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TITLE: “Delivery of Nano-Tethered Therapies to Brain Metastases of Primary Breast Cancer Using a Cellular Trojan Horse”

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Our studies provide insight that aqueous solubility is one of the keys to success for nanoparticle enabled delivery. We note that almost all the small molecule tyrosine kinase inhibitors have poor aqueous solubility, which suggests that innovative formulations of these compounds will be required. Absorption of light by the skull and scatter of light by the brain parenchyma significantly reduce the dose of light that can be delivered to intracranial lesions. This has prompted us to develop work-arounds, which include utilizing multiple NIR sources and examining various beam profiles. Our future plans also include examining the loading and unloading of therapeutics that are known DNA intercalators and which are water-soluble. We will also examine the effect of mimicking the interstitial fluid composition in our lapatinib release experiments to determine if this mitigates some of the aqueous solubility challenge.

Brain Metastases, Nanoshells, Macrophage, monocytes, blood-brain barrier, photothermal therapy, light triggered release, HER2+ breast cancer, metastatic breast cancer
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1. **INTRODUCTION:** The blood-brain barrier renders the central nervous system a sanctuary site for disease. In the era prior to the development of effective systemic therapies this fact was not of much clinical relevance as most patients succumbed to visceral metastatic disease before their brain metastases became symptomatic. However, as systemic breast cancer therapies improve and are able to successfully control non-Central Nervous System Disease, the brain is increasingly the first site of relapse. Both conventional chemotherapeutic agents and targeted monoclonal antibodies do not cross the blood-brain barrier (BBB) at concentrations sufficient to successfully treat metastatic disease. The objective of the proposed work is to use an active form of transport of therapeutics across the BBB; a transport mechanism that does not rely on passive diffusion or receptor-mediated transcytosis. We hypothesize that monocytes/macrophages are actively recruited to metastases by cytokines elaborated by the tumors and that these cells can be utilized to transport therapeutics directly to brain metastases of breast cancer. This hypothesis is support by a pilot study we published that demonstrated delivery in a mouse model of fluorescent microspheres to brain metastases by macrophages.\(^1\) Our objective in this proposal is to deliver a therapeutic small molecule to HER2+ brain metastases using a nanoshell-double-stranded DNA (dsDNA)-drug complex loaded within monocytes/macrophages. Once present within the metastasis, we aim to release the drug by transcranial irradiation at near-infrared (NIR) wavelengths.

2. **KEYWORDS:** Brain metastases, nanoshells, macrophage, monocytes, blood-brain barrier, photothermal therapy, light triggered release, HER2+ breast cancer, metastatic breast cancer

3. **OVERALL PROJECT SUMMARY:** Summarize the progress during appropriate reporting period (single annual or comprehensive final). This section of the report shall be in direct alignment with respect to each task outlined in the approved SOW.

Task 1

a. **Current Objectives**

Synthesis/assembly/characterization of lapatinib-loaded nanocomplexes and characterization of macrophage uptake and release; assessment of impact of macrophage release efficacy on adjacent breast cancer cell metastatic model.

The original proposal suggested the immobilization and near-IR triggered delivery of lapatinib from gold nanoshells. The goal was to load lapatinib onto the nanoshell surface through the intercalation of dsDNA sequences bound to the nanoshell surface. The dsDNA sequences would have one strand that was thiolated and covalently linked to the particle surface, while the complementary strand was not. Upon irradiation, the complementary sequence would be released from the particle surface thereby releasing lapatinib locally (Scheme 1). We proposed to load these nanocomplexes into macrophage / monocyte cells that could then be used to deliver them into the brain metastases.

