Delivery of Chemotherapeutic Agents with Nanotube Induced Hyperthermia

Nicole Levi-Polyachenko,[*,**] Eric Merkel,[***] Bradley T. Jones,[***]
William D. Wagner,[**] David L. Carroll,[*] John H. Stewart IV[****]

* Center for Nanotechnology and Molecular Materials, Wake Forest University
** Department of Plastic and Reconstructive Surgery, Wake Forest University
*** Department of Chemistry, Wake Forest University
**** Department of General Surgery, Section on Surgical Oncology, Wake Forest University

ABSTRACT

Carbon nanotubes are unique materials that absorb infrared (IR) radiation, especially between 700 to 1100nm, where body tissues are most transparent. A method to enhance drug localization for peritoneal malignancies is perfusion of warm (40-42ºC) chemotherapeutic agents in the abdomen. However, all tissues in the peritoneal cavity are subjected to enhanced drug delivery due to increased cell membrane permeability at hyperthermic temperatures. Here we show that rapid heating (within ten seconds) of cancer cells to 42ºC, using infrared stimulation of nanotubes as a heat source, in the presence of the chemotherapeutic agents, is as effective as two hours of radiative heating at 42ºC. This approach has the potential to be used as a rapid bench to bedside clinical therapeutic agent with significant impact for localizing chemotherapy agents during the surgical management of cancer.

Keywords: carbon nanotubes, hyperthermia, chemotherapeutics, intraperitoneal chemoperfusion, colorectal cancer

1 INTRODUCTION

Nanotubes are strong absorbers of infrared light, but body tissues are transparent to this region.[1] As nanotubes absorb IR, they emit heat, thus increasing the temperature of their surrounding media and may be used to heat tissue in a localized region. A main advantage of using carbon nanotubes as a heat source is their efficiency to heat, so fewer molecules of the heat absorber (nanotubes) are needed and the time of infrared application can be reduced.

A clinically applied method called heated intraoperative intraperitoneal chemotherapy (IPHC) is used to enhance drug localization for abdominal malignancies.[2; 3] IPHC involves surgical removal of accessible tumor followed by a two hour perfusion of the peritoneum with a chemotherapeutic agent warmed to 40-42ºC.[2-4] Figure 1 shows a schematic of the drug perfusion circuit used clinically in IPHC procedures. Although IPHC has significantly improved patient outcomes,[2; 3] the procedure has significant limitations, including lengthening the time that a patient must be anesthetized and requiring liters of drug perfusate which is costly.[2; 3]

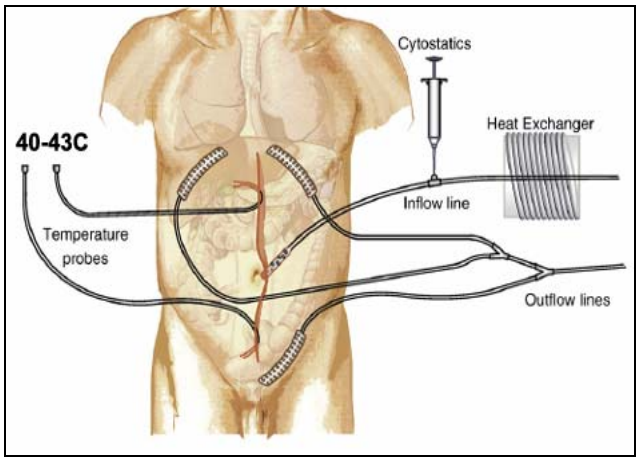

Figure 1: Perfusion circuit of chemotherapeutic agent delivery used in IPHC procedures. Reproduced from [5].

Methods for using carbon nanotubes to eliminate tumors include thermal ablation therapy, in which the nanotube is excited by infrared radiation,[1] and intracellular drug transport.[1; 6] Hyperthermia, defined as temperatures above 37ºC, is a common technique used clinically to treat a variety of malignancies, like breast, liver and colorectal cancers.[7; 8] Since carbon nanotubes absorb infrared light, and can heat a local area, they are an acceptable choice for treatment of cancer.[1; 9] Hyperthermia increases cellular metabolism and cell membrane permeability for increased drug uptake by cells. We use hyperthermia below the ablation threshold (less than 45ºC) to raise the local temperature and enhance drug uptake by cells.

