Title
Development of filter device to limit systemic toxicity from doxorubicin chemotherapy: DNA ChemoFilter

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Publication Date
2015

Peer reviewed|Thesis/dissertation

Development of filter device to limit systemic toxicity from doxorubicin chemotherapy: DNA ChemoFilter

by

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THESIS

Submitted in partial satisfaction of the requirements for the degree of

MASTER OF SCIENCE

in

Biomedical Imaging

in the

GRADUATE DIVISION

of the

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

[redacted]

Acknowledgement

I would like to thank my Thesis Committee, Drs. Steven Hetts, Alastair Martin, David Saloner, and Xiaoliang Zhang, for providing me with the support and guidance. Also, I would like to thank my teammates and friends, Leo Sze, Jeffrey Yang, Bradford Throne, Bridget Kilbride, Ashi Gautam, Andre Cote, Sravani Kondapavulur, Jeffrey Hu, Caroline Jordan, Karthik Raman, and Colin Yee for creating the most enjoyable research environment. Lastly, I sincerely thank Dr. Mariam Aboian for her mentorship, encouragement, patience, generosity, and kindness. I could not have asked for a better mentor.

Development of filter device to limit systemic toxicity from doxorubicin chemotherapy: DNA ChemoFilter

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Abstract

Objective: ChemoFilter is a new class of image-guided temporarily deployable, endovascular catheter based medical device that selectively filters chemotherapeutic agents from the bloodstream to limit systemic toxicities. We report a novel method to filter doxorubicin in blood using genomic DNA as resin.

Materials and Methods: DNA binding experiments were performed *in vitro* with doxorubicin in PBS solution and porcine serum. Genomic DNA was used for binding experiments, with the DNA either free in solutions or with the DNA encapsulated in packets made with nylon or polyester mesh. Optimum concentration of genomic DNA to filter 50 mg doxorubicin in solution was determined. We then compared the kinetics of filtering doxorubicin by genomic DNA as compared to our previously published ion exchange resin based ChemoFilter.

Results: Doxorubicin concentration was reduced by over 90% within 1 minute with free DNA in PBS and porcine serum. With packaged DNA, doxorubicin concentration was reduced by 50% within 5 minutes in PBS, and 90% within 1 minute in porcine Serum.

Doxorubicin filtering kinetics between DNA and the ion exchange resin Dowex were compared in each binding experiments and the DNA displayed more rapid kinetics in all situations.

Conclusion: We describe a new version of ChemoFilter that uses DNA as the binding agent for DNA binding drugs. DNA ChemoFilter allows selective filtration of DNA binding chemotherapeutic agents and demonstrates more rapid kinetics than previously described ion exchange version of ChemoFilter.

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Introduction

Hepatocellular carcinoma (HCC) is the third leading cause of death worldwide [1]. There are an estimated 800,000 new cases each year worldwide, and up to 80% of HCC is not surgically curable [2]. Image-guided transarterial chemoembolization (TACE) with doxorubicin is the standard of care for non-operative hepatocellular carcinoma [3, 4]. Doxorubicin is a low-cost and highly effective chemotherapeutic agent used in TACE with a standard intra-arterial dose of 50 mg. However, TACE is limited by its side effects as doxorubicin can lead to system toxicity such as bone marrow suppression, alopecia, gastrointestinal toxicity and irreversible cardiac failure [5]. Previous studies have shown that first-pass hepatic clearance of doxorubicin is between 50-70% [6]. However, clinical and experimental studies demonstrate a positive linear relationship between doxorubicin dose and tumor suppression, providing the motivation for higher-dose doxorubicin chemotherapy [7, 8].

A novel device was developed in the Hetts Laboratory, which allows selective removal of chemotherapeutic agents from the bloodstream. The original idea for ChemoFilter arose from a critical need to reduce the concentration of doxorubicin that transits through the liver and reaches the heart during intra-arterial treatment of hepatocellular carcinoma. ChemoFilter was proposed to prevent significant cardiac toxicity from doxorubicin by filtering the venous outflow from the liver and thus lowering the doxorubicin concentration that is reaching the heart (Figure 1). ChemoFilter can be placed via an endovascular approach into the venous outflow from the tumor that is being treated with intra-arterial chemotherapy (IAC). The goal is to have the filter in the vessel during treatment with chemotherapy and to remove the filter at the completion of treatment. The first version of the ChemoFilter utilizes ion exchange resin to selectively remove ionized doxorubicin from solution and is designed for treatment of hepatocellular carcinoma.

Clinically, ChemoFilter will allow administration of high concentration of intra-arterial doxorubicin for cancer therapy, while at the same time limiting the systemic toxicities of doxorubicin in a cost-effective manner.

