum cisplatin concentration 15 min after injection, with a measured C_{max} of 11,400 ng/mL. The peritoneal lavage concentration at 15 min was 13,300 ng/mL. This concentration decreased to 133 ng/mL at 1 hr post-injection. The serum and lavage cisplatin concentrations were found to be 6.85 ng/mL and 8.46 ng/mL respectively by 7 days post-injection. IP cisplatin injections of 5 mg/kg were administered weekly for the *in vivo* treatment efficacy and toxicity study because

their effective dose was comparable to that of the microdevice. The concentrations achieved in the pharmacokinetics study, where 10 mg/kg injections were administered, were halved in order to directly compare the pharmacokinetics profiles of weekly IP bolus injections (5 mg/kg) and the microdevice. It is assumed that halving the IP injection dose in turn halves the *in vivo* cisplatin concentrations, and AUC was calculated using this adjusted pharmacokinetic profile (Table 1).

Table 1. AUC and C_{max} comparison for microdevice and IP bolus injections. AUC values for peritoneal lavage and peritoneal fluid and C_{max} of cisplatin in the peritoneal cavity over the treatment period of 42 days for the two dosing regimens, IP microdevice implantation and weekly 5 mg/kg cisplatin IP bolus injections.

	Microdevice group	IP bolus injection group
Peritoneal lavage AUC over the 42 day treatment period	871 ng-day/mL	605 ng-day/mL
Actual peritoneal AUC over the 42 day treatment period	3,049 ng-day/mL	2,118 ng-day/mL
Measured cisplatin peak concentration in peritoneal lavage (C_{max})	No cisplatin concentration peak	6,500 ng/mL once every week

The IP microdevice is capable of maintaining a relatively constant cisplatin concentration of 20 ng/mL in the mouse peritoneal cavity with a peritoneal lavage AUC of 871 ng-day/mL. The peritoneal lavage AUC was calculated to be 605 ng-day/mL for an IP cisplatin injection regimen of 5 mg/kg weekly for 6 doses (42 days in total). The 5 mg/kg IP bolus injection resulted in a cisplatin C_{max} of 6,500 ng/mL, compared to the relatively constant 20 ng/mL delivered by the microdevice (Table 1).

Peritoneal lavage samples were obtained as a dilution of the mouse peritoneal fluid, and the cisplatin concentration in the lavage therefore underestimates the actual peritoneal value. A separate experiment was performed to measure the average volume of peritoneal fluid in a mouse. The results showed that the peritoneal fluid volume in nu/nu mice weighing 25 – 30 g is 0.45 ± 0.19 mL. The actual peritoneal fluid AUCs for both groups are therefore likely to be 2.5 – 4.8 times higher than the lavage AUCs. Using 3.5 as an average multiplication factor, the actual peritoneal AUC was approximated as 3,049 ng-day/mL for the microdevice group and 2,118 ng-day/mL for the IP bolus injection group. Table 1 displays the approximated peritoneal AUC after accounting for the dilution in the lavage samples.

3.5. *In Vivo Treatment Efficacy and Toxicity*

The extent of tumor burden reduction following intermittent injections or continuous dosing was first evaluated in a platinum-resistant model of ovarian cancer. Treatment efficacy was quantified using BLI and gross tumor mass at euthanasia in an orthotopic SKOV3 ovarian

cancer animal model. This model faithfully recapitulates the human disease with early intra-abdominal spread of disease [21]. The animal groups included control (no treatment) ($n = 14$), weekly 5 mg/kg IP bolus injection ($n = 19$), and a single implanted microdevice ($n = 9$). BLI normalized to its value at the start of treatment approximates the relative tumor growth after the start of treatment on day 14 following SKOV3 cell inoculation. The normalized BLI in Fig. 4A shows that both the IP bolus injection group and the microdevice group suppressed tumor growth significantly relative to the untreated control group. The normalized BLI for the control group on day 56 was 31.9, while those of the IP bolus injection and microdevice groups were 1.39 ($p < 0.001$, compared to control) and 4.64 ($p < 0.05$, compared to control) respectively. There was no statistical difference between the normalized BLI of the two treated groups ($p > 0.05$). Fig. 4B shows that the untreated control group had an average tumor burden of 249 mg by day 56, while the IP bolus injection and microdevice groups had an average tumor burden of 13.1 mg ($p < 0.001$) and 59.5 mg ($p < 0.01$) respectively. Treatment with IP injections and an implanted microdevice decreased both the size and number of tumor nodules compared to those of the untreated control animals. There was no significant difference between the tumor masses of the two treated groups ($p > 0.05$).