Iron is a common catalyst for growth of chemical vapor deposition multi-walled nanotubes (CVD-MWNT) and has the potential for imaging applications such as magnetic resonance imaging. Therefore, MWNT grown using 60mg or 400mg of iron catalyst were used to examine the effect that catalysts may have on thermodynamic potential. Oxaliplatin and mitomycin C (MMC) are two chemotherapeutic agents used in IPHC clinically for treatment of colorectal cancer. Doxorubicin is a chemotherapeutic used for many types of cancers and is

commonly used for breast. For each of these agents, a synergistic effect has been found clinically when the drugs are given at elevated temperatures.

2 MATERIALS AND METHODS

2.1 Cells and reagents

RKO colorectal cancer cells, and MDA MB231 breast cancer cells were purchased from American Type Culture Collection and cultured in McCoy's or DMEM media, supplemented with 2.5 μg/ml amphotericin, 1% L-glutamine, 1% penicillin/ streptomycin, and 10% fetal bovine serum. Cells were plated into 48-well tissue culture dishes at a seeding density of 10,000 to 20,000 cells per well. Oxaliplatin, mitomycin c, and doxorubicin were purchased from Sigma-Aldrich.

2.2 Nanotube preparation

Multi-walled nanotubes were grown by chemical vapor deposition methods according to the procedures of Czerw et al.[10] Catalyst amounts were varied by addition of ferrocene at the beginning of growth. Fifty milligrams of nanotubes were shortened and cleaned by sonication in 90 ml sulfuric acid and 30 ml of nitric concentrated acids for 20 h. Nanotubes were approximately 2 μm long, as determined by transmission electron microscopy. Nanotube suspensions were prepared by adding 1 mg of MWNT per 1 ml of water with 0.1% bio-compatible Pluronic surfactant, PC127; these suspensions were briefly sonicated, and the solution pH balanced prior to tissue culture use. The nanotube suspension was added to media at a concentration of 100 μl stock per 1 ml of media, for a final concentration of 100 μg per ml of media.

2.3 Heating methods

A Nd:YAG laser (1,064 nm) operating at 3 W of power was used to apply infrared stimulation to the nanotubes, with a beam diameter of 1 cm. A thermocouple measured the temperature of nanotube/ media solutions immediately after laser application. The time needed to raise the temperature of the wells to 42ºC varied from 8 to 11 seconds per well, depending on ambient room temperature.

A dilution series of MWNT grown with 60 or 400mg of ferrocene, cut or uncut, was done from 0.001 to 1 mg/ml to evaluate temperature increases for 30seconds of infrared exposure. This analysis enabled us to determine that 0.1mg/ml of MWNT is acceptable for rapid hyperthermia in cell culture.

2.4 Drug treatments

Five treatment variables included a control of media, a water dilution (230 μl/1 ml) of the media with 0.1% PC127, 300 μM oxaliplatin, 100 μg/1 ml of media nanotubes, and 300 μM oxaliplatin plus 100 μg nanotubes per 1 ml of media. Media with treatments was added immediately prior to laser exposure. RKO colorectal cancer cells were treated with 0.1% PC127, 0.1% PC127 plus 40 μM MMC, 100 μg of nanotubes, 100 μg of nanotubes plus 40 μM MMC, or control (no treatment). The same amount of nanotubes or water with 0.1% PC127 was used with 10 μM Doxorubicin.

2.5 Cell viability assays

One 48-well plate (with the treatment options) was held at 37ºC and another 48-well plate was held at 42ºC. A third plate was held at 37ºC until laser application. Infrared treatment was applied three times, for 8 to 11 seconds per application, over 2 hours. Following incubation, treatment media was replaced with fresh media and the three plates incubated at 37ºC for 48 h. Following incubation, cell viability was quantified over 3 h using Promega's CellTiter 96® AQ_{ueous} assay kit.

3 RESULTS

MWNT were grown with 60 or 400mg of iron catalyst to examine the effect of the catalyst on thermodynamic potential. A dilution series of MWNT, dispersed in water containing the surfactant, Pluronic, was evaluated for 30seconds of infrared laser application and the results are provided in figure 2. Not only does cutting shorten the nanotubes, but intratubular iron if also released and carboxylic acid groups form along the tube sidewalls. This leads to tubes that are more soluble in aqueous media and skews mass calculations slightly since the intratubular mass of the catalyst means there are fewer nanotube oscillators in the solution. At all dilutions, MWNT grown with minimal iron (60mg) had the highest temperature, indicating that the more efficient heat generating nanotubes are the ones grown with the least amount of catalyst.Based on the results of figure 2, we concluded that multi-walled nanotubes with minimal catalyst should be used for subsequent cellular hyperthermia procedures since temperature increases are analogous for 0.1 and 1mg/ml dilutions.

