YEDİTEPE UNIVERSITY

FACULTY of ENGINEERING and ARCHITECTURE

DEPERTMENT of GENETICS and BIOENGINEERING

"Photo-thermal Tumor Ablation in Mice Using Near Infrared-absorbing Nano Particles"

The Report of GBE 482 Presentation

Name: ÖMER AYDIN

No: 273516001

Adviser Name: Assoc. Prof. MUSTAFA ÇULHA

Photo-thermal tumor ablation in mice using near infrared-absorbing nano particles

Abstract

This study shows the feasibility of nanoshell-assisted photo-thermal therapy (NAPT). This technique can be applied in the NIR region with nanoparticles which are tunable optical absorptivities. It is actualized efficiently by enhaned permeability and retention (EPR) effect. Tumor were grown in immune-component mice by subcutaneous injection of murine colon carcinoma cells (CT26.WT). PEG coated nanoshells ,which have an absorption peak in the NIR, were injected from mice tail and allowed to circulate for 6 h. After diode laser (808 nm, 4 W/cm^2, 3 min) is used for thermal therapy. All of the nanoshell treated tumors' diameter decreased and all mice remained healthy after treatment (+90 days). But the mice in the control group and sham group were euthanized since tumors grew to a predetermined size. NAPT is an useful alternative thermal therapy but much researches are necessary for applying human.

Keywords: Hyperthermia; Nanotechnology; Nanoshell; Near infrared; Laser; Minimally invasive therapy

1. Introduction

Cancer is most common cause of death after the hearth-attact in the world. A lot of research and studies are done about the cancer in the world wide. These are early diagnosis, molecular imaging, threapy without side-effects or minimal side-effects such as surgery, chemotherapy, hormonal therapy, biological therapy radiation and thermal therapy, which are used depending on cancer types. Thermal therapy can provide a minimally invasive alternative to conventional surgical, which is an effective therapy for removal of well

defined, accessible, primary tumors located within nonvital tissue regions, treatment of solid tumors [9]. Various thermal therapies have employed using different heat sources such as; laser light, microwaves and radio frequency, magnetic thermal and focused ultrasound. Thermal therapy is prefered instead of conventional resection since it is minimally or noninvasive, simple to perform and have the potantial of treating embedded tumors in vital regions where surgical resection is not feasible. However, simple heating methods have problem discriminating between tumors and surrounding healthy tissues and heat the intervening tissue between the source and target site. Many studies are done by sevaral research groups to perform much better cancer thermal therapy using deep penetrating near infrared (NIR) lasers.

In cancer therapy, new techniques have been developed using nanotechnology. United State's National Cancer Institute has a plan for cancer nanotechnology. It discusses cancer nanotechnology in six parts:

"**Prevention and Control of Cancer**

- Developing nanoscale devices that can deliver cancer prevention agents
- Designing multicomponent anticancer vaccines using nanoscale delivery vehicles

Early Detection and Proteomics

- Creating implantable, biofouling-indifferent molecular sensors that can detect cancer-associated biomarkers that can be collected for ex vivo analysis or analyzed in situ, with the results being transmitted via wireless technology to the physician
- Developing "smart" collection platforms for simultaneous mass spectroscopic analysis of multiple cancer-associated markers

Imaging Diagnostics

- Designing "smart" injectable, targeted contrast agents that improve the resolution of cancer to the single cell level
- Engineering nanoscale devices capable of addressing the biological and evolutionary diversity of the multiple cancer cells that make up a tumor within an individual

Multifunctional Therapeutics

- Developing nanoscale devices that integrate diagnostic and therapeutic functions
- Creating “smart” therapeutic devices that can control the spatial and temporal release of therapeutic agents while monitoring the effectiveness of these agents

Quality of Life Enhancement in Cancer Care

- Designing nanoscale devices that can optimally deliver medications for treating conditions that may arise over time with chronic anticancer therapy, including pain, nausea, loss of appetite, depression, and difficulty breathing

Interdisciplinary Training

- Coordinating efforts to provide cross-training in molecular and systems biology to nanotechnology engineers and in nanotechnology to cancer researchers
- Creating new interdisciplinary coursework/degree programs to train a new generation of researchers skilled in both cancer biology and nanotechnology [13]”