![Scheme 1. Near-IR triggered delivery of lapatinib from nanoshell (NS) surface.](image-url)
b. Results:

In order to immobilize lapatinib to the nanoshell surface, we needed to determine the ideal DNA sequences to use, which would have the best binding of lapatinib but also allow efficient release upon near-IR irradiance. Since there are no reported studies on the mechanism of binding between lapatinib and DNA, we initially focused on using circular dichroism (CD) to study how two common DNA sequences interacted with lapatinib, poly adenosine (polyA) and poly cytosine (polyC); however, we found that lapatinib is very poorly soluble in water or buffer (<10 µM) and the use of dimethyl sulfoxide (DMSO) caused interference with our CD measurements. We then initiated a study to understand the interaction between lapatinib and a model dsDNA sequence, ctDNA (calf thymus DNA), to determine the general mechanism of interaction using absorbance, fluorescence quenching, and binding competition studies with ethidium bromide (EB, intercalator) and Hoechst (minor groove binder), as shown in Figure 1. There was a definite interaction between the lapatinib and ctDNA, as was seen through the increase in both absorbance and fluorescence of lapatinib upon the addition of ctDNA, Figures 1a and 1b, respectively. The increase in absorbance may be due to the fact that the lapatinib solubility increased upon binding to the DNA in solution. The increase in fluorescence emission is normally indicative of a change in local environment or rigidity of the molecule upon binding to the DNA. Our next step was to determine whether lapatinib binds to ctDNA through intercalation or groove binding using the displacement of EB or Hoechst as an indicator. When a small molecule binds to ctDNA through intercalation, the fluorescence of the ethidium bromide-ctDNA complex is expected to decrease from the replacement of EB with lapatinib and the release of the EB into solution. However, the EB fluorescence was actually found to increase with the addition of lapatinib (Fig. 1c), while Hoechst fluorescence decreased (Fig. 1d) consistent with a groove binding mechanism. The cause of the increase in EB fluorescence was shown to be from direct interaction between EB and lapatinib based on control experiments involving the two molecules alone (data not shown), which did not occur as significantly with Hoechst and lapatinib. When the interaction between lapatinib and ctDNA was calculated based on the Hoechst fluorescence quenching data, it was found that

Figure 1. Study of lapatinib interactions with ctDNA. A) Absorbance of Lap with increasing concentration of ctDNA. B) Fluorescence emission (518 nm) of 25 µM Lap with increasing concentrations of DNA. C) Fluorescence emission of EB (5 µM) +ctDNA (60 µM) complex after addition of Lap (0 to 55 µM). D) Fluorescence emission of Hoechst (5 µM) +ctDNA (60µM) complex after addition of Lap (0 to 55 µM).
the binding constant ($K_a$) was equal to $3.5 \times 10^3 \text{ M}^{-1}$, which corresponds to a pretty weak affinity between the drug and dsDNA. Based on this poor affinity and poor solubility, we have decided to pursue other chemotherapeutic options for the treatment of drug metastases in the brain via light triggered release from our nanocomplex.

In addition to our fundamental studies involving Lap interaction with ctDNA, we simultaneously attempted to create nanocomplexes containing Lap immobilized onto nanoshell surfaces with thiolated / unthiolated dsDNA complexes. These nanocomplexes were characterized with transmission electron microscopy (TEM), dynamic light scattering (DLS) / zeta potential measurements, and surface enhanced Raman scattering (SERS). The TEM results show that the nanoshells look similar before and after incubation with Lap (Fig. 2a). The plasmon resonance does not appear to shift after incubation with Lap (absorption maximum at 778 nm), suggesting that the particles do not aggregate significantly after Lap incubation. However, the hydrodynamic diameter does appear to increase after the Lap incubation and the zeta potential decreases by 6 mV, both suggestive of successful attachment of Lap (Fig. 2b). The Raman data provides the best evidence of Lap attachment to the particle surface, especially for the peak at ~1540 cm$^{-1}$.

All of the data suggest that both the dsDNA and Lap were successfully attached to the nanoshell surface (Fig. 2). Though these results were very promising, repeated attempts to release the Lap from the nanocomplex surface through either light triggered release using a CW laser at 800 nm or thermal release by melting the dsDNA complex to release the complementary strand were unsuccessful. Our current assessment is that the hydrophobicity of Lap prevents its release from the particle surface into aqueous media.