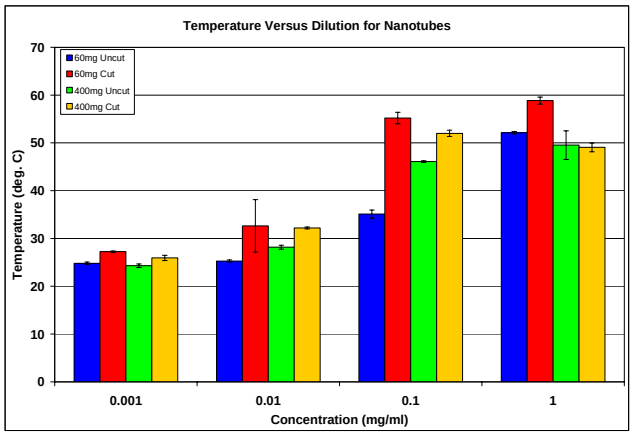

Figure 2: Dilution series of MWNT with corresponding temperature increases when infrared stimulation is applied for 30 seconds at 3W power.

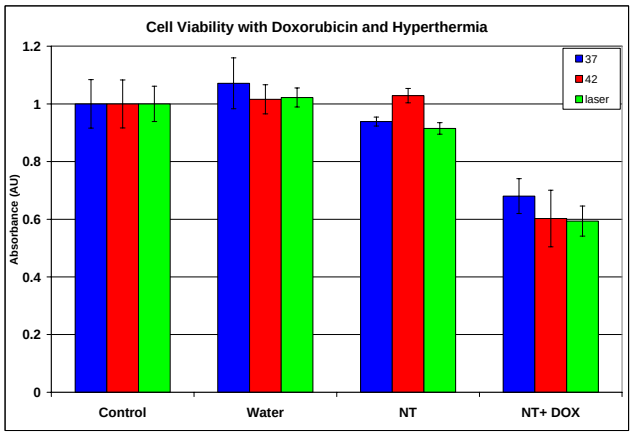

Figure 3: Doxorubicin treated breast cancer cells. Treatments consisted of incubation with culture media, media plus a water dilution, nanotubes (NT) or nanotubes and doxorubicin at 37ºC, 42ºC for two hours, or 37ºC for two hours with 10seconds of infrared laser application.

Hyperthermia induced by infrared stimulated MWNT leads to enhanced cellular metabolism and membrane permeability. Cell viability is analogous to the absorption of the MTS marker dye for analysis. When MDA MB 231 breast cancer cells were treated with Doxorubicin at 37ºC, cell death is less than for populations treated at 42ºC for two hours or rapidly heated to 42ºC using nanotubes and infrared light, as shown in figure 3. Our methods were effective in two different colorectal cancer cell lines. Cell viability assays demonstrated that when cells are stimulated with infrared light, oxaliplatin, or MMC alone, the results were comparable to cell incubation at 37ºC, as shown in figure 4a and 4b. However, once nanotubes are employed to induce hyperthermia to 42ºC via 10-seconds of infrared absorption, cell viability most closely matches that seen after cells were treated at 42ºC for 2 hours. A significant reduction in cell viability was seen in RKO cancer cells treated with nanotubes, MMC, and laser relative to treatment with these agents alone. When nanotubes were added and infrared stimulation applied for approximately 10 seconds, cell survival was reduced to a level comparable to cells incubated at 42ºC for 2 h. Most importantly, the time for increased drug uptake and decreased viability was reduced from 2 h to about 10 seconds using nanotubes.