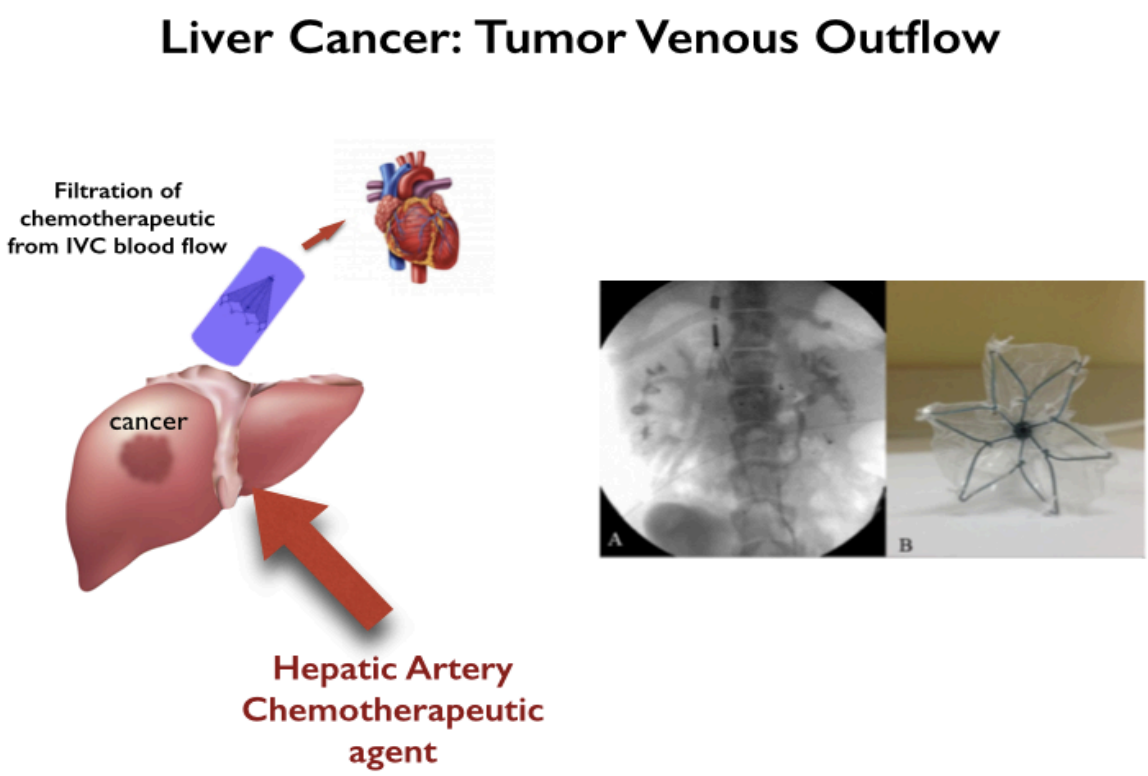

Figure 1. Role for ChemoFilter in treatment of Liver Cancer**.** From a percutaneous femoral arterial approach, a microcatheter is guided into the arteries feeding the liver tumor to directly inject Doxorubicin. From a percutaneous jugular venous approach, the ChemoFilter device is guided through the superior vena cava (SVC) and deployed in the veins draining the liver proximal to the heart, the site of dose-limiting systemic toxicity.

Our laboratory initially described an ion exchange based filtering method for ChemoFilter and showed that it is effective in filtering doxorubicin within the inferior vena cava in porcine model [9, 10]. We now report a novel method to filter chemotherapy drugs in bloodstream that have intrinsic DNA binding activity (Figure 2). We show that immobilized DNA effectively filters chemotherapy drugs with DNA binding activity and can be used as a resin in ChemoFilter. DNA

ChemoFilter allows for selective filtering of chemotherapeutic agents that have DNA binding activity such as doxorubicin and cisplatin. The latter is also a chemotherapeutic agent commonly used in IAC that functions by binding to DNA. The purpose of this preliminary study was to perform proof of concept *in vitro* experiments to show that DNA can act as an effective resin for binding of doxorubicin.

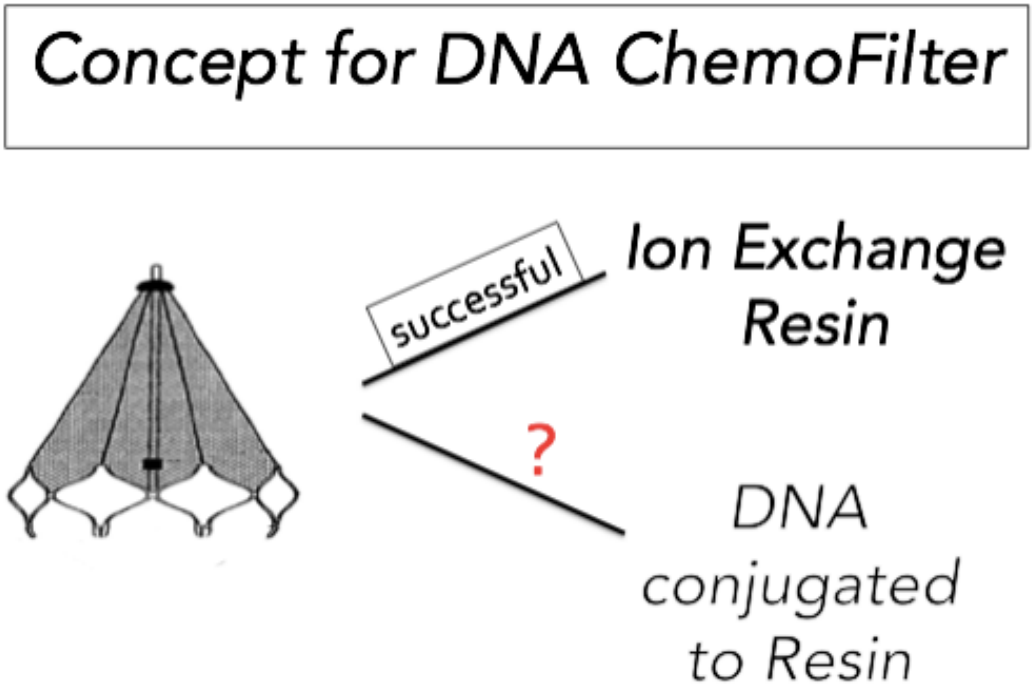

Figure 2: DNA ChemoFilter concept. An umbrella shaped object (left) is opened in a draining vein that blood must pass through before entering the systemic circulation. Entrapment of chemotherapeutic drugs can be achieved via either ion exchange or DNA based resins.

