In Vitro Binding Kinetics of ChemoFilter with Cisplatin

by

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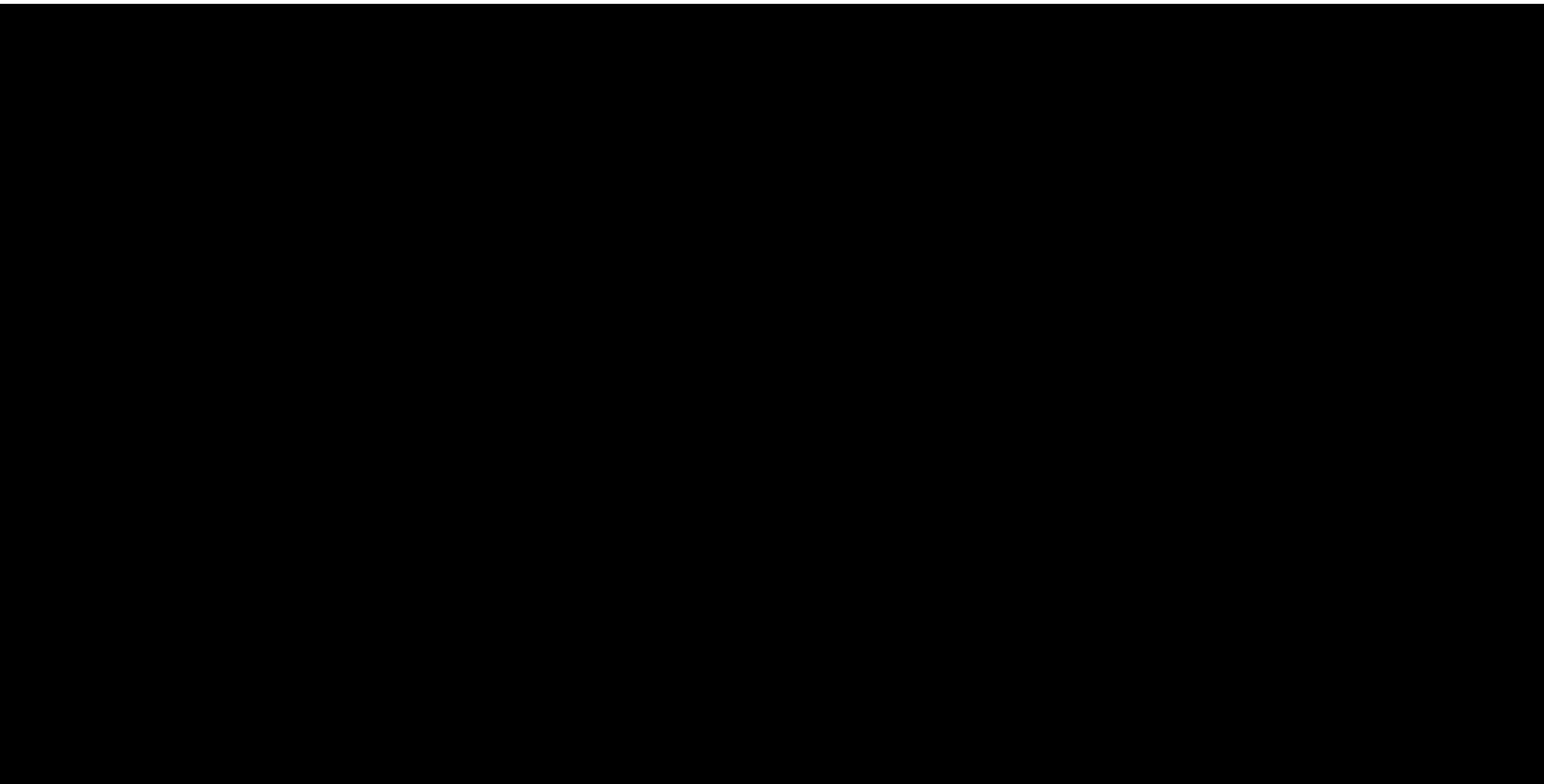
Biomedical Imaging

in the

GRADUATE DIVISION

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Abstract

Introduction: Endovascular chemotherapy treatment allows localized delivery adjacent to the target tumor; allowing an increased dosage and decreased leakage to other areas. It also allows for the opportunity to filter chemotherapy escaping the target tumor and entering the bloodstream. The ChemoFilter - a temporarily deployable, endovascular device will do just that; reducing systemic toxicity thus reducing adverse side effects from chemotherapy treatment. This will allow further increased dosage, and increased tumor suppression, along with increased tolerance to treatment. ChemoFilter has successfully filtered the chemotherapeutic Doxorubicin, but had yet to be tested in other chemotherapeutics. This study evaluates binding with new chemotherapeutics: Cisplatin, Carboplatin, and a cocktail comprised of Cisplatin and Doxorubicin.

Materials and Methods: ChemoFilter prototypes based on: 1.) Genomic DNA and 2.) Dowex (ion-exchange) resin, were evaluated for their ability to bind chemotherapy in vitro in phosphate-buffered saline (PBS). ChemoFilter was tested free in solution and encapsulated in nylon or polyester mesh packets of various dimensions. Concentrations were quantified using inductively coupled plasma mass spectrometry (IPC-MS), ultraviolet-visible spectrophotometry (UV-Vis), or fluorospectrometry. The process began of ^{11}C, ^{13}C, and/or ^{14}C radiolabeling Carboplatin for in vitro and in vivo ChemoFilter quantification. In vitro quantification can include scintillation and/or gamma counting. In vivo may include Positron Emission

Tomography (PET) imaging, Hyperpolarized ^{13}C Magnetic Resonance Imaging (MRI), and/or Magnetic Resonance Spectroscopy (MRS) for real-time visualization and metabolic studies. Reactions were verified using High Performance Liquid Chromatography (HPLC) for chemical species identification.

Results and Discussion: Results indicate significant and nearly complete, ~99% ($p<0.01$) clearance of Cisplatin using the DNA ChemoFilter sequestered in Nylon mesh, quantified with gold standard ICP-MS (evidenced at 214 nm and 265 nm). The Ion-exchange ChemoFilter has significant clearance, within seconds, of both Doxorubicin and Cisplatin mixed in a cocktail solution. However, it appears some Cisplatin is binding to the Nylon Mesh itself. Size, shape, and material of the mesh have been optimized. A potential mechanism for ^{11}C, ^{13}C, or ^{14}C radiolabeling of Carboplatin has been developed and early results have been successful. ChemoFilter works much more efficiently when sequestered in nylon packets of specific geometries. Significant improvements have been made to ChemoFilter, moving the device closer to further analysis via clinical trials.

TABLE OF CONTENTS

Introduction.....1

Materials and Methods.....8

Results.....13

Discussion 21

Conclusions.....27

References.....28

LIST OF TABLES

Table 1. Cisplatin Concentration Over Time With 50 mg Free DNA ChemoFilter, ICP-MS Results @ 214 nm ……..…14

Table 2. Cisplatin Concentration Over Time With 50 mg Free DNA ChemoFilter, ICP-MS Results @ 214 nm ……..……14

Table 3. Statistics - 50 mg DNA with Cisplatin (ICP-MS) 214 nm……..15

Table 4. Statistics - 50 mg DNA with Cisplatin (ICP-MS) 265 nm……..15

Table 5. Cisplatin Concentration Over Time With 100 mg Free DNA ChemoFilter, ICP-MS Results @ 214 nm …….……16

Table 6. Cisplatin Concentration Over Time With 100 mg Free DNA ChemoFilter, ICP-MS Results @ 265 nm …….…….….16

Table 7. Statistics - 100 mg DNA with Cisplatin (ICP-MS) 214 nm……16

Table 8. Statistics - 50 mg DNA with Cisplatin (ICP-MS) 265 nm…….....16

Table 9. Cisplatin Concentration Over Time With Sequestered DNA ChemoFilter, ICP-MS Results @ 214 nm …….....17

Table 10. Cisplatin Concentration Over Time With Sequestered DNA ChemoFilter, ICP-MS Results @ 265 nm.…….……...…..….17

Table 11. Statistics - Sequestered DNA with Cisplatin (ICP-MS) 214 nm……..….18

Table 12. Statistics - Sequestered DNA with Cisplatin (ICP-MS) 265 nm……...18

Table 13. Cisplatin Binding Nylon…...…...……..….20

LIST OF FIGURES

Figure 1. DNA ChemoFilter Binding Doxorubicin…..……3

Figure 2. Cisplatin-DNA Mechanism of Action…….....3

Figure 3. ChemoFilter Placement for (A) Liver Cancer(B) Head/Neck Cancer…….....4

Figure 4.Cisplatin & Carboplatin Molecular Structures……...6

Figure 5. ^{11}C-Decay Scheme……7

Figure 6. Genomic DNA with Beads Immobilized in Nylon Packet……..……9

Figure 7. [Cisplatin] Quantification "OPDA Method" …….....10

Figure 8. Mesh “Tea Bag” with Dowex Resin (orange)……11

Figure 9. Cisplatin → Carboplatin Mechanism……..12

Figure 10. Proof of Concept-DNA to filter Cisplatin with ICP-MS…….....13

Figure 11. 50 mg Free DNA w/ Cis ICP-MS data @ 214 nm & 265 nm……...14

Figure 12. 100 mg Free DNA w/ Cis ICP-MS data @ 214 nm & 265 nm…….15

Figure 13. 50 mg Sequestered DNA w/ Cis ICP-MS data @ 214 nm & 265 nm.....17

Figure 14. Doxorubicin and Cisplatin filtration via Ion-Exchange……...18

Figure 15. Mesh Material Optimization (A) Cisplatin (B) Doxorubicin…….……..…...19

Figure 16. Mesh Dimension Optimization (A) Cisplatin (B) Doxorubicin.....20

Figure 17. Chemotherapy cocktail concentration over 30 seconds 21

Figure 18. “Carboplatin” product HPLC 21

Figure 19. Flow model setup ……25

Figure 20. Nanotruss ChemoFilter Design……26

Introduction

Chemotherapy is an effective treatment for many types of cancers (1,2,3). It often has high success rates and affords patients a better quality of life than current alternatives (1,2,3). However, the use of chemotherapeutics is limited due to their systemic toxicity (1,2,3). Endovascular chemotherapy treatment allows localized chemotherapy delivery released adjacent to the target tumor. This more effective means of tumor suppression allows for higher doses and less systemic toxicity since there is less chemotherapy escaping the area and entering the blood stream (1,2,3,4).

