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14. ABSTRACT

Melanoma has a high rate to metastasize to the lung and the metastases are often refractory to radiotherapy and chemotherapy. In pre-clinical studies, photodynamic therapy (PDT) has shown promise as an effective method to kill melanoma cells. However, PDT is limited by the shallow tissue penetration of light. In this project, we aim to develop a novel methodology called X-ray induced PDT or X-PDT, to treat cancer cells speared to the lung. Our strategy is to use X-ray, which affords great tissue penetration, to trigger a PDT process. This is achieved using an integrated nanosystem called nanosensitizer. Upon X-ray irradiation, the scintillator core converts X-ray photons to visible light photons. The latter in turn activates the photosensitizer, producing cytotoxic singlet oxygen ($^1O_2$) and causing cell death. In the past two years, we have successfully made MC540-$SrAl_2O_4$:Eu@SiO_2 and NC-$LiGa_5O_8$:Cr@SiO_2 based nanosensitizers and confirmed their great efficacy to kill cancer cells in vitro. We showed that X-PDT can be activated from external X-ray irradiation to kill cancer cells in the lung. We showed that the nanosensitizers are low toxic and can be efficiently excreted from the host. We have also established the melanoma lung metastasis model.

15. SUBJECT TERMS
Photodynamic therapy, X-ray, tissue penetration, lung metastasis

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1. Introduction

Melanoma has a high rate of metastasis to the lung and the tumors are often refractory to radiotherapy. Photodynamic therapy (PDT) has shown promise as a new treatment modality for melanoma therapy, but its applications are limited by the shallow tissue penetration of light. In this project, we aim to develop a novel methodology called X-ray induced PDT, or X-PDT, which utilizes X-ray to initiate PDT. This will overcome the shallow penetration problem of conventional PDT, enabling eradication of metastases in the lung with external irradiation. The central piece of the technology is an integrated nanosystem called nanosensitizer, which consists of a nanoscintillator core, photosensitizer molecules whose excitation wavelength matches the emission of the nanoscintillator, and mesoporous silica coating that encapsulates the nanoscintillator and photosensitizer. Upon X-ray irradiation, the scintillator core converts X-ray photons to visible light photons, activating the photosensitizer and producing cytotoxic singlet oxygen ($^1O_2$). It is expected that a nanosensitizer can be conjugated with a targeting ligand and after systemic injection, home to tumors in the lung. X-ray of relatively low doses can then be applied externally to the lung area to trigger X-PDT, leading to efficient killing of cancer cells while minimally affecting normal tissues.

2. Keywords

Nanoparticle, photodynamic therapy, melanoma, lung metastasis, X-ray, tissue penetration

3. Accomplishments

- **What were the major goals of the project?**

Major Tasks	**Months**	**Status**
Specific Aim 1: Optimize silica coating on SAO nanoparticles and conjugate onto them NAPamide; evaluate *in vitro* treatment efficiency and selectivity of X-PDT.		
Aim 1 (a)-1: Silica coating and PEGylation of SAO nanoparticles.	1-2	finished
Aim 1 (a)-2: Investigating and optimizing nanoparticle colloidal stability, SAO integrity, and MC540 loading.	3-4	finished
Aim 1 (a)-3: Assessing MC540 loading and $^1O_2$ production.	4-5	finished
Aim 1(b)-1: Cell targeting specificity of NAPa-M-SAO@SiO_2 nanoparticles and their cytotoxicity (in the dark).	5-6	finished
Aim 1(b)-1: X-PDT induced cytotoxicity.	7-8	finished

Milestone(s) Achieved: *Select three NAPa-M-SAO@SiO_2 formulations with favorable colloidal stability, SAO integrity (>3 days), MC540 loading (>10%), and selective X-PDT toxicity against MC1R positive cells for further studies.*	1-8	
Specific Aim 2: Investigate *in vivo* targeting specificity and toxicity of NAPa-M-SAO@SiO_2 nanoparticles.		
Aim 2(a): Investigate *in vivo* targeting specificity of M-SAO@SiO_2 nanoparticles	9-12	50%
Aim 2(b): Investigate *in vivo* toxicity of NAPa-M-SAO@SiO_2 nanoparticles	13-16	finished
Milestone(s) Achieved: 1. *Select one NAPa-M-SAO@SiO_2 formulation with the highest tumor uptake.* ***2.*** *For the formulation, determine the time point with the highest tumor uptake.* ***3.*** *Assess the toxicity of the nanoparticles and X-ray when applied individually.*	9-16	
Specific Aim 3: *In vivo* therapy studies		
Aim 3: Investigate *in vivo* the efficiency of X-PDT to treat metastatic melanoma to the lung.	17-24	50%
Milestone(s) Achieved: *Evaluate in murine lung metastasis tumor models the treatment benefits and side effects of NAPa-M-SAO@SiO_2-mediated X-PDT.*	17-24	

- **What was accomplished under these goals and what are the plans for the next year?**