Materials and Methods

DNA binding experiments were performed *in vitro* with doxorubicin in phosphate-buffered saline (PBS) solution and porcine serum. Genomic DNA (salmon sperm, Sigma-Aldrich, St. Louis, MO) was used for binding experiments. Optimum concentration of genomic DNA to filter 50 mg doxorubicin in solution was determined by comparing doxorubicin-filtering kinetics between 50 mg, 20 mg, and 2 mg DNA. We then compared the kinetics of filtration of Doxorubicin between genomic DNA and the established ion exchange resin (Dowex 50-100, Sigma-Aldrich, St. Louis, MO). Kinetics comparisons were performed in PBS and porcine serum. Fifty mg genomic DNA and 1.9 g Dowex were reacted freely with 2.5 mg doxorubicin individually in 50 ml solutions in 50 ml Falcon tubes over 15 minutes and samples were collected periodically. Doxorubicin concentration in the samples was determined using a spectrofluorometer (Spectramax Gemini EM, Molecular Devices, Sunnyvale, CA). Fluorescence was induced by excitation wavelength at 480 nm, and emission was monitored at 550 nm.

To make an immobilized platform that could be engineered into an endovascular catheter, we encapsulated DNA in packets made with nylon or polyester mesh. Nylon mesh of different pore size (31 um, 70 um, 104 um, 160 um, 215 um, 255 um) was evaluated for filtering doxorubicin out of solution. Doxorubicin filtering kinetics of the encapsulated DNA and encapsulated Dowex was compared in porcine serum. Fifty mg packaged genomic DNA and 1.9 g packaged Dowex were reacted individually with 2.5 mg doxorubicin in 50 ml solutions in 50 ml Falcons tubes Doxorubicin concentration in the collected samples were measured at 480 nm using a spectrofluorometer. Leakage of DNA from nylon packets was also measured. Samples were taken at the end of the reaction to test for DNA concentration where absorbance were measured

at 260 nm using a spectrophotometer (U-2810, Digilab Hitachi, Marlborough, MA). All *in vitro* experiments were performed at room temperature.

Results

Genomic DNA is an effective Resin for filtering doxorubicin from solution

To test whether genomic DNA can act as a resin to filter doxorubicin from solution, initial experiments were performed with free genomic DNA in solution. We first tested the concentration of genomic DNA needed to remove 50 mg of doxorubicin from 50 ml PBS solution. We used a range of DNA concentrations including 1 mg/ml, 0.4 mg/ml, and 0.04 mg/ml and found that 1 mg/ml genomic DNA resulted in over 90% decrease in doxorubicin concentration from solution within 1 minute of reaction (Figure 3).

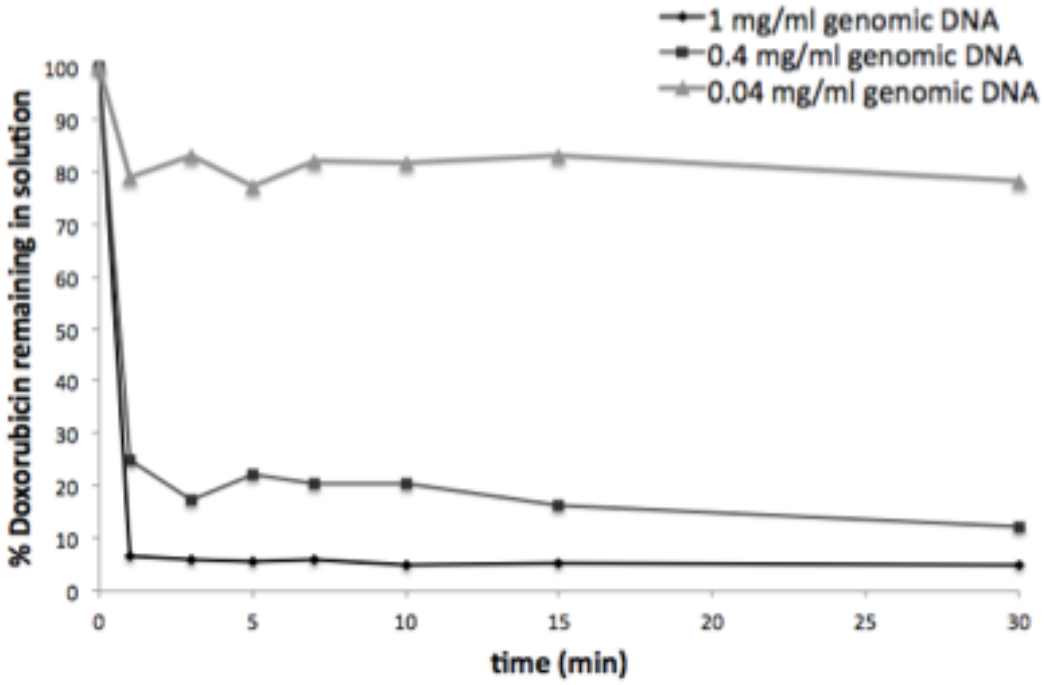

Figure 3: Filtering in Doxorubicin from solution is dose dependent on the amount of DNA.

Genomic DNA demonstrates faster kinetics in filtering doxorubicin from solution

The next step was to compare how effective DNA is in filtering doxorubicin in solution to established ion exchange resin Dowex. We performed these experiments *in vitro* in 50 ml PBS solution and used free 1 mg/ml genomic DNA and 0.19 g of Dowex 50-100 resin. Our results show that the kinetics of removal of Doxorubicin in solution is different between DNA and Dowex and that DNA shows a much more rapid reduction in Doxorubicin from PBS (Figure 4A). We also performed these experiments in porcine serum and found similar result (Figure 4B).