Despite these advancements, the side effects of chemotherapy are still a significant medical and public health concern (1,2,3,4). Common side effects of chemotherapy toxicity include: nausea, vomiting, hair loss, mucositis, weakened bone marrow, cardiomyopathy, fibrosis, renal failure (for which Cisplatin is the chemotherapeutic most responsible), neuropathy, pain, thrombosis, and others (5). These symptoms are currently being managed by primary care physicians, with many of the treatments being aimed at symptom reduction since there is no effective treatment for many of the side effects caused by chemotherapy toxicity (5,6).

Localized endovascular chemotherapy treatment allows for a unique filtration of the drug. The ChemoFilter - a temporarily deployable, endovascular device - aims to extract and filter excess chemotherapeutic agent (drug) escaping the target tumor into the bloodstream. Therefore, the chemotherapy would be administered and filtered through devices attached to the same endovascular catheter. This allows clinicians to increase the maximum dosage of chemotherapy administered to tumors while also minimizing systemic toxicity; making chemotherapy treatment more effective and more tolerable (1,2,3,4). The target is to filter 90% of the chemotherapeutic

that escapes the target tumor (1,2,3,4). Increasing chemotherapy dose has been shown to linearly increase tumor suppression (7,8).

ChemoFilter is similar in concept to Transarterial Chemoembolization (TACE), which is the standard of care for non-operative hepatocellular carcinoma in conjunction with the chemotherapeutic Doxorubicin, brand name Doxil or Adriamycin (9,10). The problem with TACE is that there are serious side effects at higher doses, including bone marrow suppression and cardiac failure (11). Furthermore TACE is not very effective at keeping the drug in the liver, with approximately 40-60% leakage into the blood stream (12). Therefore low-dose TACE and ChemoFilter could be used together to filter the most chemotherapy.

I. Using DNA to filter Cisplatin

ChemoFilter's purpose is to reduce toxicity and potential side effects while also enabling an increased dosage of chemotherapeutics for more effective treatment of tumors (1,2,3,4). Two ChemoFilter prototypes are the ion exchange ChemoFilter (Dowex Resin 50Wx2, 200-400) and the genomic DNA ChemoFilter (salmon sperm, Sigma-Aldrich, St. Louis, MO) (1,4). These were first tested on the chemotherapeutic Doxorubicin (1,4). Doxorubicin is used to treat hepatocellular carcinoma (HCC), the third leading cause of death worldwide, with an estimated 800,000 new cases each year, up to 80% of which are not surgically curable (13,14). Both ion-exchange and the DNA ChemoFilters successfully filtered Doxorubicin, however, DNA had much better kinetics, as shown in Figure 1. (4).

ChemoFilter appears to work for Doxorubicin, but what about other chemotherapeutic agents? Cisplatin, brand name Platinol, is a chemotherapeutic used in head, neck, cervical, and ovarian cancers. As shown in Figure 2, its mechanism of action is to cross link DNA and alter its structure (15,16,17). Cisplatin adducts are intra-strand d(GpG) and d(ApG) allowing protein

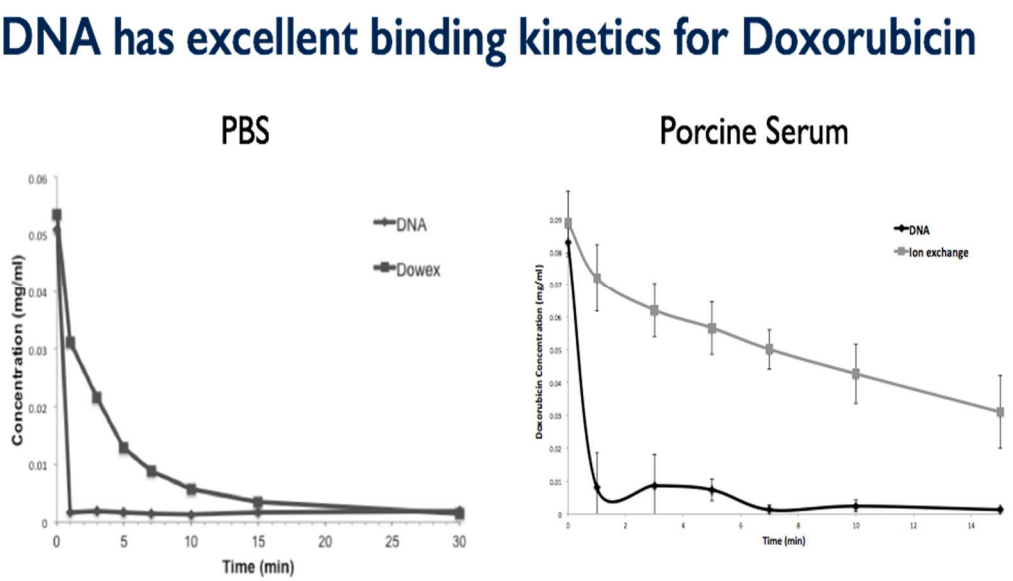


Figure 1. DNA ChemoFilter Binding Doxorubicin. Concentrion of Doxorubicin in solution over time. These in vitro experiments represent concentration of chemotherapeutic in the human blood stream. (A) Experiments were performed in PBS –phosphate buffered saline. (B) Experiments were performed in porcine serum.

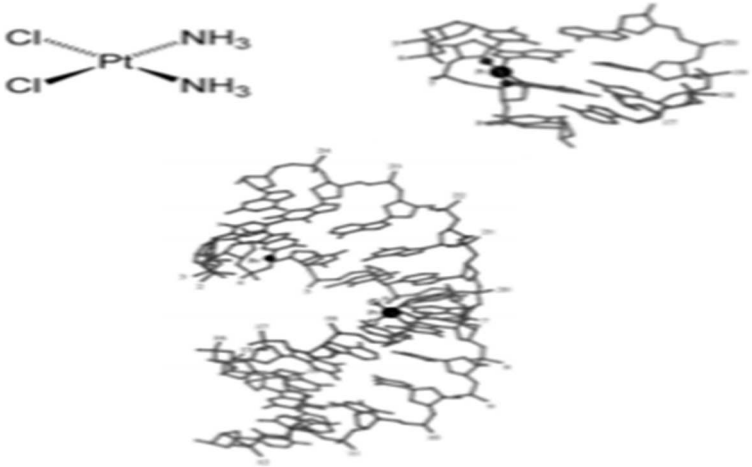


Figure 2. Cisplatin-DNA Mechanism of Action. Cisplatin Molecular structure shown top left. Top right, DNA with bloded Cisplatin bound. Bottom shows DNA bend and damaged due to Cisplatin binding.

binding within HMG domains via DNA bending (15,16). The adducts then cannot be repaired due to the newly bound proteins acting as a shield. The DNA is then damaged and bound to Cisplatin (15, 16). The binding sequence is d(CCTCTG*G*TCTCC), where the * represents Platinum intra-strand cross links and where the Cisplatin binds (16, 17). Cisplatin can also make crystal structures of single stranded DNA (16, 17).

Due to this mechanism of action, it is hypothesized that the DNA ChemoFilter, particularly genomic DNA of the aforementioned binding sequence, will clear Cisplatin. TACE however, is not commonly used for head and neck cancers (4). As shown in Figure 3, ChemoFilter may be placed in the Superior Vena Cava (SVC) for head and neck cancers and in the Inferior Vena Cava (IVC) for gynecological and liver cancers, during treatment (4).

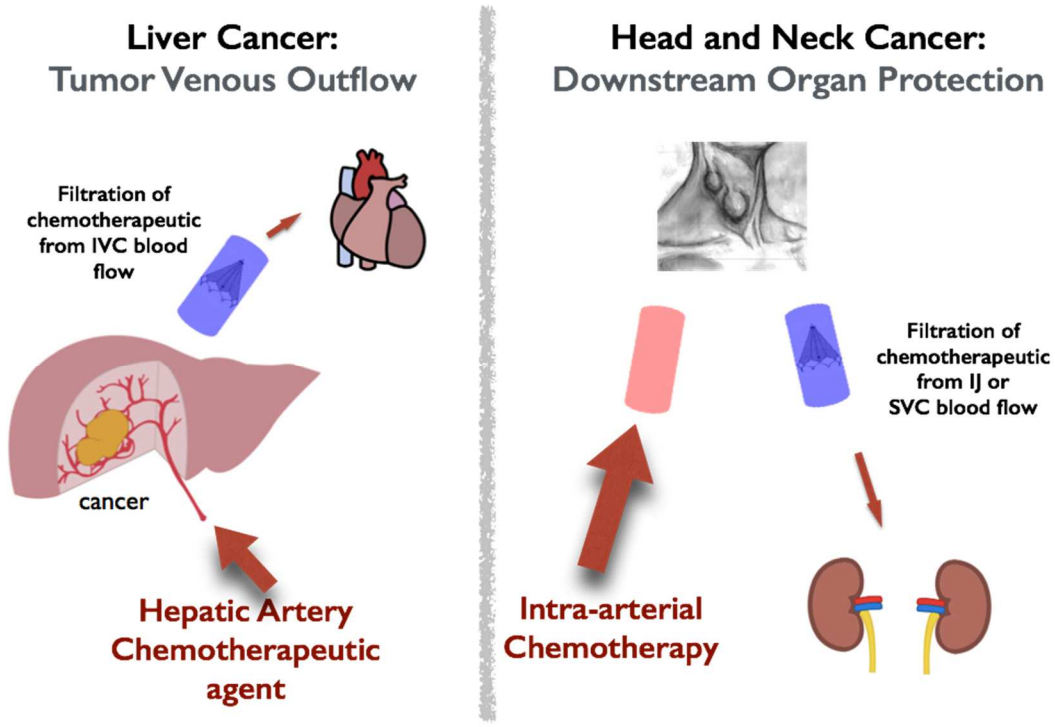


Figure 3. ChemoFilter Placement for (A) Liver Cancer (B) Head/Neck Cancer. ChemoFilter will be placed in the IVC to filter Doxorubicin while treating liver cancers. ChemoFilter will be placed in the SVC to filter Cisplatin or Carboplatin in treating head & neck cancers. These placements allow ChemoFilter to exist in the veins directly feeding the heart so that the chemotherapeutic can be filtered before reaching the heart, thus, the rest of the body.