In this study, laser-induced thermal therapy is used with nanoparticles called nanoshells. Metal nanoshells, which are a new class of optically active nanoparticles, may provide a novel methods of targeted photothermal therapy in tumor tissue, to minimize damaging of surrounding healthy tissue. Silica gold nanoshells have a silica core and core is coated with thin gold shell [4]. These particles have a highly tunable plasmone resonance, a resonant phenomenon whereby light induces oscillations of conductive metal electrons at the nanoshell surface. This determines the absorbing and scattering properties of the particle [9]. It is well known that the plasmon resonance of metal nanoparticles is sensitive to the nanoparticle size, shape, and the dielectric properties of the surronding medium. Optical properties of gold nanoparticles can be tuned by varing their size and shape [7], [8]. By changing the ratio of core and shell thickness, nanoshell can be manufactured to absorb or scatter light at a desired wavelength visible and NIR wavelength. It is seen in Figure:1, when the core/shell ratio is changed, the wavelength shifts diffrent wavelengths [2] .

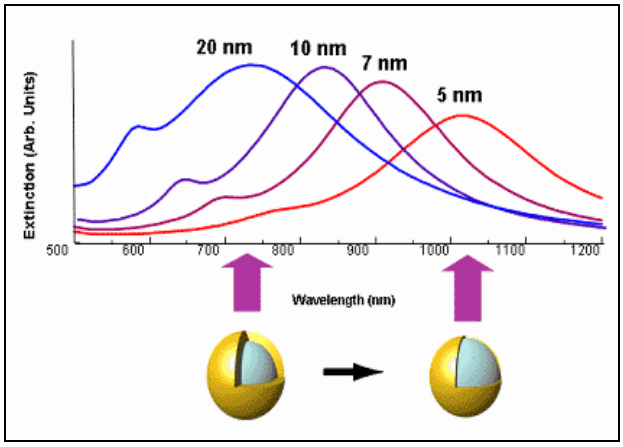

Figure 1: Show the wavelength changing, it depends on altering core/shell ratio [2]

This optical tunability permits the nanoshells with a peak optical absorption in the NIR, a region of light where optical penetration throuhg tissue is optimal [3]. NIR light has been shown to travel at least 10 cm through breast tissue, and 4 cm of skull/brain tissue or deep muscle using microwatt laser sources. With a higher power levels, light has been shown to penetrate through 7 cm of muscle and neonatal skull/brain [6].

Furthermore, the metal shell of nanoshell converts absorbed light to heat with an efficacy and stability and nanoshells have absorption cross sections that have magnitude higher than conventionally used absorbing and fluorescent dyes as indocyanine green, making this material a much stronger NIR absorber, so more more effective photothermal coupling agent [1], [7]. Photobleaching is a common problem using conventional dyes, but for nanoshells, it is not a problem since nanoshells' absorption properties are dependent rigid of metarial metallic structure. These imaging properties are demonstrated in some studies like as magnetic resonance thermal imaging (MRTI) and optical coherence tomography (OCT) [9], [10], [11].

As a biomaterial, gold nano particles have an important advantage because of their nontoxicity. The metal surface of nanoshell is covered with gold which is an inert metal and well known as biocompatibility. For improving their biocompalibity, they are coated with poly

ethylene glycol (PEG). PEG provides stealthing liposome properties and metarials with PEG suppresses immungenic responses therefore improving blood circulation times and overall material/implant performance.

Delivery of nanoparticles to cancerous tissue is provided by leaky tumor vasculature, which is highly permeable to macromolecules relative to normal tissue and a dysfunctional lympathic drainage system, whicih results in enhanced fluid retention in the tumor interstitial space. As a result of these characteristics, the concentration of nanoparticles and macromolecules found in tumor tissues can be up to 100x higher than in normal tissue. The small particles (60-400 nm) extravasate and accumulate in tumors. This is a passive mechanism referred to as the “enhanced permeability and retention (EPR) effect”. The extent of nanoparticle extravasation depends on the size of open interendothelial gap junctions and trans-endothelial channels [12].

In this study, nanoshell is used because of its the optical, chemical physical and deep penetrating properties of NIR light for a targeted, minimally invasive photothermal therapy. Subcutaneous tumors were grown in mice, PEG-coated nanoshells were injected intravenously and accumulation in the tumor was monitoring with surface temprature, measurement tumor growth/regression and animal survival time in three groups. The first group is injected nanoshells and applied laser group, the second group is nanoshells free but applied laser called as sham group and the last group is control group no nanoshells and no laser.