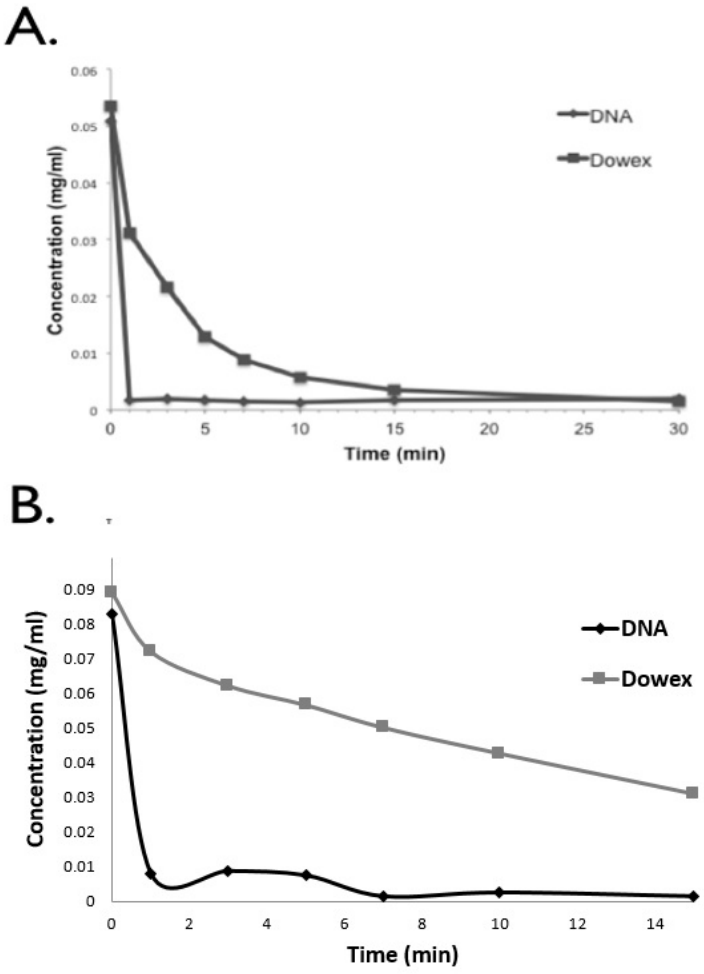

Figure 4: Comparison of DNA to ion exchange resin Dowex for filtering Doxorubicin in PBS (A), porcine serum (B).

Genomic DNA packaged in packets demonstrates minimal leakage of DNA into solution

The next step was to determine how to can package DNA into a ChemoFilter design. The first prototype that we tested was a "packet" model (Figure 5A). Using the packet model, we showed that leakage of DNA from the 160 micron nylon packet was only 0.5% of the DNA (Figure 5B). After 60 minutes reaction of encapsulated DNA and doxorubicin in PBS, samples of the solution were collected and measured for DNA concentration by detecting absorbance at 260 nm.

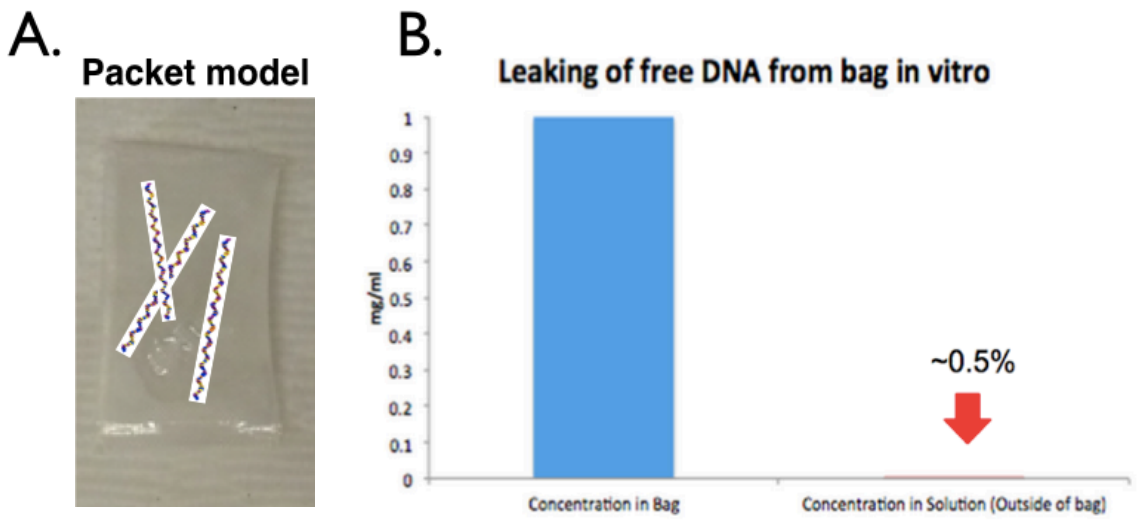

Figure 5: Packet model of DNA within nylon or polyester membrane (A). Genomic DNA is encapsulated in a sealed packet made with nylon or polyester mesh. Leaking of DNA into the solution is minimal after 60 minutes of reaction between encapsulated DNA and doxorubicin (B).

Genomic DNA encapsulated in nylon or polyester packets demonstrates reduced kinetics in filtering doxorubicin from solution

To test whether the packet model of sequestering DNA for use in ChemoFilter is effective we compared different types of nylon mesh (Figure 6). We found that 160 um packets were effective in reducing doxorubicin concentration to approximately 50% within 5 minutes of reaction in PBS (Figure 6).