The percentage body weight loss plotted over time reflects the overall health condition of the mice as an estimate for treatment toxicity (Fig. 4C). The control animals gained weight over the course of the experiment. The IP bolus injection group underwent temporary weight losses of at least 4% after each IP bolus dose on days 14, 21, 28, 35, 42 and 49. The animals in the IP bolus group had significant percentage weight loss compared to the control and microdevice groups by the end of the treatment period ($p < 0.001$ and $p < 0.05$ respectively), with a final average loss of 7.9%. The body weights of the animals from the microdevice group were relatively stable during the entire course of treatment, with no significant difference from those of the control group at day 56.

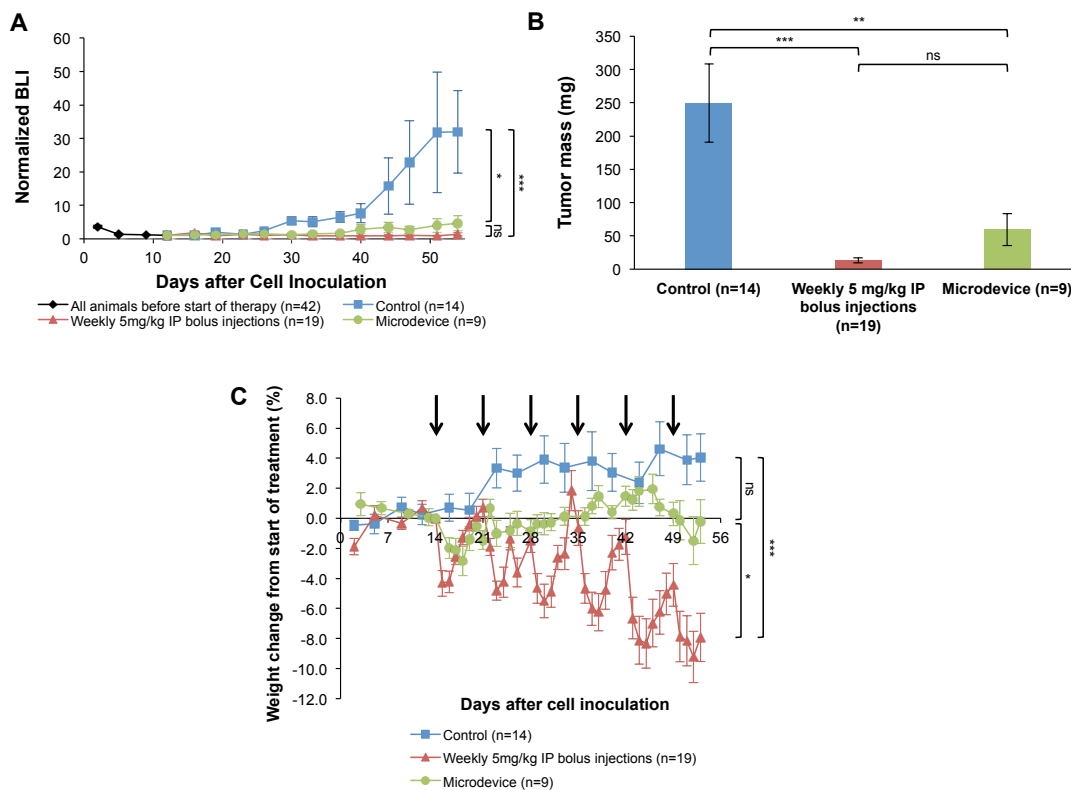


Fig. 4. Treatment efficacy and toxicity of the microdevice and bolus injections in an SKOV3 ovarian cancer mouse model. (A) The microdevice group showed comparable BLI signal to the weekly 5 mg/kg IP bolus treatment group at the end of 56 days (not statistically different, $p > 0.05$). Both the microdevice and IP bolus treatment groups demonstrated statistically significant reductions in BLI signal as compared to the control group ($p < 0.05$ and $p < 0.001$ respectively). (B) The control group had significantly more tumor mass than both the IP bolus ($p < 0.001$) and microdevice ($p < 0.01$) groups. The arithmetic mean tumor mass was different for the microdevice and IP groups but the difference was not statistically significant ($p > 0.05$). (C) The IP bolus animals lost weight in cycles corresponding to the cisplatin doses (arrows). There was greater weight loss in the IP bolus group compared to both the control ($p < 0.001$) and microdevice ($p < 0.05$) groups by day 56. Significance is indicated as follows: ns $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Higher continuous doses were then administered via multiple microdevices to evaluate whether tumor burden could be further reduced with less resulting toxicity than the standard of care (Fig. 5). A platinum-sensitive orthotopic UCI101 ovarian cancer animal model was used to broaden the applicability of our conclusions. The animal groups included