Quantification of Cisplatin concentration, [Cisplatin], utilized Inductively Coupled Plasma-Mass Spectrometry (IPC-MS), which is considered the gold standard (18). IPC-MS uses a plasma source to create time varying magnetic fields to electrically separate (ionize) the chemical species (18). Then, the chemical species are separated based on mass-to-charge ratio (18). However, IPC-MS does introduce interfering species such as Argon (18). [Cisplatin] can be

identified at either 214 nm or 265 nm wavelengths. Atomic Absorption Spectroscopy (AAS) is also considered gold standard for Cisplatin quantification. ICP-MS is utilized in these experiments, in collaboration with the Balsara lab at UC Berkeley. Aside from availability, ICP-MS also offers greater speed, precision, and sensitivity (19).

II. Using Dowex Resin to filter a Cocktail of Doxorubicin and Cisplatin

Cancer is commonly treated with a cocktail of various chemotherapeutics combined into a single solution. Doxorubicin and Cisplatin are not used together in cocktails, since they treat different cancer types. However, it is believed to be worthwhile to study the ability of ChemoFilter to filter a cocktail (both drugs simultaneously) increasing the clinical relevance of the device.

Fluorescence spectroscopy (aka spectrofluorometry or fluorometry) is utilized to quantify [Doxorubicin] as previously reported (4)**.** This method involves shooting a beam of light, in the UV & visible range of the electromagnetic spectrum, ~100 – 800 nm, at a sample. If the sample is fluorescent, a specific wavelength within that range excites the sample (20). The sample will then emit light at a different wavelength (20). The excitation and emission wavelengths for Doxorubicin are 480 nm and 550 nm respectively (20).

Ultraviolet-visible spectroscopy (UV-Vis), aka ultraviolet-visible spectrophotometry are utilized to quantify [Cisplatin] as previously reported (21)**.** When light passes from one medium to another, parts of the beam are absorbed, reflected, refracted, or diffracted. Refraction is where the beam passes through but its path changes angle while diffraction is similar but where the beam also experiences scattering. UV-Vis takes advantage of these properties of light (20). A beam of light is shot at a sample and either the absorbance or reflection is measured (20). Our UV-Vis measured absorbance. For cocktail experiments, UV-Vis is utilized for quantification

over the gold standards for Cisplatin quantification – ICP-MS and/or AAS. UV-Vis is used for convenience, since measuring [Doxorubicin] and [Cisplatin] from the cocktail is time sensitive and the UV-Vis is located at the same site as the flourometer allowing quantification for Doxorubicin and Cisplatin simultaneously without any of the product evaporating. Both fluoremetry and UV-Vis offer high sensitivity, reliability, and affordability (20).

ChemoFilter is sequestered into a mesh packet, to maximize filtration and minimize escaped/leaked Chemotherapy. This is known as the tea bag model. This tea bag model is tested against a model of free flowing ChemoFilter in solution. Two materials utilized are polyester and nylon due to in vivo applications and availability. Optimal ChemoFilter geometries for human venous system use are under investigation.

III. Radiolabeling Carboplatin

Carboplatin, brand name Paraplatin, is a platinum containing chemotherapeutic agent similar to Cisplatin, as shown in Figure 4. Like Cisplatin, Carboplatin is used for head, neck, and gynecological cancers utilizing a similar DNA adduct binding mechanism that can be taken advantage of with the DNA ChemoFilter (22). Since Carboplatin contains Carbon atoms it can ^{11}C, ^{13}C, or ^{14}C radiolabeled into a radiopharmaceutical. Radiolabeling allows for new in vitro

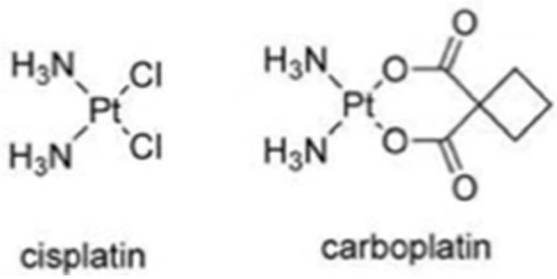


Figure 4. Cisplatin & Carboplatin Molecular Structures. Both are built around Pt. However, carboplatin is much larger and is organic with a Carbon ring.

quantification methods as well as methods for in vivo assessment of ChemoFilter.

As shown in Figure 5, ^{11}C decays mostly via positron (β+) emission (~99%), therefore this is a useful chemical species for positron emission tomography imaging (PET). This would allow real-time in vivo visualization of the chemotherapy while the endovascular catheter can be visualized using either computed tomography imaging (CT) or magnetic resonance imaging (MRI). Thus, multi-modal imaging such as PET/CT or PET/MRI will allow real-time visualization of chemotherapy delivery and filtration in the body. ^{11}C also allows for an in vitro quantification method, using gamma counting, however, the half-life is only ~20 minutes. ^{13}C, a non-radioactive isotope, may be used in Hyperpolarized ^{13}C MRI or Magnetic Resonance Spectroscopy (MRS) for additional chemotherapy and ChemoFilter metabolic studies (molecular imaging). ^{14}C (half- life 5730 years) provides an additional in vitro quantification method using scintillation counting with minimal radiation. Combining previous studies, a mechanism for

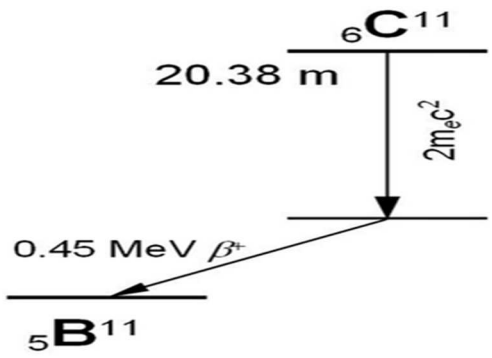


Figure 5. 11C-Decay Scheme. ^{11}C to ^{11}B is ~99% positron (β+) emission allowing for positron emission tomography (PET)

radiolabeling Carboplatin is developed and tested (23, 24, 25). High Performance Liquid Chromatography (HPLC) as previously reported is utilized for chemical species determination utilized (26). HPLC pumps a sample with high pressure through a column that separates based on chemical properties such as polarity, pH, etc.

IV. Study Goals

The goals are to further the development of the genomic DNA and ion-exchange ChemoFilters and their encasings, to test their binding with new chemotherapeutics Cisplatin, Carboplatin, and a cocktail comprised of Cisplatin and Doxorubicin, and to begin to radiolabel Carboplatin for in vitro and in vivo ChemoFilter quantification.

Materials and Methods

Preliminary binding studies were performed in a simple beaker model. There are 3 sets of experiments: *I.* Binding Kinetics of Cisplatin filtration using genomic DNA ChemoFilter (salmon sperm, Sigma-Aldrich, St. Louis, MO), using IPC-MS for Cisplatin quantification. *II.* Binding Kinetics and Optimization of free and sequestered ion-exchange ChemoFilter (Dowex Resin 50Wx2, 200-400) with chemotherapy cocktail (quantification via UV-Vis and fluorimeter). *III.* Preliminary steps in Carboplatin radiolabeling.

All in vitro binding kinetic studies were performed in phosphate-buffered saline (PBS) or deionized water (diH_2O). All binding experiments, besides proof of concepts, were performed in triplicate (which is a set of 3 trials in which all experimental conditions are kept constant). Three trials was reported to be a large enough sample size for preliminary studies (1,2,3,4). Paired samples t-tests were performed using IBM SPSS Statistics 21.

I. Using DNA to filter Cisplatin

1. **Proof of Concept:** 5 mL Cisplatin (0.05 mg/mL) was stirred in 100 mL PBS or diH_2O in a 200 mL beaker for a few minutes. 50 mg genomic DNA was added. 5 mL samples were taken @ time 0, before DNA addition, and 46 minutes after.
    i. Cisplatin concentration in the samples was quantified using IPC-MS (Perkin Elmer Optima 7000 DV) at the Balsara Lab at UC Berkeley. Samples were initially processed at UCSF then driven to Berkeley for quantification. Later, samples were processed and quantified at Berkeley.
    ii. Standard curves were generated prior to each experiment to convert parts per million to Cisplatin concentration (mg/mL).
2. **Determining Binding Kinetics of DNA with Cisplatin:** Same as # 1 but only in PBS and with time points: 0, 5, 15, 30, 45 minutes
3. **Increasing [DNA]:** Same as #1 but with 100 mg DNA.
4. **Tea Bag Model:** Same as #1 but with 50 mg DNA linked to beads in a 1x6 cm nylon Packet, as shown in Figure 6 Beads are larger than mesh pore size to prevent leakage (4).

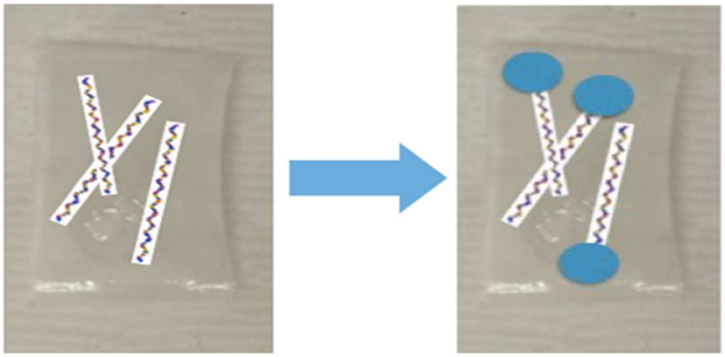


Figure 6. Genomic DNA with Beads Immobilized in Nylon Packet. Bead is larger than pore size of nylon mesh to prevent leakage.