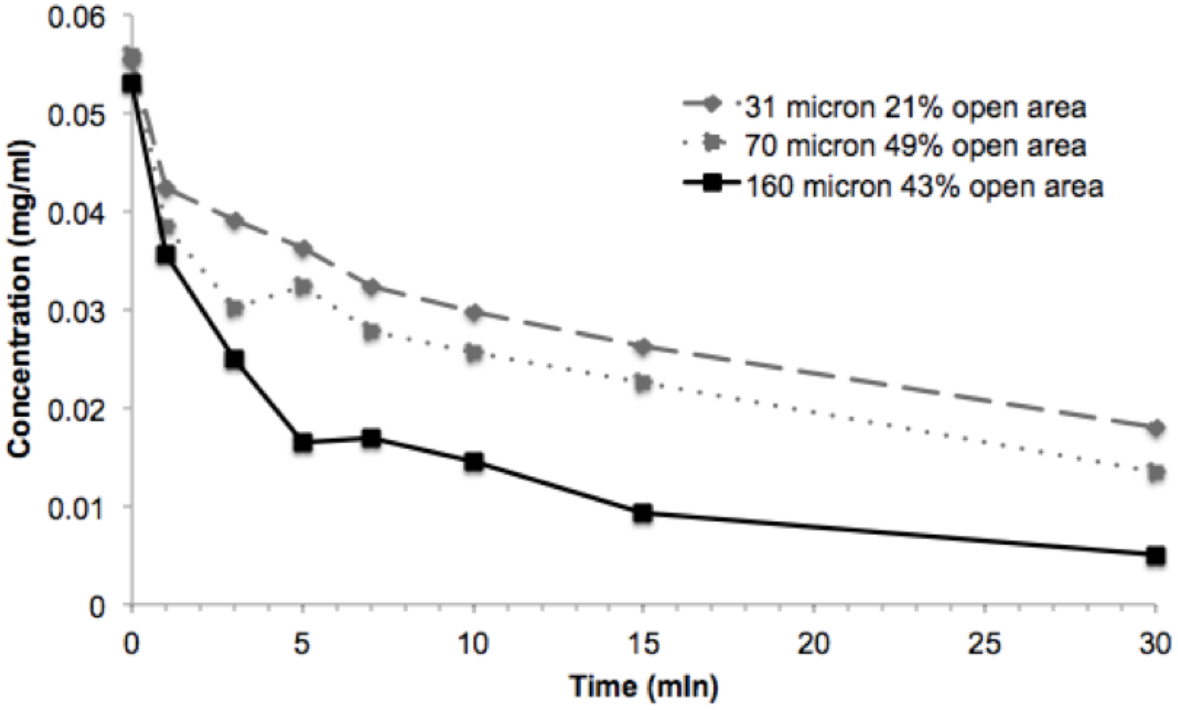

Figure 6: Comparison of doxorubicin filtering kinetics of DNA encapsulated in nylon mesh of different pore size and open area.

Genomic DNA packaged in nylon or polyester packets demonstrates faster kinetics in filtering doxorubicin from solution than ion exchange Dowex

A comparison was made of the efficacy of DNA in filtering doxorubicin in solution relative to ion exchange resin Dowex in packet. We performed these experiments *in vitro* in 50 ml porcine serum and used nylon and polyester mesh packaged 1 mg/ml genomic DNA and 0.19 g of Dowex 50-100 resin. Our results show that the kinetics of removal of doxorubicin in solution is different between packaged DNA and Dowex and that DNA shows a much more rapid reduction in doxorubicin from solution in both nylon and polyester mesh packets (Figure 7, 8).