control (no treatment) ($n = 5$), weekly 4 mg/kg IP bolus injections ($n = 14$), 4 implanted microdevices ($n = 14$), and 6 implanted microdevices ($n = 12$). The IP bolus dose was reduced from 5 mg/kg to 4 mg/kg, because it was the maximum tolerated dose in this animal model. Fig. 5A shows that the untreated control group had an average tumor burden of 4.74 g after 3 weeks from the start of treatment, which was significantly higher than that of the IP bolus injection (1.69 g, $p < 0.001$), 4-microdevice (1.89 g, $p < 0.001$), and 6-microdevice groups (1.35 g, $p < 0.001$) at this time point. Treatment with IP injections and 4 and 6 implanted microdevices decreased both the size

and number of tumor nodules compared to those of the untreated control animals. The average tumor mass in the IP bolus injection group (0.72 g) was significantly lower than that of the 4-microdevice group after 6 weeks of treatment (1.18 g, $p < 0.001$), but there was no difference in tumor mass between the IP bolus injection and 6-microdevice groups (0.72 g, $p > 0.05$).

CBC was evaluated as a more sensitive metric of treatment-induced toxicity, in addition to weight loss. Both IP bolus injections and treatment with 4 and 6 microdevices caused significant weight loss in comparison to the untreated control animals by the end of the third week of therapy, but IP therapy induced significantly more weight loss than both microdevice treatments by the end of 6 weeks (Supplementary Fig. S2). A portion of this weight loss can be attributed to tumor shrinkage with cisplatin treatment, due to the substantial tumor burden in the UCI101 animal model, so weight loss was a less accurate predictor of systemic toxicity in this case. There was, however, a significantly lower degree of myelosuppression in the animals that received treatment with microdevices than those that received IP bolus injections. White blood cells (WBC) were the only cell population that showed significant depletion in response to chemotherapy. The IP bolus group had a statistically lower average percentage of WBC relative to the number of cells before treatment (47.5%) than the 4-microdevice (63.7%, $p < 0.001$) and 6-microdevice (58.1%, $p < 0.01$) groups after 6 weeks of treatment (Fig. 5B).