Given the challenges of delivering Lap with dsDNA, a strategy that was based on the assumption that Lap would bind to dsDNA through intercalation, we have also investigated the use of protein coronas for the immobilization and delivery of Lap to brain metastases. Protein coronas have been successfully used by other groups to deliver chemotherapeutics to tumors via light triggered release from gold nanorods.(5–7)
addition, albumin has been successfully used as a FDA approved drug carrier system (Abraxane™) and another group showed that it was possible to load bovine serum albumin (BSA) with lapatinib to create nanoparticles.(8)

By controlling incubation time and temperature, we showed that we could successfully create protein coronas around nanoshells without causing significant particle aggregation (Fig. 3a), even after the incubation with Lap. SERS was used to confirm the association between the protein-coated nanoparticles and Lap (Fig. 3b). After these particles were successfully synthesized, Drs. Susan Clare and MiRan Choi of Northwestern University came to Rice University to train Professor Halas’ students on the isolation of monocytes from human blood (Buffy coat). Upon successful isolation of monocytes, the Halas group tested whether coating NS with HSA would inhibit their uptake by blood derived monocyte / macrophage cells (Fig. 4). This study demonstrated that the optimal uptake / particle incubation time without significantly impacting cell viability was between 2 and 3 hrs. However, many attempts were made to release Lap from the particle surface using both heat and near-IR laser excitation, all of which were unsuccessful. We believe that the insolubility of Lap prevents it from being released into solution from the particles’ surfaces, using either strategy (dsDNA or protein coronas) via light triggered release.

c. Changes to originally proposed methods:

Task 1. At this point, we have decided that based on the challenges with solubility and light triggered release of lapatinib, that it is time to focus on different therapeutic that has shown efficacy in brain metastases and that will have fewer issues with solubility. For the next reporting period, we will focus on the delivery of doxorubicin, a known DNA intercalator, which is also very important clinically in treating triple negative breast cancer. Triple negative breast cancer is also known to have a high incidence rate for brain metastases. If doxorubicin can be delivered via light triggered release, then we can avoid many of the
challenges of working with this drug, including the inability to pass the blood-brain barrier and cardiotoxicity.

Task 2

a. Current Objectives:
Determination of attenuation of laser power by the skull:
Developing tools for simulation and local measurements of NIR photon transport in heterogeneous medium.

b. Results:
Voxel-based Monte Carlo code: Past MC code developed in Dr. Stantz’s lab (Purdue) simulated 3D objects with differing optical properties. To integrate simulation and CT/MRI images, a voxel-based approach has been developed. First, MCX code, a Monte Carlo code (developed at Harvard) has been developed modified for this application. The base code was developed using GPU based CUDA platform to utilize the parallel computational capabilities of GTX Nvidia card, with an acceleration of 300X over the traditional CPU based Monte Carlo models. To improve the applicability of the code to our studies, the code was modified by Dr. Stantz’s team to accommodate different user defined beam profiles including flat, convergent and divergent Gaussian (super-Gaussian) beams as specified by the laser and accompanying optics; and optimized for speed by writing sections of Matlab interface code into CUDA and improving upon the threading of the code for our application. Based on our bench marks, the execution time was reduced by a factor of 30, thus providing simulated data with sufficient statistics at depths of 10 cm within the time-frame of hours (as opposed to weeks). Additional Nvidia cards have been added, and the code is threaded out to multiple GTX processors. To date, the MCX code does not appear to have been validated by measured data. The results in our proposal are possibly the first.

Empirical Photon Transport Code: MC codes are considered the gold standard; however, they are prohibitively slow if optimization algorithms are to be applied. Instead, a voxel-based empirical model implementing near-neighbor (and next-to nearest-neighbor) methods calibrated to MC simulated data have been employed. Executions speeds are on the order of seconds to minutes, but rescaling of the voxel size is necessary based on density and category of the voxel.