2. Materials and methods

2.1 Synthesis of thiolated polyethylene glycol (PEG-SH)

PEG with a terminal thiol group (PEG-SH) was synthesized by reacting PEG-amine with 2-iminothiolane for 1 hour. For removing excess agent, the product was dialyzed against deionized (DI) H_2O for 6–8 hours. Finisihing of PEG-SH synthesis was understood owing to

show 412 nm colorimetry peak after reaction with Ellman's Reagent (5,50-Dithiobis(2-Nitrobenzoic Acid)). The product was stored in aliquots at -20 °C.

2.2 Gold-silica nanoshell fabrication

110 nm silica nanoparticles were obtained and suspended in ethanol. Reaction of the silica core nanoparticles with 3-aminopropyl triethoxysilane provided amine groups on the surface of the core to allow for adsorbtion of gold colloid. This colloid was stayed at 6°C, 4-14 days then concentrated and mixed with the aminated silica particles, allowing small gold colloid, attached to the larger silica nanoparticle surface, to act as nucleation sites in the subsequent reduction step. Growing the nanoshell was accomplished by the reduction of gold from $HauCl_4$ in the presence of formaldehyde [10]. At the end nanoshell solution has an 8-10 nm thick gold shell, generating a peak optical absorption at 805-810 nm. Nanoshell surfaces were coated with PEG by combining PEG-SH with nanoshells in DI water, followed by centrifugation to remove residual PEG-SH from the nanoshell formulation. PEG nanoshells were suspended again in sterile saline solution then sterilized syringe filter.

2.3 Tumor inoculation

All animals were handled and cared for in accordance with the 'Guide for the Care and Use of Laboratory Animals' 25 female albino BALB/cAnNHsd mice (5–6 weeks age, 15–20 g) were obtained. Each was shaved on the right dorsal flank prior to subcutaneous inoculation with 1.5×10^5 (50 µl injection volume) CT26.WT murine colon carcinoma tumor cells (ATCC) .

2.4 Nanoshell injection and laser treatment

Mice were selected for treatment when the subcutaneous tumors reached 3-5.5 mm diameter as measured with a digital caliper. Laser's spot size is 5.5 mm and it determines the diameter of tumor. Seven of the mice had two tumors and 17 had one, these were randomly distributed between 3 groups. For injecting nanoshells, each mouse was anesthetized then

nanoshell was injected via the tail vein for mice in the treatment group. For sham treatment group, sterile saline injection instead of nanoshells. The control group did not recieve any intravenous injections and subsequent laser treatment. Nanoshell group and shall group performed laser treatment 6h, after injection to allow delivered nanoshell time to collect in the tumors. The using NIR light is 808 nm diode laser, 800 mW at 4 W/cm^2 for 3 min.

Infrared thermometer was used for measuring the cutaneous temprature. It measures across a 5mm diameterspot. Post treatment tumor size were taken daily using a digital caliper. If tumor is reached 10 mm, mice euthanize and survival time of mice was monitored.

The results of this study was evaulated with some statical analysises which are p value, Kaplan-Meier analysis. Mean of the tumor size, survival time, surface temprature are calculeted.

3. Result and discussion

Surface temprature was measured during each NIR laser treatment for nanoshell and sham treatment groups (15 mice). The overall temprature of the nanoshell treatment group's was (~50 °C) higher than NIR treated nanoshell free.

Tumor size and animal survival time were monitored during 90 days following treatment. After the treatment was performed, in 10 days nanoshell tumor treatment showed complete necrosis but other groups go on increasing the tumor size, this can be seen figure 2.