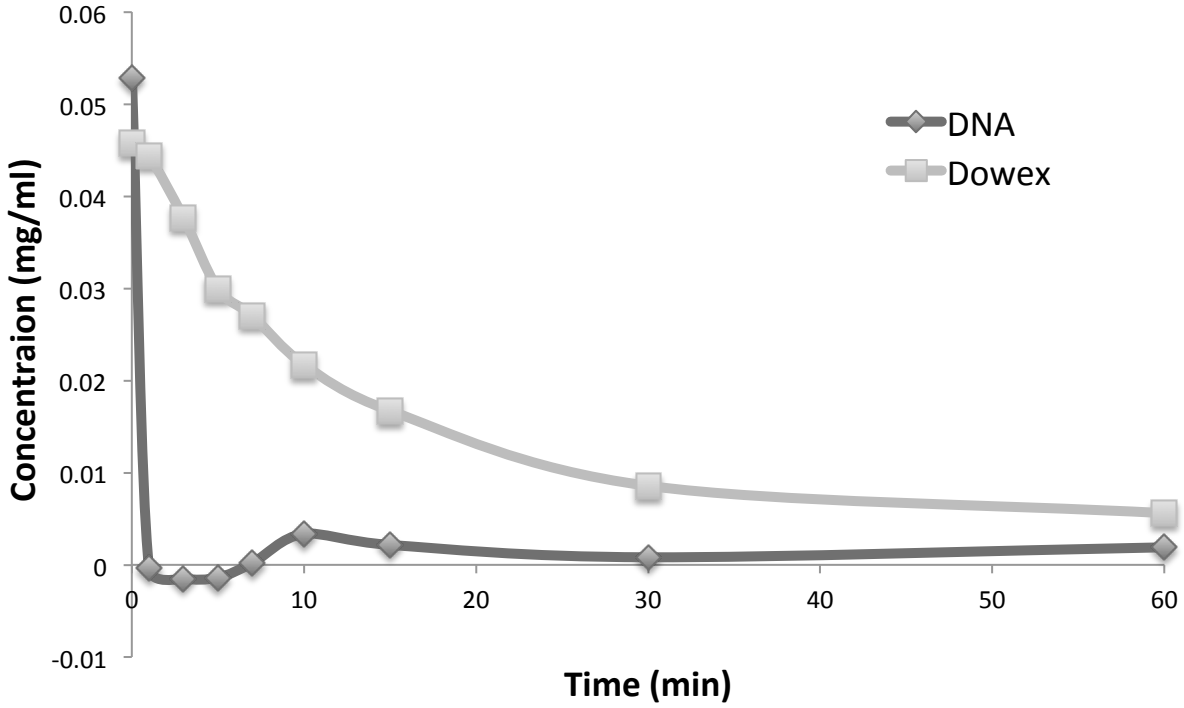

Figure 7: Comparison of DNA to ion exchange resin Dowex encapsulated in 160 nm nylon packets for filtering doxorubicin in porcine serum.

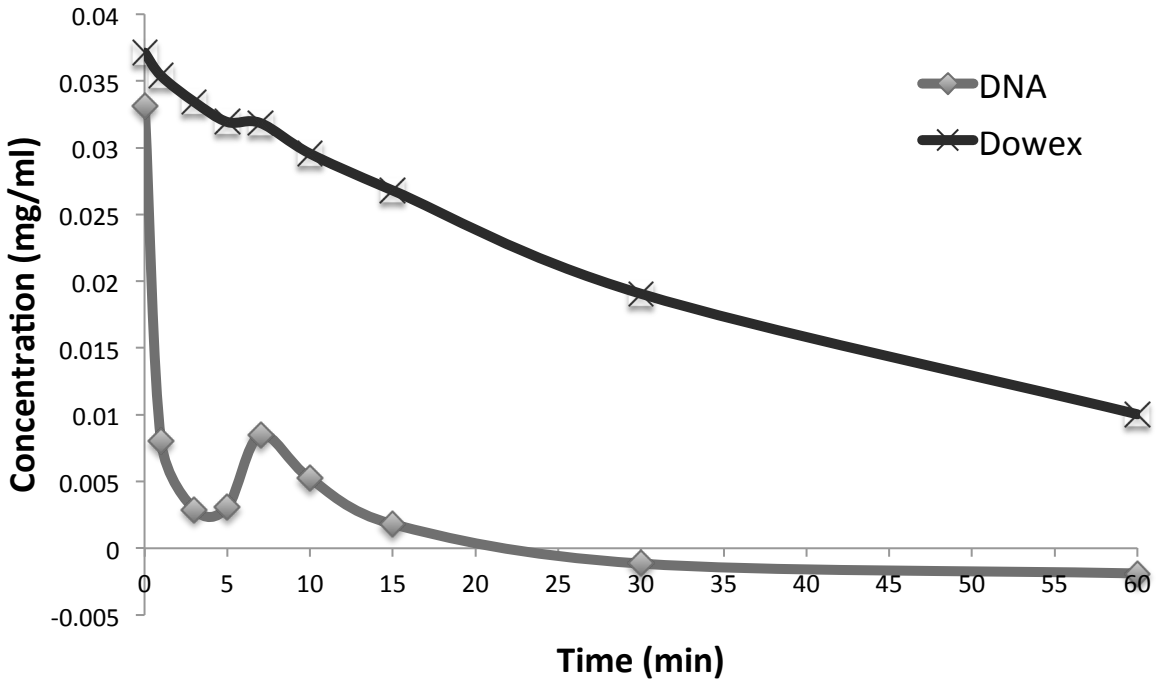

Figure 8: Comparison of DNA to ion exchange resin Dowex encapsulated in 160 nm polyester packets for filtering doxorubicin in porcine serum.

Discussion

In vitro proof of concept experiments showed that DNA in solution has rapid binding kinetics to doxorubicin and performs as an effective filter for doxorubicin; we demonstrated that 95% of doxorubicin is filtered from solution in PBS and 94% of doxorubicin is filtered from solution in porcine serum within 1 minute of reaction time. DNA binding kinetics to doxorubicin is dose dependent. One mg genomic DNA per mg doxorubicin was shown to be an effectively dose for filtering doxorubicin from solution. Also, DNA based filtration method for doxorubicin demonstrated faster kinetics compared to the ion exchange resin filtration method.

When genomic DNA was encapsulated in packets, doxorubicin-filtering kinetics in PBS was reduced (Figure 6). The reduction in filtering kinetics was likely due to the physical constraint of the packaging material that reduced the surface area for efficient exchange of solutions. However, compared to filtering kinetics of packaged DNA in porcine serum, packaged DNA showed more rapid filtering kinetics in porcine serum than in PBS. This could be a result of improved laboratory techniques such as improved pipetting and preparation efficiency over time as the packaged DNA in porcine serum experiments were performed at a later period of the study.

Moreover, a small variability was seen in the doxorubicin filtering kinetics of DNA in both Figure 8 and 9 where the concentration of doxorubicin dropped below 0. We speculate that the spectrophotometer used to detect fluorescence of doxorubicin was unable to precisely measure a very low concentration of doxorubicin. Although DNA leakage from nylon packets was shown to be minimal (0.5%), in the future we aim to limit leakage by binding DNA to resin that is larger than the packet pore size (Figure 12).

Initially, a polyester mesh packet was tested for doxorubicin filtering kinetics in addition to a nylon mesh packet due to inconsistent results observed in DNA filtering kinetics when packaging genomic DNA in nylon mesh. An experiment was performed to test for doxorubicin filtering kinetics for different mesh materials where we found that the packets made with the newly purchased nylon mesh worked poorly while the polyester mesh packets were effective and consistent (Figure 9). Further investigation of additional mesh materials that allow good exchange of doxorubicin solution with DNA is warranted.