II. Using Dowex Resin to filter a Cocktail of Doxorubicin and Cisplatin

1. **Proof of concept**: To determine if Dowex could be used as a potential binding substrate for a chemotherapy cocktail, 1 g of Dowex was added to 2.5 mL of Cisplatin (1 mg/mL), and 1.25 ml Doxorubicin (1mg/mL) in 46.25 mL of PBS. Two samples (200 µL for Dox, 300 µL for Cis) were taken at each time point (0 and 15 minutes). The samples were processed and quantified separately.
    a. Cisplatin quantification: As shown in Figure 7, 600 µL o-phenylenediamine (OPDA) was pipetted into the samples, which were then boiled for 10 minutes; increasing the conjugation, shifting the compound's absorbance into the UV spectrum, specifically at 706nm, giving it a green color. 2.1 mL DMF was pipetted in to stop the reaction. The absorbance of each sample was then measured using UV-Vis (U-2810 Spectrophotometer, Digilab Hitachi, AJN Scientific, Stoughton, MA) at 706 nm, which was then converted to Cisplatin concentration using a standard curve. Due to its unreliability, this method has now been replaced with ICP-MS, the gold standard for Cisplatin quantification.

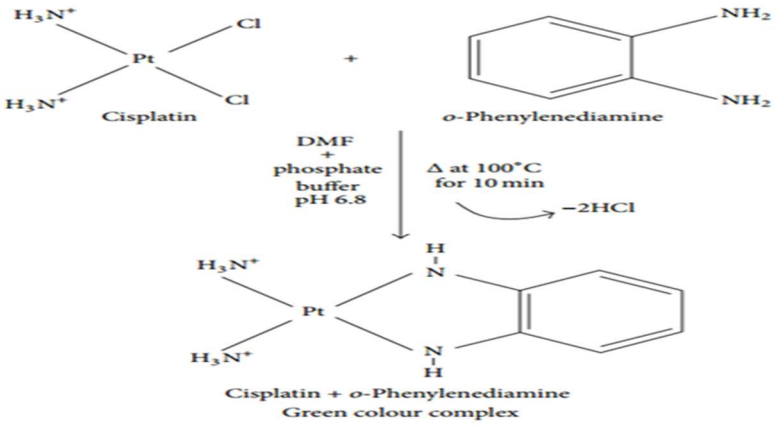


Figure 7. [Cisplatin] Quantification "OPDA Method". Cisplatin + o-Phenylenediamine (OPDA) yields a green colored complex that can be read on UV-Vis at 706 nm.

b. Doxorubicin quantification: 40 µL of the sample was pipetted into 160 µL of PBS. 100 µL of this solution was pipetted into a well in a Costar 96 black well plate and quantified using a spectrofluorometer (Spectramax Gemini EM, Molecular Devices, Sunnyvale, CA) with excitation 480 nm, emissions 550 nm, for Doxorubicin concentration.

2. **Determining Binding Kinetics of Dowex with cocktail:** Same as #1 but with time points 0, 30 seconds, 1, 3, 5, 7, 10, 12, and 15 minutes.

3. **Optimizing packet material for Dowex with cocktail:** Same as #1 but Dowex was sequestered into either polyester or nylon mesh (2x6 cm) as shown in Figure 8.

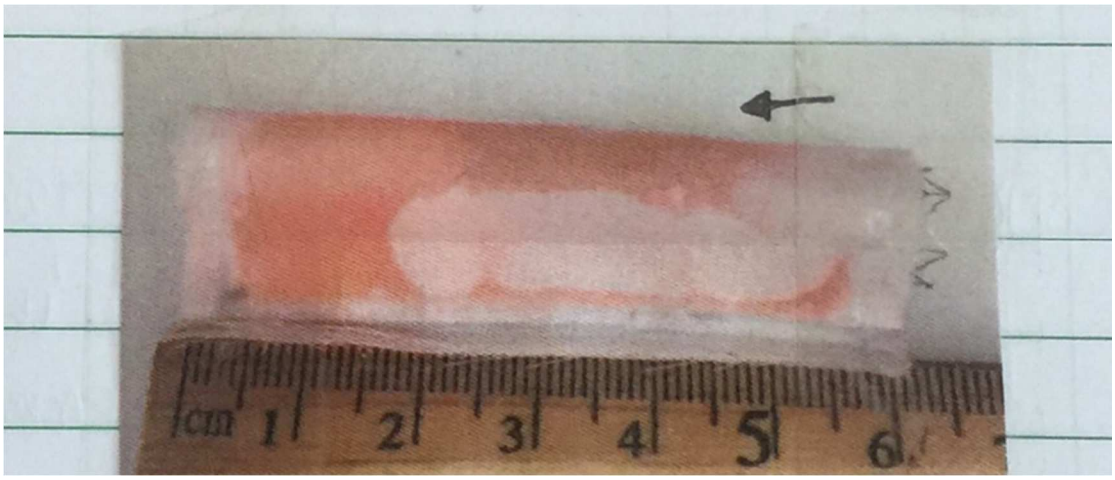


Figure 8. Mesh "Tea Bag" with Dowex Resin (orange).

4. **Optimizing packet Dimensions:** Same as #1 but Dowex was sequestered into either 2x3 cm or 2x6cm Nylon Packets.

5. **Nylon Packet Negative Control:** Same as #1a but with an empty Nylon Packet (No ChemoFilter)

6. **Sub-minute time points:** Same as #1 but with 10, 15, 20, & 30 second time points.

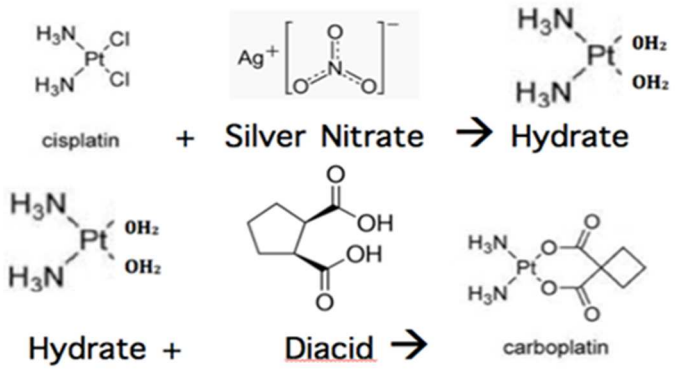


Figure 9. Cisplatin → Carboplatin Mechanism. This 2 step reaction allows for synthetization of Carboplatin from Cisplatin.

III. Radiolabeling Carboplatin

1. **Synthesis of Carboplatin product:** Following the reaction in Figure 9,
    a. Silver nitrate (11.2 mg, 2 mmol) was added to 1 mmol Cisplatin (pharmaceutical grade), continuously stirred with temperature of 60^{o} C for 1 hour. The product of this reaction, Cisplatin hydrate, was refrigerated overnight. This product was subsequently filtered using filter paper to remove any solids (retain only liquids).
        i. Second trial utilized 272.8 mg silver nitrate to react with 9 parts sodium chloride and 1 part Cisplatin within pharmaceutical grade Cisplatin.
    b. For the second reaction,1,1-dicarboxylic acid ("Diacid" in Figure 9) (158.2 g, 1 mmol) was added to the filtered product from (a). This was stirred for one hour and filtered with filter paper. This was our "Carboplatin" product.
2. **Quantification of product:** HPLC was used for quantification with 27.2 g potassium phosphate monobasic. Specifications: Hitachi Elite LaChrome with organizer, autosampler, pump, column oven, and diode array detector utilizing UV detection using the Column - Synergi 4 um Hydro RP 80 Angstrom, 250x3mm and EZ Chrome Elite software.

Cisplatin and Carboplatin Standards. Cisplatin and Carboplatin (pharmaceutical grade) were quantified via HPLC. Reaction products (assumed to be Carboplatin) were compared to prepared standards of Carboplatin and Cisplatin.

Results

I *Using DNA to filter Cisplatin*

1. **Proof of Concept:** Figure 10 shows DNA clears ~85% of Cisplatin from solution in 46 minutes.

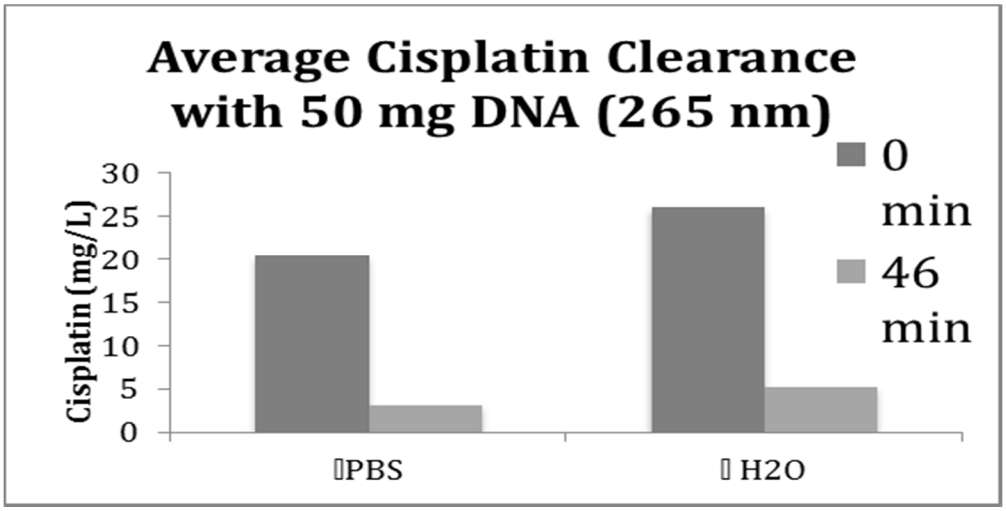


Figure 10. Proof of Concept-DNA to filter Cisplatin with ICP-MS. DNA ChemoFilter binds much of the Cisplatin within 46 minutes (~85%).