Optical dosimetry probe (ODP): An optical dosimetry probe was fabricated to provide point-measurements of laser power with high sensitivity. The probe consists of 1.5mm sphere of epoxy and titanium dioxide positioned onto the tip of optical fiber and designed to have an isotropic acceptance over nearly 4π, and calibrated to a calorimeter was determined using a flat beam (integrating sphere) (Fig.1).
Preliminary data with just the skull bone: To determine the attenuation of the skull bone, a sheep skull (1.1 cm thickness; sourced from local butcher) was exposed to a pulsed laser beam (1.0 cm diameter; 13.5mW per pulse) and the laser energy along the central axis was measured to be 3.3% (0.3mW per pulse) as measured by the ODP. This is approximately 98.8% per cm.

c. **Current Objectives:**
   Determination of attenuation of laser power by a phantom of the brain:

   d. **Results:**
   Phantom work of white and gray matter: Water and agar-based phantoms with the optical properties of white \((\mu_a=0.05\text{cm}^{-1}; \mu_s'=83\text{cm}^{-1})\) and gray \((\mu_a=0.35\text{cm}^{-1}; \mu_s'=25\text{cm}^{-1})\) matter were developed. To determine \(\mu_a\) and \(\mu_s'\), all solutions and agar based media was measured using a photospectrometer to validate mixtures. Flat and divergent beams were measured and used as input to the MC code. ODP probes measurements of the beam profiles at various depths were measured (data not shown), and the normalized photon fluence rate as a function of depth along the central axis was measured (Fig.2). The energy loss for white matter was 86.0% per cm and gray matter was 93.2% per cm. The agreement between the ODP measurements and MC simulated data was very excellent.

Skull plus phantom: With the same setup, the photon fluence rate in the brain cavity through 1.0 cm thickness of skull bone was measured at a depth of 3 cm within agar-based white mater phantom mimicking brain tissue. Photon fluence rate before entering the skull was measured to be 0.387mW/mm², and in brain cavity (1.0 cm bone and 2.0 cm tissue) was 0.000949 mW/mm², or 0.25%.

Task 3

a. **Current Objectives:** *in vivo* studies

b. **Results:**
   These studies have not commenced; they were forecast to begin in the second year after loading/unloading and *in vitro* studies inform and enable the optimization of the various parameters, e.g., laser power, beams, duration, etc.
The animal protocol has been written and submitted to the Northwestern Institutional Animal Care and Use Committee.

4. KEY RESEARCH ACCOMPLISHMENTS: Bulleted list of key research accomplishments emanating from this research. Project milestones, such as simply completing proposed experiments, are not acceptable as key research accomplishments. Key research accomplishments are those that have contributed to the major goals and objectives and that have potential impact on the research field. If there is nothing to report, simply state "Nothing to report."

- MCX (Monte Carlo eXtreme) code, a Monte Carlo code, has been validated by measured data, to our knowledge, the first time this has ever been done.
- Binding of lapatinib to dsDNA was demonstrated to be groove-binding and weak.
- Lapatinib was successfully bound to the nanoshell surface within an albumin corona, however, the insolubility of lapanib in aqueous solution virtually prohibits release from the corona.
- Attenuation of NIR laser power by the skull, and white and grey matter phantoms was quantitated.

5. CONCLUSION: Summarize the importance and/or implications with respect to medical and/or military significance of the completed research including distinctive contributions, innovations, or changes in practice or behavior that has come about as a result of the project. A brief description of future plans to accomplish the goals and objectives shall also be included.

Our studies provide insight into those therapeutics that are best suited for nanoparticle delivery. Aqueous solubility will be one of the keys to success. We note that almost all the small molecule tyrosine kinase inhibitors have poor aqueous solubility, which suggests that innovative formulations of these compounds will be required if nano-enabled delivery is to flourish. Absorption of light by the skull and scatter of light by the brain parenchyma significantly reduce the dose of light that can be delivered to intracranial lesions. This has prompted us to develop work-arounds, which include utilizing multiple NIR sources and examining various beam profiles. Our future plans also include examining the loading and unloading of therapeutics that are known DNA intercalators and which are water-soluble. We will also examine the effect of mimicking the interstitial fluid composition in our lapatinib release experiments to determine if this mitigates some of the aqueous solubility challenge.

6. PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS:
   a. List all manuscripts submitted for publication during the period covered by this report resulting from this project. Include those in the categories of lay press, peer-reviewed scientific journals, invited articles, and abstracts. Each entry shall include the author(s), article title, journal name, book title, editors(s), publisher, volume number, page number(s), date, DOI, PMID, and/or ISBN.

   1. Lay Press: Nothing to report
   2. Peer-Reviewed Scientific Journals: Nothing to report
3. Invited Articles: Nothing to report

4. Abstracts:


b. List presentations made during the last year (international, national, local societies, military meetings, etc.).

Use an asterisk (*) if presentation produced a manuscript.


2. INVENTIONS, PATENTS AND LICENSES:

Nothing to report

3. REPORTABLE OUTCOMES:

Nothing to report

4. OTHER ACHIEVEMENTS: This list may include degrees obtained that are supported by this award, development of cell lines, tissue or serum repositories, funding applied for based on work supported by this
award, and employment or research opportunities applied for and/or received based on experience/training supported by this award.

For each section, 4 through 8, if there is no reportable outcome, state "Nothing to report."

Based upon the work supported by this award, we have submitted a proposal to the NIH in response to a funding opportunity: Image-guided Drug Delivery in Cancer (R01); PAR-13-185.

5. REFERENCES:

6. APPENDICES:
Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

c. Abstract: Materials Research Society fall 2014 meeting
d. Abstract: SPIE Bios 2015
An Optical Therapeutic Protocol to treat brain metastasis by mapping NIR activated drug release: A Pilot Study

Akshay Prabhu Verleker, Qianqian Fang, Mi-Ran Choi, Susan Clare and Keith M. Stantz

Abstract

Treatment of Central Nervous System (CNS) metastasis poses a critical clinical challenge due to limitations in drug uptake across the blood brain barrier and blood-cerebrospinal fluid barrier. Recent research has shown the efficacy of using macrophages as drug carriers to target metastatic sites in the brain, which can then be activated by illuminating with near infrared radiation. The goal of this study is to design an optical therapeutic protocol to treat brain metastasis by calibrating a GPU based 3D Monte Carlo simulation tool to effectively predict the photon distribution and subsequent drug activation in brain tissues. We designed and calibrated an optical dosimetry probe with a linear and isotropic response, which was used to quantify photon fluence and validate the Monte Carlo in an optical brain phantom, resembling gray and white matter. The voxel based Monte Carlo software was integrated with a segmentation software to convert CT (Computed Tomography) image densities of the brain to optical properties, which were used by the Monte Carlo to predict photon fluence in the brain. The Computed Tomography image volume was used to segment tissue types and bone-tissue boundaries, and coregistered with the Monte Carlo generated drug-release maps. An optical treatment plan, using fast Monte Carlo software, optimized with CT segmented image volumes, would significantly reduce the treatment time and allow targeted drug activation while sparing healthy tissues.
An Optical Therapeutic Protocol to treat brain metastasis by mapping NIR activated drug release: A Pilot Study

Akshay Prabhu Verleker, Qianqian Fang, Mi-Ran Choi, Susan Clare and Keith M. Stantz