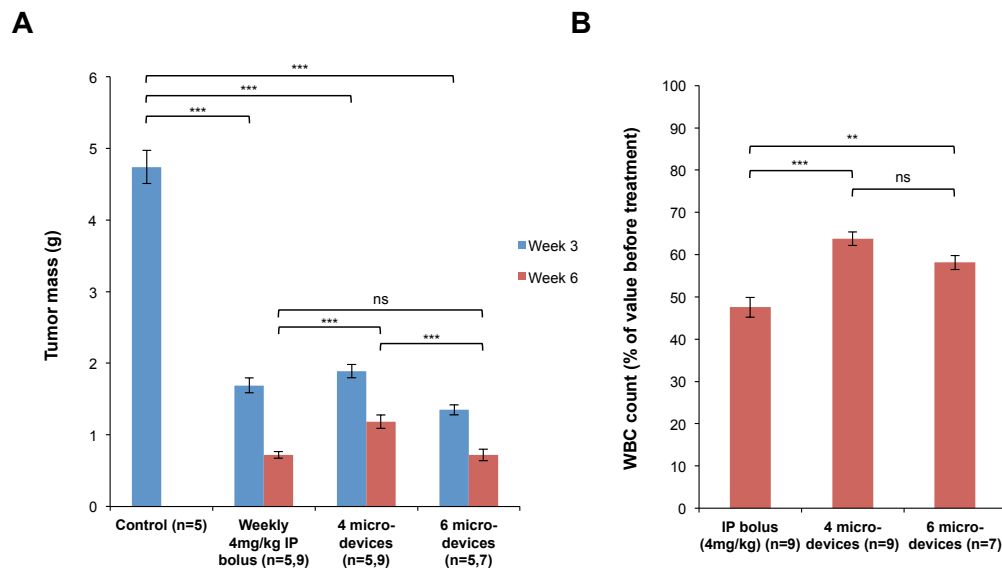


Fig. 5. Treatment efficacy and toxicity of multiple microdevices and bolus injections in a UCI101 ovarian cancer mouse model. (A) The control group had significantly more tumor mass than the IP bolus ($p < 0.001$), 4-microdevice ($p < 0.001$), and 6-microdevice ($p < 0.001$) groups after 3 weeks of treatment. The 4-microdevice group had significantly more tumor mass than the IP bolus ($p < 0.001$) and the 6-microdevice ($p < 0.001$) groups after 6 weeks of treatment, but the arithmetic mean tumor masses in the IP bolus and 6-microdevice groups were the same ($p > 0.05$). (B) The IP bolus group had a significantly lower average percentage of WBC relative to the number of cells before treatment (47.5%) than that of the 4-microdevice (63.7%, $p < 0.001$), and 6-microdevice (58.1%, $p < 0.01$) groups after 6 weeks of treatment. There was no statistical difference between the degree of WBC depletion in the 4 and 6 microdevice groups ($p > 0.05$). Significance is indicated as follows: ns $p > 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

4. Discussion

The goal of this work was to develop an IP chemotherapy regimen for ovarian cancer that is at least as effective as, but less toxic than, the current standard of care. A drug-release microdevice for mice was designed and used to compare the efficacy and toxicity of low but prolonged IP cisplatin exposure (low C_{\max}) to the standard of care of periodic IP cisplatin spikes (high C_{\max}). A single compartment drug delivery device has been previously shown by our group to effectively deliver temozolomide at a constant rate intracranially for gliosarcoma treatment in rat models, and a device designed by our group to deliver drugs to the bladder is being evaluated in humans [22, 23].

It is hypothesized that continuous delivery enhances the efficacy of chemotherapy by limiting treatment-free periods, during which clones of resistant tumor cells have time to grow [13, 24]. De Souza *et al.* demonstrated improved therapeutic efficacy of continuous IP docetaxel delivery *in vivo* in murine ovarian cancer models. The drug was contained in an injectable biodegradable chitosan egg-phosphatidylcholine (PoLi_{gel}) matrix developed by the authors, which was injected in the peritoneal cavity [11]. The matrix had a final drug to material ratio of approximately 1:8 w/w. This kind of drug-polymer composite is not tenable for human dosing because of the large volume assumed by the polymeric matrix. De Souza *et al.* injected a 30 μ L depot in each mouse to deliver docetaxel over a 3-week period, which would scale to a 100 mL depot in a 70 kg human. This volume is an underestimate because the standard clinical chemotherapy regimen is 18 weeks (6 infusions separated by 3-week recovery periods). The viscosity of the docetaxel-PoLi_{gel} was also shown to increase with drug payload, so it is unlikely that such a large depot could feasibly be injected into the IP space [25]. Previous formulations of the PoLi_{gel} in the form of a surgically implantable film, as in the work of Vassileva *et al.*, face similar issues of scalability [15]. A 10x10 mm² film was used to deliver 4.5 mg of paclitaxel in each mouse. The film would have to be more than 3x3 m² to deliver the maximum tolerated dose of 280 mg/kg/wk in humans over a 3-week period. Zhidkov *et al.* demonstrated the efficacy of continuous carboplatin delivery *in vivo* in murine ovarian cancer models, as compared to intermittent bolus injections. Carboplatin has not been approved for IP chemotherapy and has exhibited significantly less tumor penetration than cisplatin *in vivo* [26, 27]. Zhidkov *et al.* also limited the duration of treatment to 2 weeks [13]. Our studies presented here spanned 6 weeks to allow administration of 6 cycles of bolus therapy as per the current standard of care. Our microdevice maintains constant cisplatin release for the full 6-week duration. The size of the commercially available osmotic pump used by Zhidkov *et al.* (roughly 19x the volume of our microdevice) may cause significant morbidity to the animals if the treatment period is extended. Any implant-generated fibrous capsule can take at least 4 weeks to develop [28]. This may affect the *in vivo* release of drug from the pump, which will likely be compromised if treatment is administered for a longer period. Zhidkov *et al.* evaluated drug-induced toxicity using total body weight analysis [13]. Our studies evaluated CBC in addition to body weight for a more thorough toxicity assessment. Zhidkov *et al.*, finally, limited their studies to a platinum-resistant model. Our data demonstrate a treatment advantage with continuous release in both platinum-sensitive and platinum-resistant models of ovarian cancer.