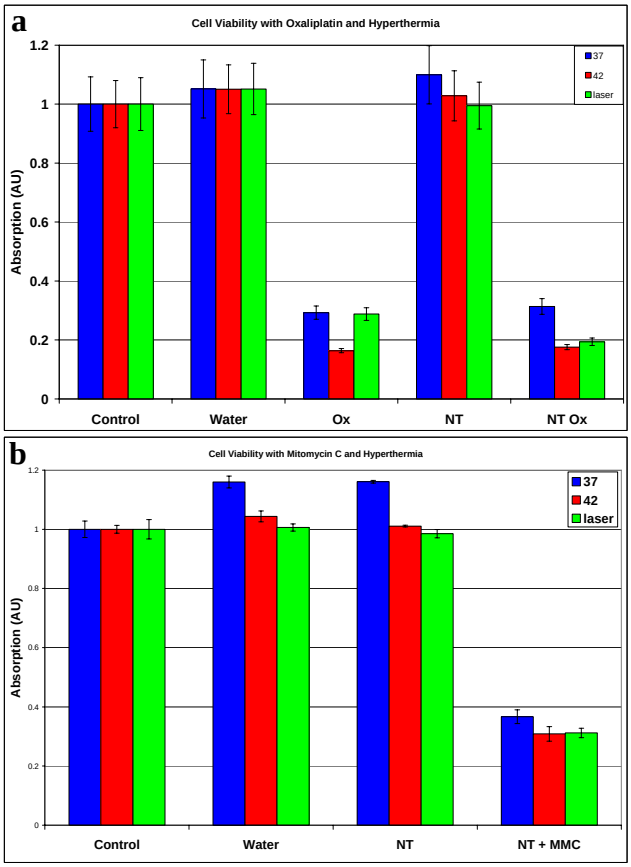

Figure 4: RKO colorectal cancer cells treated with culture media, media plus a water dilution, nanotubes (NT) or nanotubes and oxaliplatin (a) or mitomycin c (b) at 37ºC, 42ºC for two hours, or 37ºC for two hours with 10seconds of infrared laser application.

4 CONCLUSIONS

We have focused on intraperitoneal hyperthermic chemotherapy delivery (IPHC) as the foundation for our analysis, because it combines chemotherapy and surgical technique, and infrared radiation could be applied easily. Since only low hyperthermic temperatures (42ºC) via near-infrared stimulation of the nanotubes are employed, the chances for thermal damage is reduced. Although the rapid hyperthermic chemotherapy method outlined here uses multi-walled carbon nanotubes as the absorbing source, other nanoparticles such as single-walled carbon nanotubes, nanohorns or metallic nanoshells could be used as the heat generating particle. We have shown that nanotubes are useful for hyperthermic chemotherapy delivery by causing rapid heating of the bulk solution containing oxaliplatin, doxorubicin, or mitomycin C to aid in cellular uptake of the drugs. Rapid hyperthermic chemotherapy using carbon nanotubes may be utilized as a rapid bench-to-bedside technique since it can be applied during surgical procedures and all the nanomaterial can be removed from the body following delivery. Most importantly, delivery of chemotherapeutic agents with nanotube induced hyperthermia may significantly reduce treatment times by increasing the amount of chemotherapeutic agent delivered locally.

REFERENCES

1. KAM N.W.S., O'CONNELL M., WISDOM J.A. & DAI H.J. (2005) *PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA* 102, 11600-11605

2. LEVINE E.A., STEWART J.H., RUSSELL G.B., GEISINGER K.R., LOGGIE B.L. & SHEN P. (2007) *JOURNAL OF THE AMERICAN COLLEGE OF SURGEONS* 204, 943-953

3. STEWART J.H., SHEN P. & LEVINE E.A. (2005) *ANNALS OF SURGICAL ONCOLOGY* 12, 765-777

4. STEWART J.H., SHEN P., RUSSELL G.B., BRADLEY R.F., HUNDLEY J.C., LOGGIE B.L., GEISINGER K.R. & LEVINE E.A. (2006) *ANNALS OF SURGICAL ONCOLOGY* 13, 624-634

5. REINGRUBER B., BOETTCHER M.I., KLEIN P., HOHENBERGER W. & PELZ J.O.W. (2007) *JOURNAL OF PEDIATRIC SURGERY* 42, E17-E21

6. KAM N.W.S. & DAI H.J. (2006) *PHYSICA STATUS SOLIDI B-BASIC SOLID STATE PHYSICS* 243, 3561-3566

7. HANAJIRI K., MARUYAMA T., KANEKO Y., MITSUI H., WATANABE S., SATA M., NAGAI R., KASHIMA T., SHIBAHARA J., OMATA M. & MATSUMOTO Y. (2006) *HEPATOLOGY RESEARCH* 36, 308-314

8. BASTIDE C., GARCIA S., ANFOSSI E., RAGNI E. & ROSSI D. (2006) *EUROPEAN JOURNAL OF SURGICAL ONCOLOGY* 32, 980-983

9. HEPPLESTONE S.P. & SRIVASTAVA G.P. (2006) *APPLIED SURFACE SCIENCE* 252, 7726-7729

10. CZERW R., LIU J.W. & CARROLL D.L. (2004) *NEW JOURNAL OF PHYSICS* 6,