Abstract—Treatment of Central Nervous System (CNS) metastasis poses a critical clinical challenge due to limitations in drug uptake across the blood brain barrier and blood-cerebrospinal fluid barrier. Recent research has shown the efficacy of using macrophages as drug carriers to target metastatic sites in the brain, which can then be activated by illuminating with near infrared radiation. The goal of this research is to develop an optically targeted therapeutic treatment of metastasis, specifically for metastatic breast cancer of the brain. As a first step towards accomplishing this, we developed a 3D Monte Carlo photon transport code capable of simulating phantoms with optical properties of brain and tumor; and validated the Monte Carlo simulated photon fluence within brain phantoms using an optical dosimetry probe. The phantom studies showed good correlation (correlation coefficient $R=0.977$) between the probe measurements and the Monte Carlo simulation in a white matter phantom (reduced scattering coefficient $\mu_s=8.25\, \text{mm}^{-1}$, absorption coefficient $\mu_a=0.005\, \text{mm}^{-1}$). Our future steps will be to implement the Monte Carlo to map out photon energy distribution in the brain, and subsequent drug release, by segmenting & translating head CT images to corresponding optical properties of brain tissues. To access the therapeutic response, changes in the vascular physiology of the brain due to Her2 inhibition will be measured using dynamic contrast-enhanced imaging (e.g. DCE-CT), and with Monte Carlo based optical fluence maps. An optical treatment plan, using fast Monte Carlo software, optimized with CT segmented image volumes, would significantly reduce the treatment time and allow targeted drug activation while sparing healthy tissues.

I. INTRODUCTION

TREATMENT of Central Nervous System (CNS) metastasis poses a critical clinical challenge due to limitations in drug uptake in the brain (across the blood brain barrier and blood-cerebrospinal fluid barrier) and adverse neurotoxic effects of mainstay therapies such as whole brain radiation therapy (WBRT) and stereotactic radiosurgery. With non-CNS metastasis being treated successfully with receptor targeted drug delivery, aided by improved imaging and localized radiotherapy techniques, the brain remains a sanctuary for metastasis (e.g. triple negative and HER2+ breast cancer metastasis with median survival rates of 2 to 16 months). This study is part of a project which demonstrated a macrophage based “Trojan Horse” delivery of fluorescent molecules into brain metastasis and subsequent release of the fluorescent molecules on activation in-vivo by NIR radiation. The larger goal of this collaborative project is to deliver lapatinib-gold nanocomplex laden macrophages to brain metastasis and design a therapeutic protocol to optically stimulate drug release in target tissues in the brain. In the current project, the purpose is to design an optical simulation protocol (using Monte Carlo simulations) to effectively predict the photon distribution and subsequent drug activation in the brain tissue, and validate it by using an optical dosimetry probe. With the rate/quantity of drug release being directly dependent on the optical power delivered, the Monte Carlo simulation protocol, with the ability to predict photon distribution in heterogeneous brain tissue, can be used to generate an equivalent drug release map. The optical properties of the brain can be mapped by converting the CT image densities of the brain into the corresponding optical properties of the tissue (e.g. white/gray matter, CSF, etc.), which would serve as an input to the Monte Carlo routine. The optical drug release map generated by Monte Carlo can be co-registered with CT images to improve the therapeutic efficacy of the protocol.

In order to predict the photon energy distribution in heterogeneous brain tissue, we used a GPU based 3D Monte Carlo simulation code, which was modified to generate user defined beam profiles to emulate the NIR (800 nm) laser source used in our lab. Monte Carlo simulation has been used as the gold standard for photon propagation studies (in 3D heterogeneous media), with the GPU version having acceleration speeds 100 to 300 times faster than corresponding CPU versions. In order to validate and quantify the Monte Carlo generated fluence in brain tissue phantoms, we used an optical dosimetry probe to measure the photon fluence in low optical fluence regions. An optimized treatment plan using a fast Monte Carlo software to predict in vivo drug release, would significantly reduce the treatment time and allow the targeted drug activation while sparing healthy tissues.