2. **Determining Binding Kinetics of DNA with Cisplatin:** As shown in Tables 1 & 2 and Figure 11, 50 mg DNA reduces [Cisplatin] by approximately 43% at 214 nm and 45% at 265 nm within 5 minutes. There is an additional ~18% drop from 5 minutes to 30 minutes. Otherwise the concentration remains relatively stable. As shown in tables 3 & 4, the only significant drops ($p<0.05$) are 0-30 minutes and 0-45 minutes for 214 nm and 0-5, 0-30, and 0-45 for 265 nm.

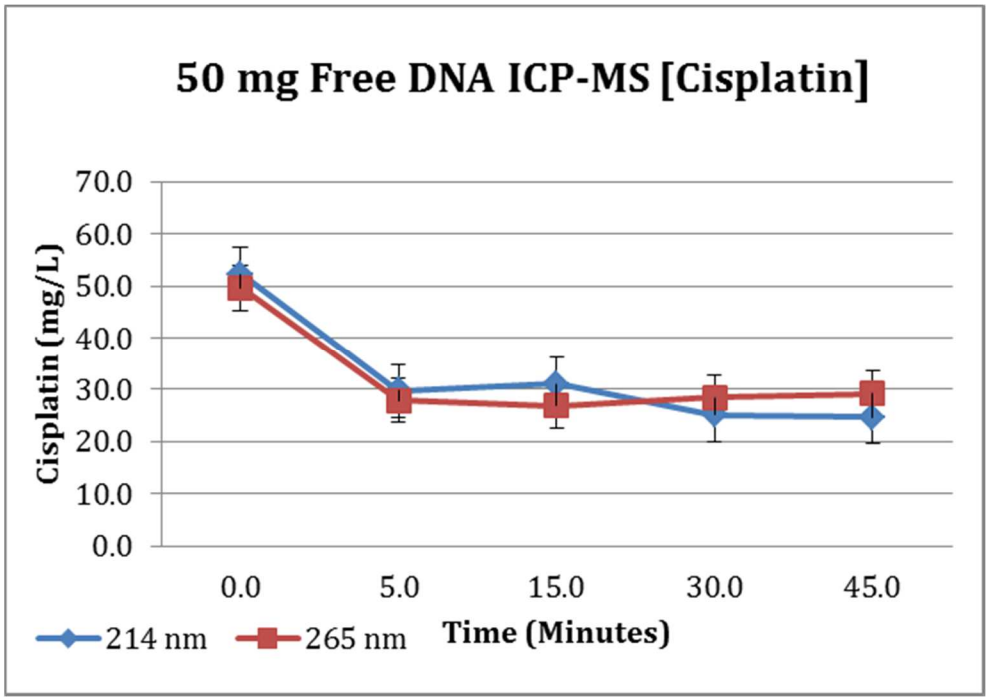


Figure 11. 50 mg Free DNA with Cisplatin. ICP-MS Results @ 214 & 265 nm

Table 1. Cisplatin Concentration Over Time With 50 mg Free DNA ChemoFilter, ICP-MS Results @ 214 nm

Time	0	5	15	30	45
Trial 1	48.9	35.6	38.6	20.1	17.8
Trial 2	58.8	26.4	27.5	26.8	26.6
Trial 3	49.1	27.1	27.3	28.6	29.6
Average	52.3	29.7	31.1	25.2	24.7

Table 2. Cisplatin Concentration Over Time with 50 mg Free DNA ChemoFilter, ICP-MS Results @ 265 nm

Time	0	5	15	30	45
Trial 1	49.8	33.8	39	20.6	17.5
Trial 2	53.2	26.4	26.7	26.5	26.9
Trial 3	49.6	27.9	26.8	28.5	29.2
Average	50.9	29.7	30.8	25.2	24.5

Table 3. Statistics - 50 mg DNA with Cisplatin (ICP-MS) 214 nm

Paired Samples Test 50 mg Free DNA 214 nm

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	0 – 5 min	22.6	9.51	5.49	-1.05	46.2	4.11	2	.054
Pair 2	5 min – 15 min	-1.42	1.42	.823	-4.96	2.12	-1.72	2	.227
Pair 3	15 min – 30 min	5.96	10.8	6.28	-21.1	33.0	.949	2	.443
Pair 4	30 min – 45 min	.493	1.63	.944	-3.56	4.56	.522	2	.653
Pair 5	0 – 15 min	21.2	10.5	6.06	-4.91	47.2	3.49	2	.073
Pair 6	0 – 30 min	27.1	6.00	3.46	12.2	42.0	7.83	2	.016
Pair 7	0 – 45 min	27.6	7.04	4.06	10.1	45.1	6.80	2	.021
Pair 8	5 min – 30 min	4.54	9.49	5.48	-19.0	28.1	.829	2	.494
Pair 9	5 min – 45 min	5.03	11.1	6.39	-22.5	32.6	.788	2	.513
Pair 10	15 min – 45 min	6.45	12.5	7.21	-24.5	37.5	.896	2	.465

Table 4. Statistics - 50 mg DNA with Cisplatin (ICP-MS) 265 nm

Paired Samples Test 50 mg Free DNA 265 nm

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	0 – 5 min	21.5	5.38	3.11	8.13	34.9	6.92	2	.020
Pair 2	5 min – 15 min	-1.47	3.30	1.91	-9.67	6.73	-.771	2	.521
Pair 3	15 min – 30 min	5.65	11.1	6.42	-22.0	33.3	.880	2	.472
Pair 4	30 min – 45 min	.687	2.06	1.19	-4.43	5.80	.577	2	.622
Pair 5	0 – 15 min	20.0	8.17	4.72	-.259	40.3	4.25	2	.051
Pair 6	0 – 30 min	25.7	4.19	2.42	15.3	36.1	10.6	2	.009
Pair 7	0 – 45 min	26.4	5.95	3.43	11.6	41.1	7.69	2	.017
Pair 8	5 min – 30 min	4.18	7.85	4.54	-15.3	23.7	.921	2	.454
Pair 9	5 min – 45 min	4.87	9.92	5.73	-19.8	29.5	.850	2	.485
Pair 10	15 min – 45 min	6.33	13.2	7.61	-26.4	39.1	.833	2	.493

Approaching significance, p<.05, p<.01, p<.001

3. **Increasing [DNA]:** As shown in Figure 12 and tables 5 & 6, 100 mg free DNA reduces Cisplatin concentration within 5 minutes by approximately 52% & 48% at 214 nm & 265 nm, respectively. Beyond 5 minutes, the concentration remains relatively stable. As shown in tables 7 & 8, the only significant ($p<0.001$, $p<0.01$, or $p<0.05$) drops are from the starting time to all other time points.

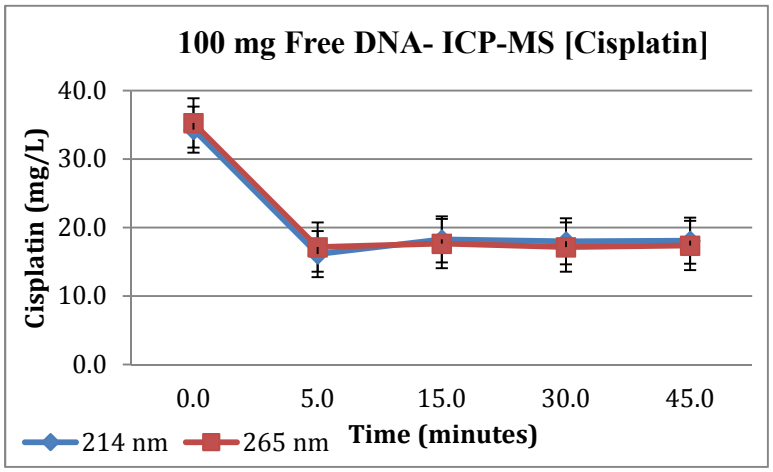


Figure 12. 100 mg Free DNA with Cisplatin. ICP-MS Results @ 214 nm & 265 nm

Table 5. . Cisplatin Concentration Over Time With 100 mg Free DNA ChemoFilter, ICP-MS Results @ 214 nm

Time	0	5	15	30	45
Trial 1	33.3	14.8	20.8	18.0	18.8
Trial 2	34.7	16.9	17.0	18.8	19.8
Trial 3	34.9	16.6	17.0	17.2	15.7
Average	34.3	16.1	18.2	18.0	18.1

Table 6. . Cisplatin Concentration Over Time With 100 mg Free DNA ChemoFilter, ICP-MS Results @ 265 nm

Time	0	5	15	30	45
Trial 1	34.7	17.3	20.1	17.5	18.2
Trial 2	37.6	17.1	16.7	17.4	18.4
Trial 3	33.5	17.0	16.2	16.5	15.6
Average	35.3	17.1	17.7	17.2	17.4

Table 7. Statistics - 100 mg DNA with Cisplatin (ICP-MS) 214 nm

Paired Samples Test 100 mg Free DNA 214 nm

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	0 – 5 min	18.2	.339	.196	17.3	19.0	92.9	2	.000
Pair 2	5 min – 15 min	-2.12	3.33	1.92	-10.4	6.16	-1.10	2	.386
Pair 3	15 min – 30 min	.260	2.30	1.33	-5.45	5.97	.196	2	.863
Pair 4	30 min – 45 min	-.073	1.39	.802	-3.52	3.37	-.091	2	.935
Pair 5	0 – 15 min	16.1	3.07	1.77	8.45	23.7	9.07	2	.012
Pair 6	0 – 30 min	16.3	1.24	.713	13.25	19.4	22.9	2	.002
Pair 7	0 – 45 min	16.3	2.57	1.48	9.86	22.6	10.9	2	.008
Pair 8	5 min – 30 min	-1.86	1.33	.765	-5.15	1.44	-2.42	2	.136
Pair 9	5 min – 45 min	-1.93	2.57	1.48	-8.31	4.45	-1.30	2	.323
Pair 10	15 min – 45 min	.187	2.60	1.50	-6.28	6.66	.124	2	.913

Table 8. Statistics - 100 mg DNA with Cisplatin (ICP-MS) 265 nm

Paired Samples Test 100 mg Free DNA 265 nm

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	0 – 5 min	18.1	2.05	1.18	13.1	23.2	15.4	2	.004
Pair 2	5 min – 15 min	-.526	1.92	1.11	-5.29	4.23	-.476	2	.681
Pair 3	15 min – 30 min	.503	1.74	1.01	-3.84	4.84	.499	2	.667
Pair 4	30 min – 45 min	-.237	1.01	.584	-2.75	2.27	-.406	2	.724
Pair 5	0 – 15 min	17.6	3.09	1.78	9.94	25.3	9.88	2	.010
Pair 6	0 – 30 min	18.1	1.78	1.03	13.7	22.5	17.6	2	.003
Pair 7	0 – 45 min	17.9	1.35	.778	14.5	21.2	23.0	2	.002
Pair 8	5 min – 30 min	-.023	.394	.227	-1.00	.955	-.103	2	.928
Pair 9	5 min – 45 min	-.260	1.40	.811	-3.75	3.23	-.321	2	.779
Pair 10	15 min – 45 min	.267	1.75	1.01	-4.09	4.62	.263	2	.817

Approaching significance, p<.05, p<.01, p<.001

4. **Tea bag Model:** As shown in Figure 13 and tables 9 & 10, 50 mg DNA sequestered within a mesh packet reduced [Cisplatin] by 98.7% and 96.2% for 214 nm and 265 nm, respectively, within 5 minutes. As shown in tables 11 & 12, the drops from the initial time point are all significant ($p<0.01$).