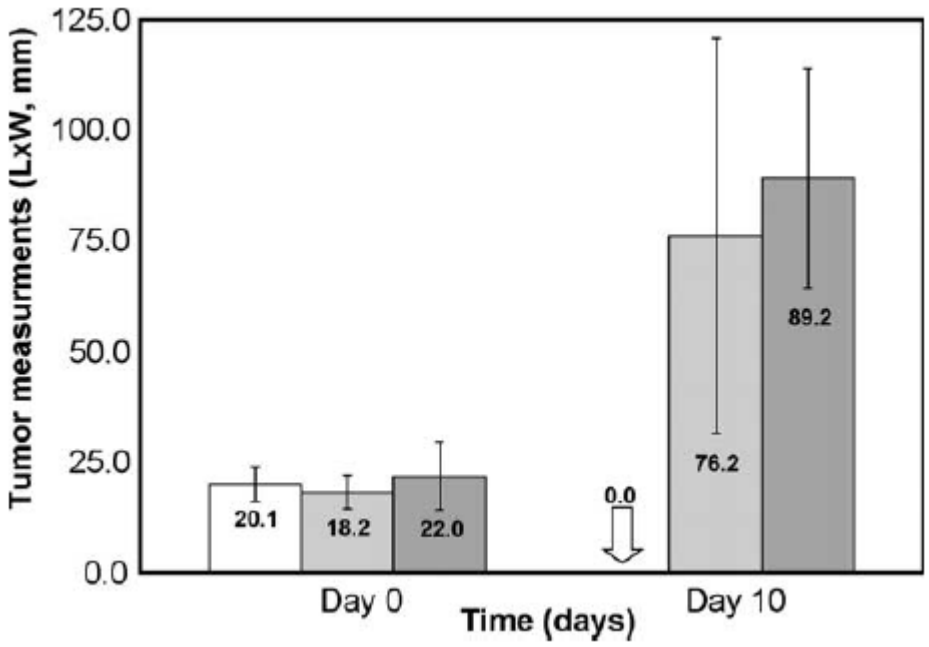

Figure 2: Mean tumor size measured on treatment day and 10 days later for 25 tumors. All tumor which were treated using NAPT showed complete necrosis by day 10. One standart deviation is shown. NAPT (n=7), sham (n=8), control group (n=9).

After 90 day treatment, all mice ,wihich was used nanoshell treatment, had healthy again but sham groups and control groups' tumors were increasing. Mice were euthanized when tumor diameter exceeded 10 mm. By day 12 all control group mice were euthanized and all mice in the sham group were euthanized by day 19 as seen in figure 3.

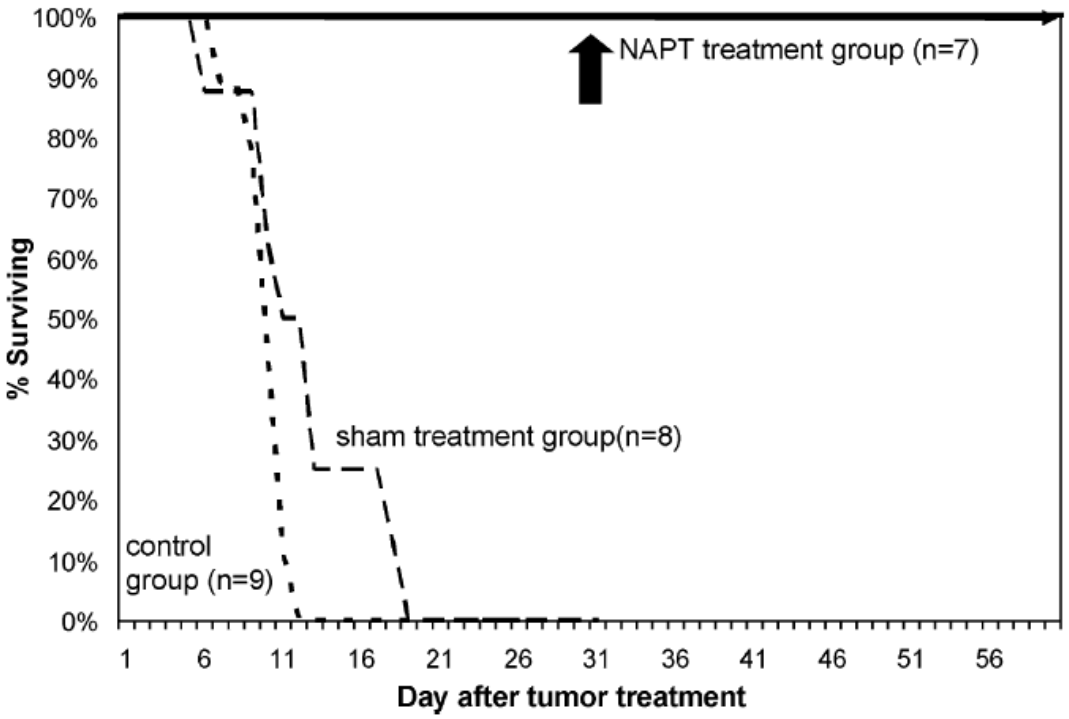

Figure 3: A survival time plot for the three groups for 60 days. The mean survival time of control group was 10.1. The mean survival time of sham group was 12.5. The mean survival time of the treatment group was significantly higher compared to other groups by 18 days.

Main reference source is **"D. Patrick O'Neal, Leon R. Hirsch, Naomi J. Halas, J Donald Payne, Jennifer L. West, Photo-thermal tumor ablation in mice using near infrared-absorbing nano particles, Cancer Letters, 2004**

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