In addition to designing the Optical therapeutic protocol, we also looked into the feasibility of using Dynamic Contrast Enhanced CT (DCE-CT) imaging to monitor and quantify the efficacy of treatment. The vascular structure of a tumor is complex and heterogeneous, and is seen to vary between individual tumors and during different phases of tumor growth. This heterogeneity in tumor vasculature, characterized by hypoxia, anoxia and hypoglycemia, leads to differing response to treatment due to formation of barriers to transport and distribution of drug molecules.
treatment with anti-angiogenic or cytotoxic drugs, the tumor vasculature undergoes complex physiological and biological changes some of which are adaptive in nature. DCE-CT has been successfully used to simultaneously quantify physiological parameters such as perfusion and vascular plasma volume in tumors after treatment with therapeutic drugs. Past research has proved the feasibility of using DCE-CT in measuring the physiological response of breast tumors to anti-angiogenic therapies using the two-compartmental model. In the present study we used the above model to obtain parametric maps of perfusion, permeability, fractional plasma volume and fractional interstitial volume in glioma tumors grown in mice; thus demonstrating the feasibility of DCE-CT imaging to monitor brain tumor response to treatment.

II. METHODS

Step 1: The optical dosimetry probe (1.5mm diameter) was calibrated using a calorimeter (Gold standard) in low fluence regions. The probe measurements were used to validate Monte Carlo generated photon fluence in brain phantoms resembling white and gray matter, made with specific concentrations of India ink (absorber) and Intralipid (scatter), as measured by photo-spectrometer (Gold standard), consistent with brain tissue and tumors (values) as shown in Fig 1. The source of light used was an integrating sphere connected to a pulsed NIR laser with wavelength 800 nm, frequency 200MHz, and energy 10mJ/pulse. The broad beam NIR output power emanating from the integrating sphere was 0.2083mW/mm².

Step 2: In order to apply the Monte Carlo (MC) simulation protocol to the brain, we designed a segmentation software to determine tissue boundaries in the brain using Computed tomography images. The CT image densities in Hounsfield units (HU) of the brain were translated into corresponding optical properties (absorption coefficient, scattering coefficient, and anisotropy factor) using lookup tables (Fig 2). The lookup tables were prepared from earlier studies which derived the Hounsfield units and optical properties of brain tissues such as white/grey matter. The segmentation software generated an optical map of the brain, which was given as input to the Monte Carlo. The photon energy map generated by the Monte Carlo can be co-registered with the CT image to visualize the efficacy of drug release.

Step 3: “Optical treatment protocol”, voxel-based optical properties of a human head (obtained from CT scans) were input into MC code to simulate 3D fluence maps from multiple (optically coupled) light sources. This study also looked into the feasibility of monitoring effectiveness of targeted drug release by monitoring the physiology at target sites and surrounding tissues. An Orthotopic model of glioblastoma tumors were grown in mice and parametric images of perfusion, permeability, fractional plasma volume and fractional interstitial volume were derived using the two compartmental model.

III. OBSERVATIONS & RESULTS

The Monte Carlo generated photon fluence was validated by probe measurements in optical brain phantoms (e.g. white matter in Figure 1). The optical fluence measured by the probe was in turn, calibrated using a calorimeter (Gold standard) and showed a linear and isotropic response to input NIR fluence at different incidence angles (<5% variation). Our MC simulation studies showed that 99% of photons are attenuated by the bone (1cm), which was confirmed by probe measurements in an empty goat skull. The segmentation code (with iterative Monte Carlo) can be used to identify regions of least bone thickness and determine the path of least attenuation to couple maximum power to brain tissues. This study proved that an optimized Monte Carlo could be successfully used to quantify photon propagation in the brain and thus predict the rate of drug release in target tissues to depths up to 5cm in brain tissues.
Our preliminary longitudinal study has indicated that DCE-CT is an effective tool to study tumor progression and physiology (Fig 3) of glioma tumors. We propose to monitor these effects by studying the change in fractional plasma volume and fractional interstitial volume, both of which are excellent indicators of vascular physiology (Fig 3).

In this study, we have assumed that the number of drug release is proportional to the optical power coupled to the tissue within clinically relevant time frames.