Table 9. Cisplatin Concentration Over Time With Sequestered DNA ChemoFilter, ICP-MS Results @ 214 nm

Time	0	5	15	30	45
Trial 1	16.0	0.2	0.2	0.2	0.2
Trial 2	15.7	0.2	0.2	0.2	0.2
Trial 3	18.1	0.2	0.2	0.2	0.2
Average	16.6	0.2	0.2	0.2	0.2

Table 10. Cisplatin Concentration Over Time With Sequestered DNA ChemoFilter, ICP-MS Results @ 265 nm

Time	0	5	15	30	45
Trial 1	16.0	0.6	0.6	0.6	0.6
Trial 2	15.8	0.6	0.6	0.6	0.6
Trial 3	18.0	0.6	0.6	0.7	0.6
Average	16.6	0.6	0.6	0.6	0.6

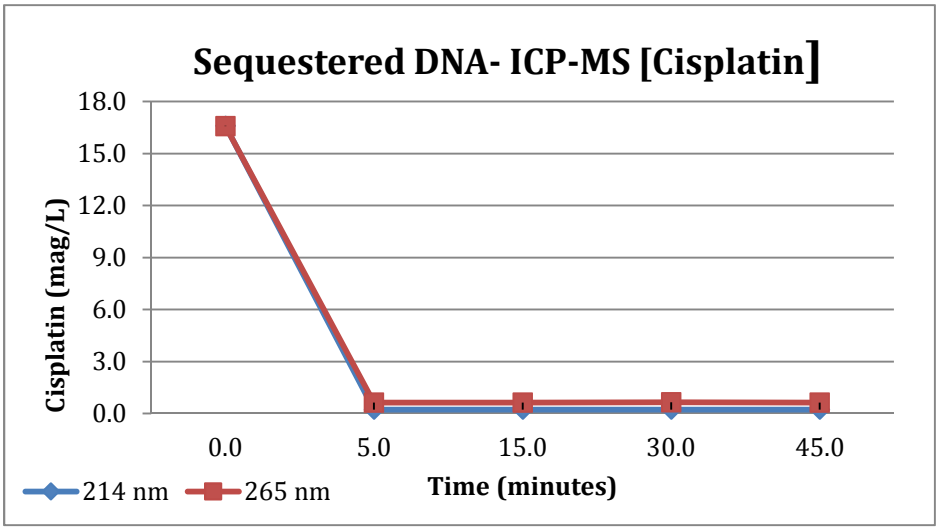


Figure 13. Sequestered DNA with Cisplatin. ICP-MS Results @ 214 nm & 265 nm

Table 11. Statistics - Sequestered DNA with Cisplatin (ICP-MS) 214 nm

Paired Samples Test 1x6cm Packets 50mg DNA 214 nm

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	0 – 5 min	16.4	1.31	.757	13.1	19.6	21.6	2	.002
Pair 2	5 min – 15 min	.001	.002	.001	-.003	.007	1.10	2	.383
Pair 3	15 min – 30 min	.001	.005	.003	-.011	.014	.468	2	.686
Pair 4	30 min – 45 min	.002	.008	.005	-.017	.023	.571	2	.625
Pair 5	0 – 15 min	16.4	1.31	.756	13.1	19.6	21.7	2	.002
Pair 6	0 – 30 min	16.4	1.31	.756	13.1	19.6	21.7	2	.002
Pair 7	0 – 45 min	16.4	1.31	.754	13.1	19.6	21.7	2	.002
Pair 8	5 min – 30 min	.003	.003	.002	-.005	.010	1.60	2	.251
Pair 9	5 min – 45 min	.005	.011	.006	-.022	.033	.842	2	.488
Pair 10	15 min – 45 min	.004	.013	.008	-.028	.036	.533	2	.647

Table 12. Statistics - Sequestered DNA with Cisplatin (ICP-MS) 265

Paired Samples Test 1x6cm Packets 50mg DNA 265 nm

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
					Lower	Upper			
Pair 1	0 – 5 min	16.0	1.25	.721	12.8	19.1	22.1	2	.002
Pair 2	5 min – 15 min	.000	.003	.002	-.007	.007	.000	2	1.00
Pair 3	15 min – 30 min	-.009	.016	.009	-.049	.030	-.998	2	.423
Pair 4	30 min – 45 min	.014	.012	.007	-.016	.045	2.03	2	.179
Pair 5	0 – 15 min	16.0	1.25	.721	12.9	19.1	22.1	2	.002
Pair 6	0 – 30 min	15.9	1.23	.712	12.9	19.0	22.4	2	.002
Pair 7	0 – 45 min	15.9	1.25	.719	12.9	19.1	22.2	2	.002
Pair 8	5 min – 30 min	-.009	.017	.010	-.051	.032	-.944	2	.445
Pair 9	5 min – 45 min	.005	.005	.003	-.006	.017	1.96	2	.190
Pair 10	15 min – 45 min	.005	.005	.003	-.007	.018	1.80	2	.214

Approaching significance, p<.05, p<.01, p<.001

II. *Using Dowex Resin to filter a Cocktail of Doxorubicin and Cisplatin*

1. **Proof of concept – Dowex with Cocktail:** 99% drop in [Cisplatin] from 0-15 minutes, 75% drop in [Dox] from 0-15 minutes, as shown in Figure 14.

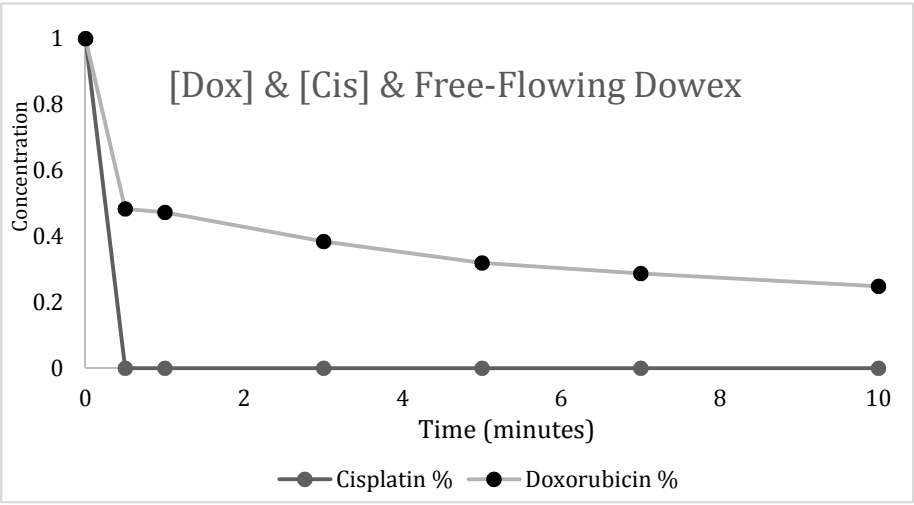


Figure 14. Doxorubicin and Cisplatin Filtration via Ion-Exchange

2. **Determining Binding Kinetics of Dowex with cocktail:** Within 30 seconds filters clearance of ~75% for Doxorubicin and ~100% Cisplatin.
3. **Optimizing packet material for Dowex with cocktail:** As shown in Figure 15(A), Cisplatin clears ~100% within 30 seconds for both polyester and nylon 2x6cm packets. However, as shown in Figure 15(B) for Doxorubicin, polyester cleared more in earlier time points (31.15% compared to 92.33% within 30 seconds) but over the 15 minutes, nylon had a higher clearance (~63.8% compared to ~52.7%).

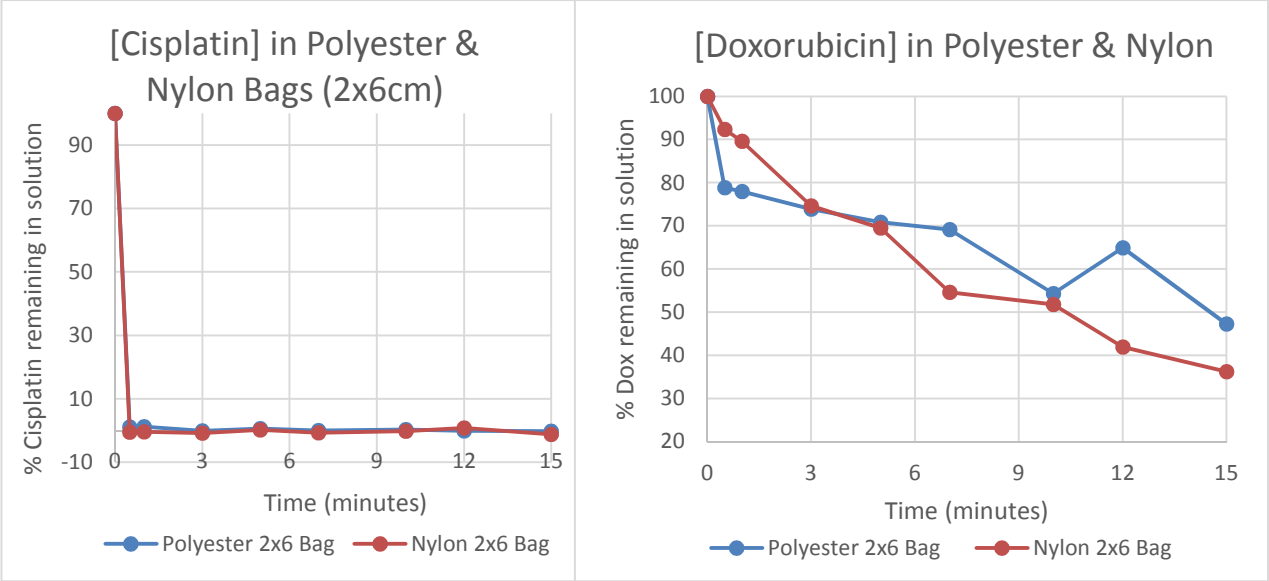


Figure 15. Mesh Material Optimization (A) Cisplatin (B) Doxorubicin. Immediate drop for Cisplatin. For Doxorubicin, polyester cleared more in earlier time points (31.15% compared to 92.33% within 30 seconds) but over the 15 minutes, nylon had a higher clearance (~63.8% compared to ~52.7%).