An *in vitro* cytotoxicity study demonstrated that continuous exposure to cisplatin at a low concentration was as toxic to the ovarian cancer cells as short exposure to cisplatin at a high concentration (Fig. 2). A therapeutic AUC range of 500 ng-day/mL to 2,500 ng-day/mL was elucidated for four types of ovarian cancer cells, including both cisplatin-resistant and cisplatin-sensitive cell lines. The microdevice maintained a constant IP cisplatin concentration of 20

ng/mL over 42 days, with a minimal C_{max} (Fig. 3C). This concentration resulted in a peritoneal AUC of 3,049 ng-day/mL (Table 1); the 5 mg/kg IP bolus injection dosing regimen with a weekly C_{max} of 6,500 ng/mL measured in the peritoneal lavage resulted in a peritoneal AUC of 2,118 ng-day/mL. These doses were expected to cause significant tumor kill based on the range of cytotoxic AUCs determined in our *in vitro* assay. It is certain that there are substantial differences between the required therapeutic doses *in vitro* and *in vivo*, most likely requiring higher concentrations *in vivo* because drug is continuously cleared from the peritoneal cavity.

The *in vivo* studies demonstrate that the IP microdevice and weekly IP bolus injection treatment regimens are equally effective in orthotopic animal models of the disease, but that continuous dosing with IP microdevices results in significantly less toxicity. Implanted microdevices were allowed to move freely within the peritoneal cavity during treatment. They did not cause any visible damage on gross inspection, and there was no intestinal obstruction, compression of IP organs, signs of inflammation, or scar tissue formation at euthanasia. The results of the first efficacy study illustrate that when a comparable peritoneal AUC is administered, treatment outcomes are not significantly different following intermittent and continuous dosing. The IP microdevice and IP bolus injection groups in the SKOV3 xenograft model showed similar treatment efficacy outcomes in terms of both normalized BLI and tumor mass at the end of treatment (Fig. 4A and Fig. 4B), despite the vastly different C_{max} (the microdevice does not produce any significant cisplatin concentration peaks, Fig. 3C). The results support that delivering continuous, low-dose cisplatin over a prolonged period of time has benefits over IP bolus cisplatin injections by reducing the degree of treatment-induced toxicity. The study performed in the UCI101 xenograft model demonstrates that administration of higher continuous doses further reduces tumor mass to *match* the efficacy of bolus therapy (Fig. 5A, comparing 6 implanted microdevices to 4 mg/kg IP bolus injections). These results indicate that drug administered via microdevices penetrates into tumor tissue to the same degree as drug injected intraperitoneally. Multiple toxicity endpoints were used in the two studies to evaluate the ability of continuous dosing to reduce chemotherapy-induced side effects. Previous experiments revealed that IP injections of cisplatin at the administered doses do not induce significant nephrotoxicity, according to examination of H&E stained kidney sections by a trained pathologist and serum creatinine levels. Nephrotoxicity was, therefore, not used in these studies to evaluate cisplatin-induced side effects. Grade 3 or 4 adverse renal or genitourinary events were also only observed in 7% of patients receiving IP therapy in the GOG-172 clinical trial, whereas 76% of patients experienced high-grade leukopenia [7]. Overall weight loss (Fig. 4C) was significantly reduced in the microdevice arm of the SKOV3 study, in comparison to the degree of weight loss observed in the IP bolus arm of the study. The weight loss observed in the microdevice arms of the UCI101 study was more drastic than in the SKOV3 study (Supplementary Fig. S2), because administered cisplatin doses were much higher than those of the single microdevice and tumor shrinkage contributed to total weight loss. WBC depletion (Fig. 5B) was, however, significantly reduced following continuous drug administration in comparison to bolus injections. The lower toxicity associated with the microdevices demonstrates that continuous drug delivery limits drug concentrations in the serum compared to IP injections, despite achieving equivalent tumor kill. These results highlight the advantage of a continuous dosing regimen.