Future studies will perform flow experiments with packaged DNA to test for doxorubicin filtering kinetics under an environment that simulates blood flow *in vitro* with a flow model (Figure 10). Ultimately this will involve *in vivo* experiments in porcine animal models.

In parallel, we are currently collaborating with the Greer Lab at California Institute of Technology in building ChemoFilter with nanotruss (Figure 11). Amino groups can be incorporated into the surface of nanotruss and bind to DNA covalently. Computer modeling and crystallographic studies have suggested that sequences $d(TATATA)_2$, $d(TGAT)_n$, and d(CGATCG) have high affinity for binding doxorubicin and thus would be excellent candidates for drug sequestration [11-13]. In theory, ChemoFilters built with nanotruss can greatly improve the surface area for doxorubicin binding.

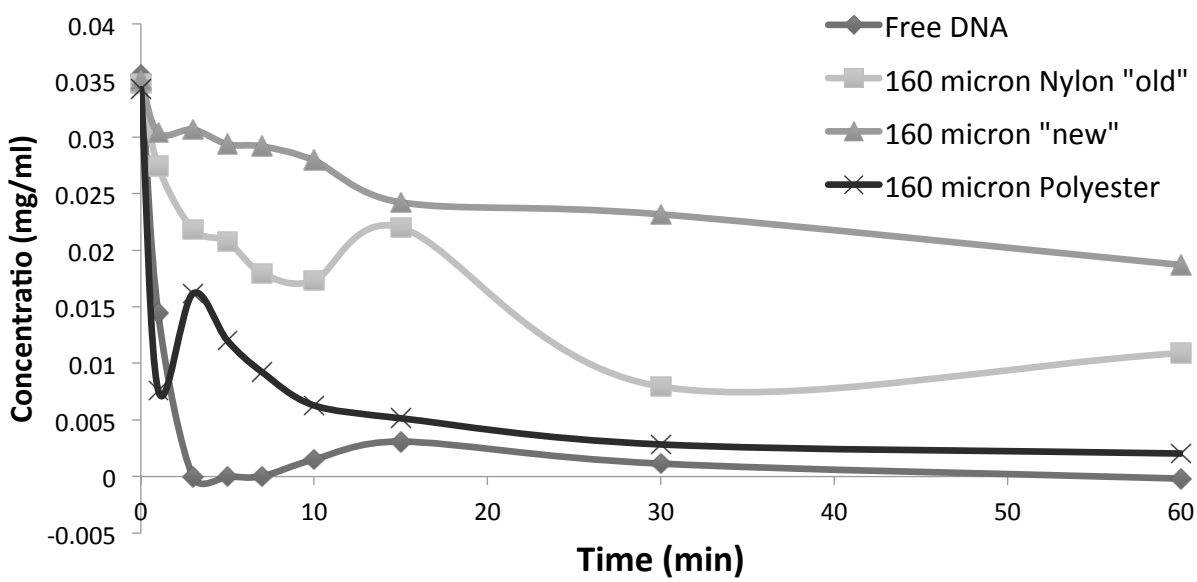

Figure 9: Comparison of doxorubicin filtering kinetics of DNA encapsulated in packets made with different mesh materials.

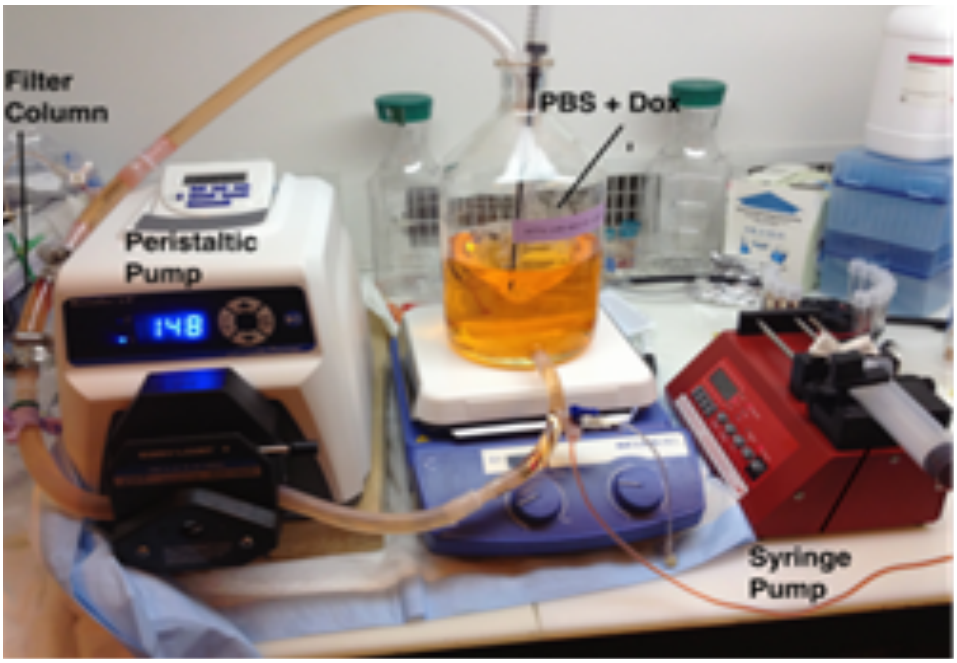

Figure 10: The set-up of the flow model that simulates blood flow *in vitro*.