4. **Optimizing packet Dimensions:** As shown in Figure 16 (A), Cisplatin clearance is nearly 100% within 30 seconds for both dimensions. However, as shown in Figure 16(B), the 2x6cm packet cleared more Doxorubicin at nearly every time point (12% vs 0% within 1 minute and 63.8% vs. 56.6% for 2x3 and 2x6cm respectively).

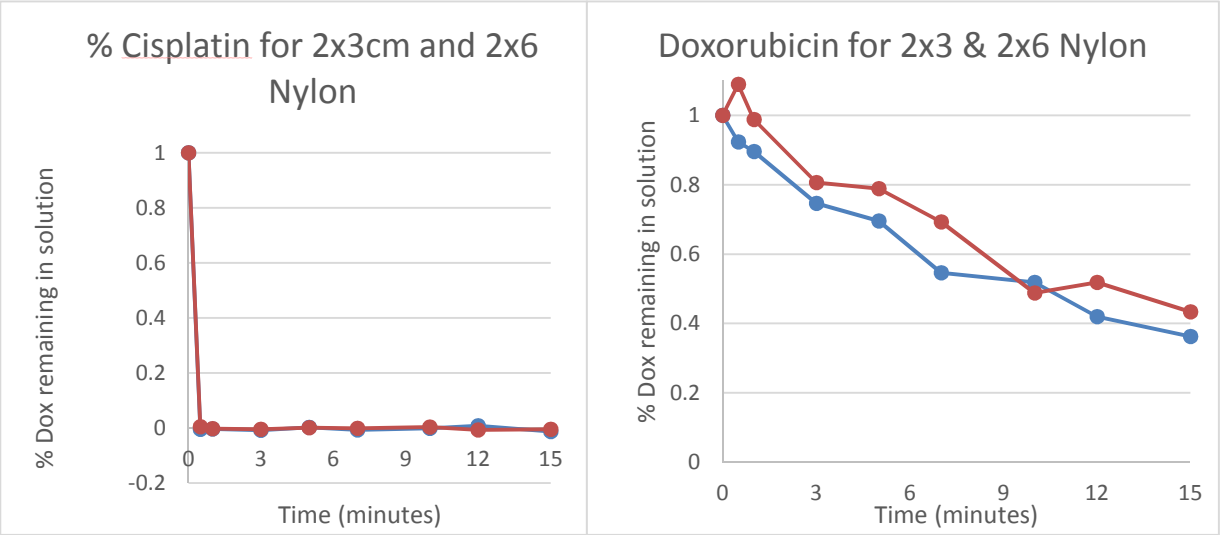


Figure 16. Mesh Dimension Optimization (A) Cisplatin (B) Doxorubicin. Cisplatin clearance is nearly 100% within 30 seconds for both dimensions. For Doxorubin, the 2x6cm packet was more efficient at each time point.

Table 13. Cisplatin Binding Nylon. Negative Control. Cisplatin absorbance, and relative concentration reduced by ~30% in 5 minutes.

time	absorbance
0 min	0.945
2.5 min	0.804
5 min	0.628

5. **Nylon Negative Control:** Table 13 shows that an empty nylon bag reduced Cisplatin concentration by 31.7% in 5 minutes
6. **Sub-minute time points:**, Cisplatin cleared 23%, 45%, 65%, and 92% within 10, 15, 20, and 30 seconds respectively & Doxorubicin cleared 6, 13, 20, and 25% within 10, 15, 20, and 30 seconds, as shown in Figure 17.

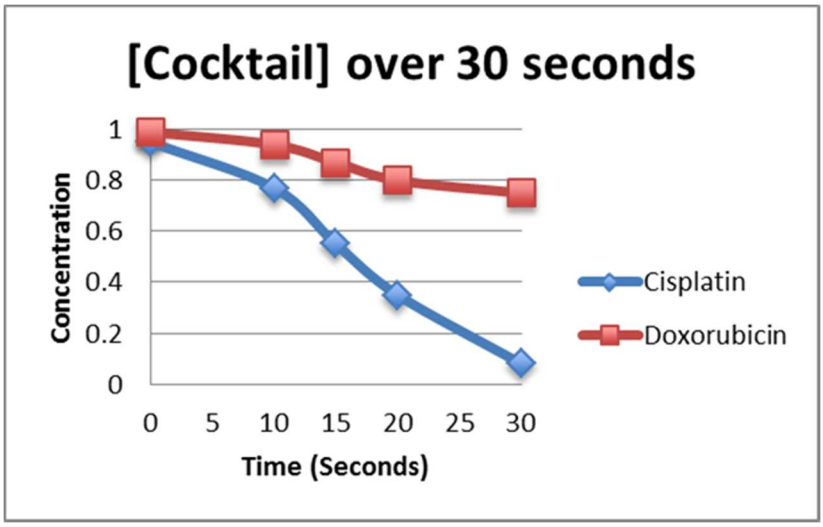


Figure 17. Chemotherapy cocktail concentration over 30 seconds

III. Radiolabeling Carboplatin

Quantification of "Carboplatin" product: The HPLC standard of pharmaceutical grade Cisplatin indicated a large peak at ~4.5 minutes. Our Carboplatin standard indicated a peak in similar size at ~9.5 minutes. As shown in Figure 18, our product had one large peak at ~4.5 minutes and another smaller peak at ~9.5 minutes.

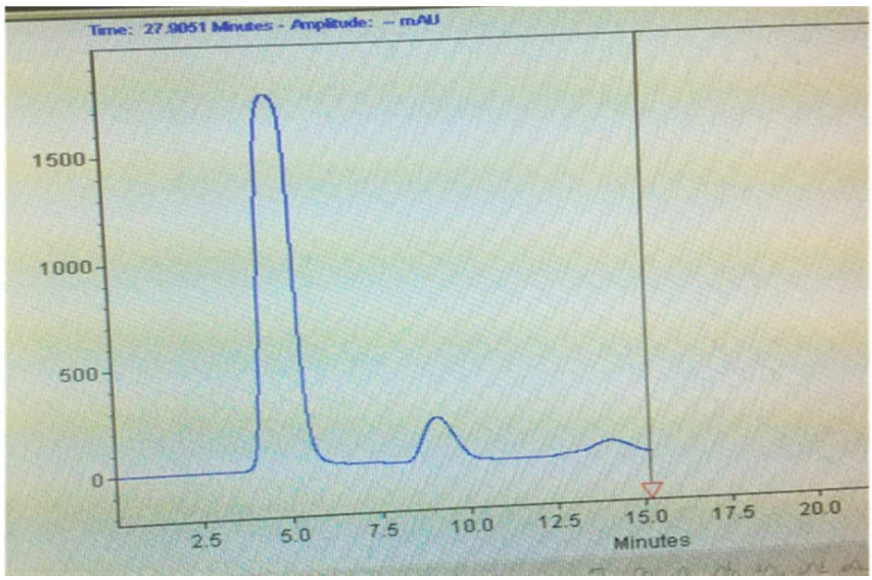


Figure 18. HPLC of the "Carboplatin" Product. Large peak at ~4.5 represents Cisplatin while smaller peak at ~9.5 represents Carboplatin.

Discussion

I. Goals

Cisplatin and a cocktail comprised of Cisplatin and Doxorubicin successfully bound to Genomic DNA and Dowex Resin free in solution and encapsulated in nylon and/or polyester mesh packets of various geometries. Successful steps were taken in the process of ^{11}C, ^{13}C, and/or ^{14}C labelling Carboplatin for in vitro ChemoFilter quantification and for later use in PET imaging.

II. Interpretation of Results

1. Using DNA to filter Cisplatin

It appears as though a higher quantity of DNA ChemoFilter filters Cisplatin better but more importantly, once DNA is sequestered in a mesh bag, the binding becomes near complete. The results obtained are expected, and some even better than expected, based on previously reported results (1,2,3,4). Excellent clearance of Cisplatin (~99%) is reported using DNA ChemoFilter sequestered in a 1x6cm nylon packet within 5 minutes, using ICP-MS for quantification.

2. Using Dowex Resin to filter a cocktail of Doxorubicin and Cisplatin

Nylon mesh had better binding kinetics than polyester meshes and 2x6 cm had better kinetics than 2x3cm. These findings still need confirmation for Cisplatin using ICP-MS. However, it is hypothesized that more rectangular shaped "tea bags" work better than more square shaped. Future studies will test this and other geometries for optimal filtration efficiency (binding kinetics) within the human venous system, given that the IVC ranges in diameter from ~1 cm to ~2.5 cm (4).

3. Radiolabeling Carboplatin

Since the ~4.5 minute large peak represented Cisplatin and the smaller peak at ~9.5 minutes represented Carboplatin, it appears that some Carboplatin was produced, however, a large amount of the solution is unreacted Cisplatin. This is likely due to the use of pharmaceutical grade Cisplatin (9 parts sodium chloride (salt) per 1 part Cisplatin). Since an abundance of salt appears problematic, future studies will use pure chemical cisplatin (99%) for its lack of salinity.