Translation of the proof-of-concept microdevice into a human device will address design and functionality limitations to ensure clinical viability. These include: choice of device configuration and material for laparoscopic deployment and patient safety; drug payload and

release rate for antitumor efficacy in patients; and *in vivo* validation of the human devices in large animals. The human device can be a fully implanted tubular reservoir similar to existing peritoneal catheters. The device will be sized to allow deployment and retrieval through a laparoscopic port. The PLLA microdevices presented in this manuscript were free-floating and migrated towards the mouse pelvic region over the course of treatment (Fig. 1C). This migration along with drug clearance from the peritoneal cavity can lead to nonuniform drug distribution. Adequate intracavitary drug distribution is necessary to treat distant tumor nodules such as subdiaphragmatic metastases. Strategies for uniform drug distribution include (i) design of the device as a long tube to maximize its reach within the peritoneal cavity and (ii) implantation of multiple smaller devices localized to different cavity quadrants. Fixation of the human device to the abdominal wall can prevent unwanted migration, similar to laparoscopic internal fixation of peritoneal dialysis catheters to the abdominal rectus muscle [29, 30].

The device will require a single implantation for the complete course of treatment. Agents such as cisplatin with high radiopacity will enable non-invasive monitoring of such a device with x-rays or CT scans to minimize complications and aid in surgical device removal. Device retrieval will occur following the end of treatment or any adverse reaction. Safe retrieval necessitates use of nonresorbable materials to maintain the mechanical integrity of the device. A nonresorbable flexible polymer such as medical-grade silicone elastomer – widely used for peritoneal dialysis catheters – will minimize damage to surrounding tissues and formation of adhesions [31, 32]. The human device will contain a payload sufficient to administer drug over 18 weeks, recapitulating the current standard of care. An excess of drug will be loaded in the device to maintain a saturated drug solution throughout the treatment period. The length of a tubular device is estimated to be of the order of 30-60 cm (or 1-2 feet), to contain the required drug payload calculated from the mouse microdevice studies using a standard conversion equation [19]. If a single long device cannot achieve uniform drug distribution, a maximum of three smaller devices will be implanted in the peritoneal cavity. The size and limited number of the implanted device(s) will allow their removal at the end of therapy and prevent any permanent device accumulation. Safety of the human device will be confirmed in large animals.

5. Conclusions

Treatment approaches with minimized cisplatin concentration peaks are herein shown to reduce drug-related toxicities while achieving significant tumor kill. The mouse models included here provided anatomically relevant platforms with which to demonstrate that a better ovarian cancer treatment outcome can be attained with a device for continuous drug delivery compared to the currently administered IP bolus injections. Administering higher cisplatin doses over the 42-day treatment period can result in improved efficacy over IP bolus therapy with less severe side effects. These promising preclinical results highlight the potential for this novel IP dosing regimen to improve the treatment of late-stage ovarian cancer and set the stage for development of the proposed human device for implementation in patients.

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