**REFERENCES**


**V. CONCLUSION**

This study has successfully demonstrated the potential of using a fast voxel based Monte Carlo based routine to predict the rate of drug release in heterogeneous brain tissue by using Computed Tomography images to segment and translate tissue boundaries of the brain. An optimized therapeutic protocol with a fast Monte Carlo routine can be used to determine the best irradiation conditions for targeted drug release in brain tissue.
Nanoshell Mediated Light-Triggered Delivery of Lapatinib for Treatment of Brain Metastasis

Amanda M. Goodman1, Carrie Li1, Oara Neumann2, Kamilla Norregaard3, Sandra Bishnoi2, Mi-Ran Choi4, Susan Clare4, Naomi Halas1 2 5.

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Treatment of brain metastases from primary breast cancer is a critical clinical challenge. Approximately 30% of all breast cancer patients have central nervous system (CNS) metastases, based on autopsies. This rate is thought to be increasing as a function of the aging population, better treatment of non-CNS disease, and improved imaging techniques. Drug uptake into the brain is limited by numerous factors, such as the blood-brain barrier (BBB). More than 98% of small molecule drugs (< 400-500 Da) and nearly all large-molecule therapeutics do not cross the BBB. Lapatinib is a small molecule therapy targeted at the over-expression of HER2+ in breast cancer. However, lapatinib currently shows low efficacy in the treatment of CNS disease due to inadequate delivery across the BBB.

In this study, we investigate a macrophage-based “Trojan horse” light-triggered delivery of lapatinib to treat brain metastases. Macrophages, originating as blood monocytes, can infiltrate brain metastases through an intact blood brain barrier and can be readily loaded with nanoparticles. Here, nanoshells are functionalized with double stranded DNA, which serves as a protective host carrier for lapatinib delivery. It is important to be able to remotely trigger the release of the therapeutic cargo once the macrophages have relocated to the site of the metastases. Drug release is triggered using low levels (1-2 W/cm²) of continuous wave near-infrared light, which induces the dehybridization of the double helix and subsequently releases lapatinib. The optimal DNA sequence is determined by circular dichroism and loading is confirmed with surface enhanced Raman scattering. Loading capacity and release are quantified with LC-MS. Intracellular drug release will be demonstrated and the effects of near-infrared triggered lapatinib release on neighboring HER2+ brain-seeking breast cancer cells will be investigated.
An empirical approach to estimate near-infra-red photon propagation and optically induced drug release in brain tissues
Akshay Prabhu Verleker, Qianqian Fang, Mi-Ran Choi, Susan Clare and Keith M. Stantz

ABSTRACT

Purpose: The purpose of this study is to develop an alternate empirical approach to estimate near-infra-red (NIR) photon propagation and quantify optically induced drug release in brain metastasis, without relying on computationally expensive Monte Carlo techniques (gold standard).

Introduction: Targeted drug delivery with optically induced drug release is a non-invasive means to treat cancers and metastasis. This study is part of a larger project to treat brain metastasis by delivering lapatinib-drug-nanocomplexes and activating NIR-induced drug release. The empirical model was developed using a weighted approach to estimate photon scattering in tissues and calibrated using a GPU based 3D Monte Carlo. The empirical model was developed and tested against Monte Carlo in optical brain phantoms for pencil beams (width 1mm) and broad beams (width 10mm).

Materials and Methods: The empirical algorithm was tested against the Monte Carlo for different albedos along with diffusion equation and in simulated brain phantoms resembling white-matter ($\mu_s'=8.25\text{mm}^{-1}$, $\mu_a=0.005\text{mm}^{-1}$) and gray-matter ($\mu_s'=2.45\text{mm}^{-1}$, $\mu_a=0.035\text{mm}^{-1}$) at wavelength 800nm. The goodness of fit between the two models was determined using coefficient of determination (R-squared analysis).

Results: Preliminary results show the Empirical algorithm matches Monte Carlo simulated fluence over a wide range of albedo (0.7 to 0.99), while the diffusion equation fails for lower albedo. The photon fluence generated by empirical code matched the Monte Carlo in homogeneous phantoms ($R^2=0.99$). While GPU based Monte Carlo achieved 300X acceleration compared to earlier CPU based models, the empirical code is 700X faster than the Monte Carlo for a typical super-Gaussian laser beam.