4. ChemoFilter

ChemoFilter can significantly reduce Cisplatin and Doxorubicin concentration. Optimizations of ChemoFilter prototypes have been performed. Preliminary studies were performed to test ChemoFilter's effectiveness in reducing [Carboplatin] by radiolabeling this chemotherapeutic, which also allows future in vivo investigation and clinical trials using PET.

III. Limitations

Our OPDA method for quantification did not seem to accurately reflect Cisplatin concentration. We propose two potential reasons for this. 1.) Our UV-Vis machine may be incorrectly calibrated. To test this theory we plan to test a different UV-Vis machine from another lab. 2.) Adding ChemoFilter makes the sample unreadable at 706 nm by shifting the absorbance of the molecule. This quantification method has been replaced with ICP-MS since it is a more reliable machine and is considered the gold standard.

While previous studies found that a triplicate was satisfactory for preliminary studies, it appears insufficient for studies beyond the preliminaries (1,2,3,4). When comparing and optimizing various metrics, the kinetics are too similar to be distinguished with 3 trials and additional trials are recommended.

IV. Implications

From these successful results, ChemoFilter may work better than a TACE. With TACE as much as 40-60% of the drug administered to the hepatic artery enters the systemic circulation, resulting in systemic toxicity. However, our results indicate that with ChemoFilter, <40% of the drug would enter systemic circulation and contribute to toxicity. ChemoFilter and TACE may be able to work together to maximize filtration (1,2,3,4). It is promising that sequestered DNA ChemoFilter filters ~99% of Cisplatin within 5 minutes. There are also many planned improvements being made to ChemoFilter and various prototypes being developed. Enveloped DNA ChemoFilter has shown to bind to nearly all of Cisplatin in solution suggestion that none of the drug would enter systemic toxicity. DNA ChemoFilter worked much better at clearing Doxorubicin and also filters Cisplatin at ~99% within 5 minutes (4).

V. Recommendations for Future Research

1. DNA/Cisplatin

Future research into the DNA ChemoFilter will focus on replicating and extending the data using ICP-MS. Optimization of DNA concentration is necessary. Tea Bag geometries will be further explored. These experiments would all be performed in PBS, porcine serum, and porcine whole blood. The final in vitro steps include a flow model, as shown in Figure 19

2. Ion-Exchange/Cocktail:

Mesh bag material and geometrical optimizations will continue. The number of chemotherapeutic agents in experimental cocktails will be increased, as will their clinical relevance by using chemotherapeutic agents that are used together to fight specific cancers.

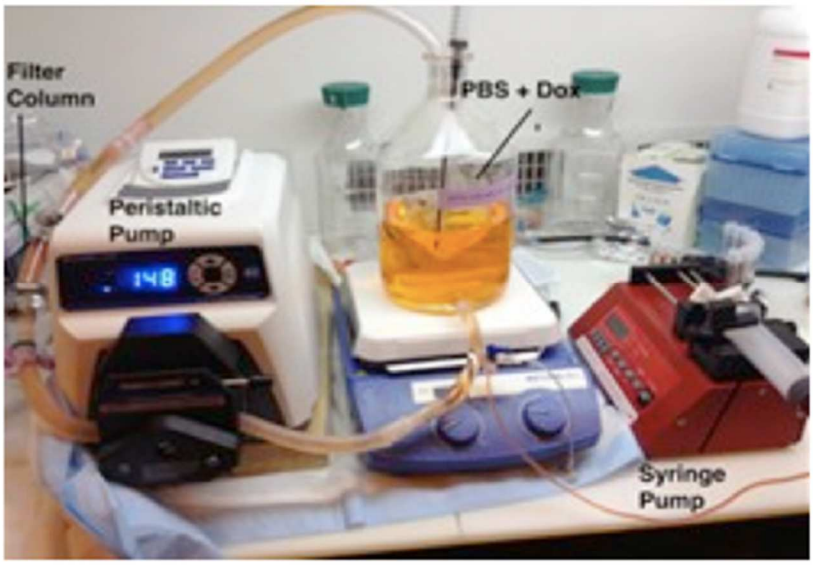


Figure 19. Flow Model Setup. Peristaltic Pump pumps the solution (PBS, Porcine Serum, or whole Porcine Blood. The solution also contains chemotherapy and ChemoFilter. Samples are taken at specific time points.

3. Carboplatin Radiolabeling

Pure chemical Cisplatin, as opposed to pharmaceutical grade, will be used in a mechanism to radiolabel using $^{11}CO_2$. Once Carboplatin is radiolabeled, testing will begin on the binding kinetics of various ChemoFilter prototypes, in vitro using gamma or scintillation counting. IPC-MS and/or HPLC can be a secondary quantification of [Carboplatin]. In vivo studies will begin in porcine. They will involve endovascular administration of chemotherapy and subsequent filtration via ChemoFilter with real-time visualization of the ^{11}C-Carbopatin. This will verify the effectiveness of ChemoFilter in vivo.

4. ChemoFilter Device Design

Device prototypes are being developed in collaboration with the Greer Lab at the California Institute of Technology. The current design, as shown in Figure 20, is a synthetic nanotruss onto which we can attach, via covalent bonds, to ChemoFilter prototype(s) such as genomic DNA or

Dowex resin. Blood will flow through the device and come in contact with a large amount of genomic DNA to maximize binding efficiency. This nanotruss will also attach to an endovascular catheter. Another design is permeable membranes of specific geometry for human vascular entry including numerous bonding sites (27). Both designs allow for increased surface area, many ChemoFilter binding sites, more efficient drug filtration, and take human venous blood flow in to consideration. Based on these experiments, the importance of sequestering ChemoFilter, of specific geometries is better understood, giving support for the membrane design. This study gives evidence that a rectangular mesh tea bag type model increases efficiency. Finally, membranes may be able to bind to points on a nanotruss, thereby combining these two designs.

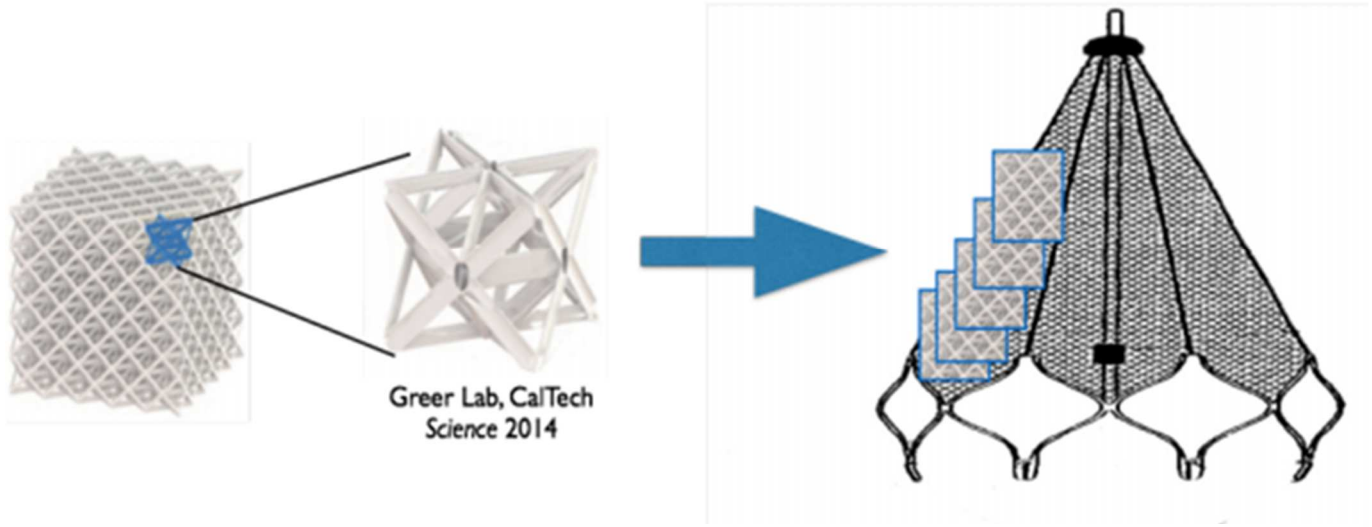


Figure 20. Nanotruss ChemoFilter Design. Has a geometry allowing many covalent bonds togenomic DNA and/or various ion-exchange resins, such as Dowex. It increases surface area by offering many binding sites. Blood can flow through and bind to ChemoFilter. This device will also be attached to an endovascular catheter that can be removed, removing the chemotherapy. This device is designed to be inserted into the human blood stream.

Conclusion

Chemotherapy is highly effective and affords patients a better quality of life than any of the current alternatives. However, the use of chemotherapeutics is limited due to their systemic toxicity. ChemoFilter is a novel device aimed at limiting this toxicity and thus the side effects associated with chemotherapy. With this reduced toxicity there is potential for increased dosage and improved outcomes. Two ChemoFilter prototypes include the DNA ChemoFilter and the Ion-Exchange ChemoFilter. The effectiveness of both prototypes in filtering Doxorubicin has been previously reported **(**1,2,3,4). However, their ability to filter other chemotherapeutics had been unknown. Cisplatin, a chemotherapeutic used in head and neck cancers, was filtered by both ChemoFilter prototypes with excellent binding kinetics. Our OPDA method using UV-Spectroscopy for Cisplatin quantification does not appear to be as accurate or reliable as ICP-MS. Mesh envelopes for ChemoFilter were tested, with materials and dimensions being optimized. Radiolabeling Carboplatin is in its early stages with the hopes of molecular metabolic imaging via PET. The DNA ChemoFilter shows the most promise as it has excellent binding kinetics for Doxorubicin and Cisplatin and is involved in the mechanism of action for these drugs. Based on these experiments the importance of sequestering ChemoFilter is better understood. Based on these experiments the importance of sequestering ChemoFilter is better understood and further designs considerations will be tested. Significant improvements have been made to ChemoFilter. This device is now one-step closer to making chemotherapy more efficient and more tolerable.

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