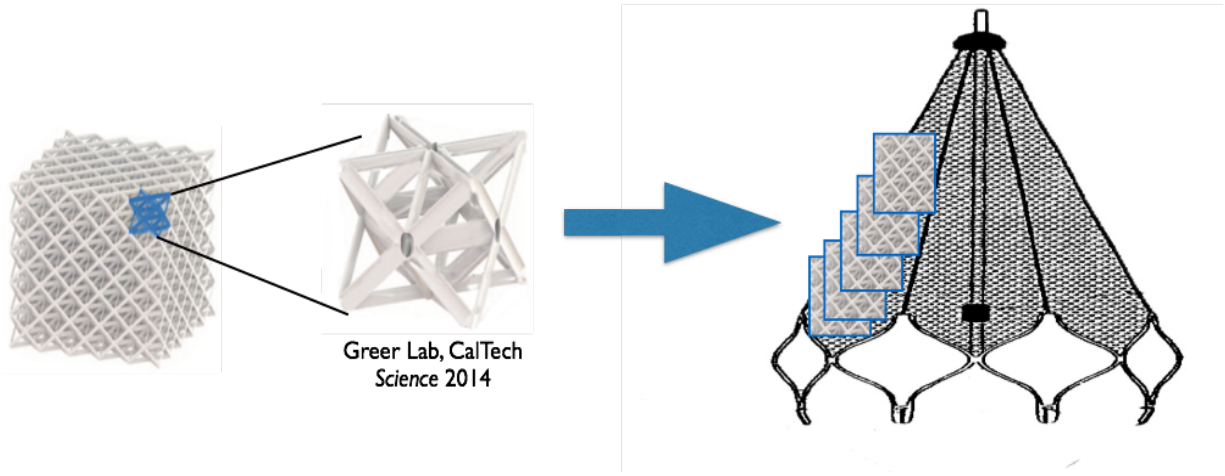

Figure 11: A proposed method of engineering nanotruss onto ChemoFilter. Binding DNA onto nanotruss increases surface area for doxorubicin filtering.

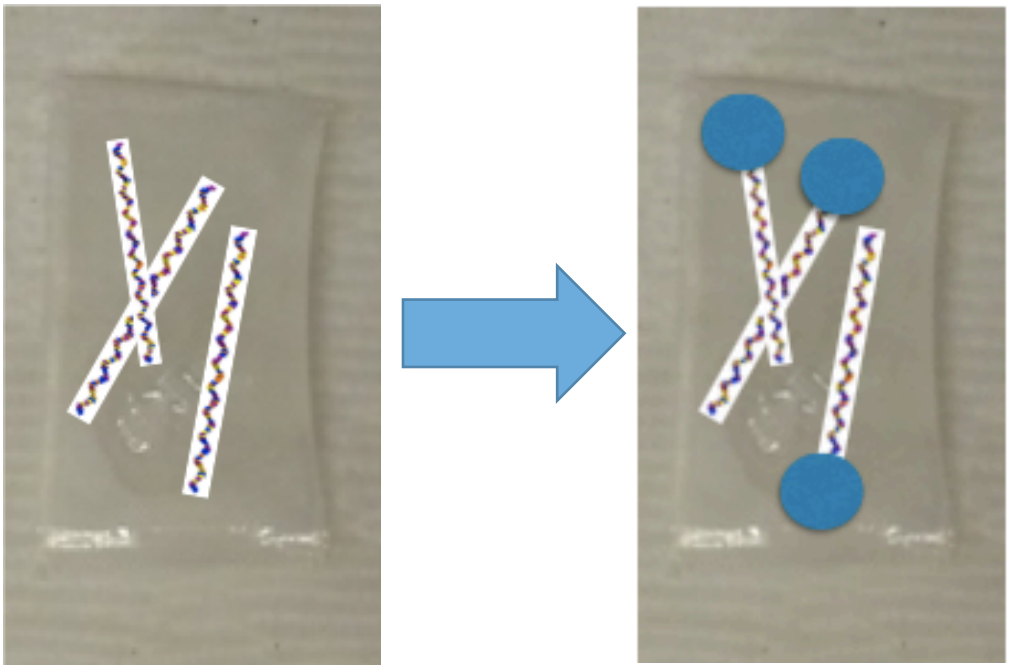

Figure 12: Genomic DNA immobilized in nylon or polyester packet with beads. Genomic DNA is linked to beads that are larger than the mesh pore size to prevent leakage.

Conclusion

Many highly effective chemotherapy drugs have been developed, but their use is significantly limited by their systemic toxicity. TACE with doxorubicin is currently used for hepatocellular carcinoma as a way to administer higher concentrations of the drug to the tumor while limiting systemic side effects. One of the limiting factors in TACE of hepatocellular carcinoma is high liver bypass rate. As high as 50-70% of the drug administered to the hepatic artery enters the systemic circulation, resulting in systemic toxicity. ChemoFilter is a new class of image-guided temporarily deployable, endovascular catheter based medical device that selectively filters chemotherapeutic agents from the bloodstream to limit systemic toxicities. We describe a new version of ChemoFilter that uses DNA as a binding agent for DNA binding drugs. We found DNA based resin to be superior to ion exchange resin in doxorubicin binding volume and the rate at which it could extract doxorubicin from solution. Nylon and polyester mesh were explored as a platform to contain DNA resin on the ChemoFilter. With DNA encapsulated in packets made with either nylon or polyester mesh, DNA was also found to extract doxorubicin from solution with greater efficiency than ion exchange resin. In sum, DNA ChemoFilter allows selective filtration of DNA binding chemotherapeutic agents and demonstrates more rapid kinetics than the previously described ion exchange version of ChemoFilter.

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