We have successfully prepared MC540(photosensitizer)-$SrAl_2O_4$:Eu (scintillator)@SiO_2 (coating) nanoparticles (M-SAO:Eu@SiO_2 nanoparticles), and 2,3-naphthalocyanine (photosensitizer)-$LiGa_5O_8$:Cr (scintillator)@mSiO_2 (coating) (NC-LGO:Cr@mSiO_2 nanoparticles), and assessed their capacity to mediated X-PDT. Many of these data were reported last year. We have confirmed that these nanoparticles can efficiently mediate X-PDT to eradicate B16 melanoma cells. We have also transfected B16 cells with firefly luciferase and have established lung tumor models with the cells line (Fig. 1). We have also conjugated NAPamide peptide to the surface of the M-SAO@SiO_2 and NC-LGO:Cr@mSiO_2 nanoparticles and started testing their tumor targeting capacity in vivo. However, so far, the tumor targeting efficiency was slow. We saw a large amount of nanoparticles in the liver and their lung tumor uptake was not high. This will lead to poor therapy outcomes. We are working on addressing the issue. One is to further reduce the nanoparticle size. For M-SAO@SiO_2, in particular, because the particles have two layers of silica (one solid silica coating to protect the scintillator core from degradation in water and one mesoporous silica coating to load photosensitizers), it is difficult to make the overall nanoparticle size below 100 nm. We are also working on using longer PEG chains to

PEGylate the nanoparticle surface, which may also help suppress serum protein adsorption and improve the particle circulation. In addition to these efforts, we are also exploring alternative radiosensitizer materials. In particular, we have recently prepared a zinc phthalocyanine (ZnPc, which is a potent photosensitizer) nanocrystal and PEGylated the particle surface (Fig. 2a). Our preliminary studies show that while these nanoparticles are not toxic in the dark (Fig. 2b), they can efficiently enhance radiation-induced cancer cell death through X-PDT (Fig. 2c). Given that these nanoparticles don't need a scintillator core, the size can be potentially be tuned very small (e.g. 20 nm). This will greatly improve tumor accumulation and penetration. We will explore these nanoparticles as an alternative nanosensitizer in future studies.

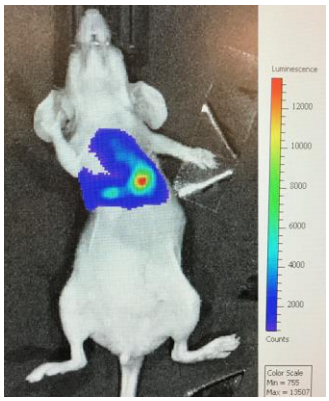

Figure 1. B16 lung metastasis model. The cells were transfected with firefly luciferase so we can track the tumor growth.

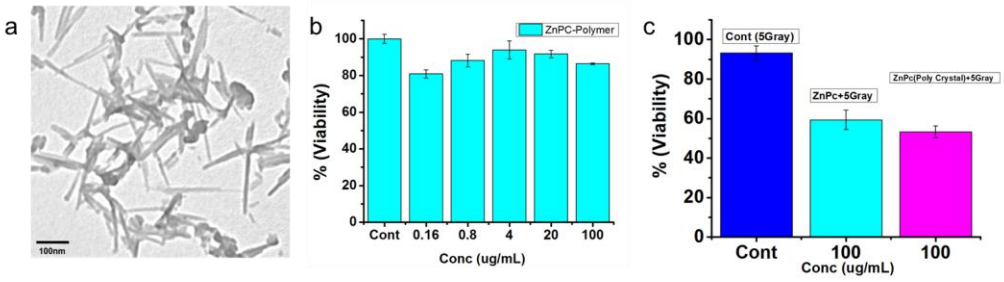

Figure 2. a) TEM of PEGylated ZnPc nanocrystals. b) MTT assays, with ZnPc nanocrystals of different concentrations. c) 72 h MTT assays after 5 Gy irradiation. ZnPc nanocrystals can significantly enhance radiation therapy efficacy.

One problem is that we are experiencing some personnel changes. Dr. Hongmin Chen, the postdoc who has been working on this project, has got a faculty position and left the group. Jeff Wang and Wei Tang,

two graduate students on the grant, recently graduated. On the other hand, we have hired new hands to work on the project. These include our new postdoc, Dr. Wen Jiang, who has extensive experience in nanoparticle synthesis and surface modification. We have applied for a one-year no-cost extension and the request is approved. We hope to address the delivery issue in the coming year.

- **What opportunities for training and professional development has the project provided?**

  This highly interdisciplinary project provides training opportunities at different levels. For instance, Dr. Hongmin Chen had been a postdoc on this grant and was well trained. He's got a faculty position recently. Ms. Wei Tang, who was a graduate student on this grant, is currently a postdoc at NIH. Two undergraduate students, Jessica Aldrich and Jenny Hen, worked with us in the past summer. A high school student, Gouri Rajesh, helped on this project in the past summer through the UGA Young Dawgs program.

- **How were the results disseminated to communities of interest?**

  We want to make ensure that the related technology (materials and final research data) is widely available in timely fashion to the research community in compliance with policies and regulations governing research awards from the DoD CDMRP. We have actively attended national meetings to disseminate our research results. I was invited to give a presentation at 2017 SPIE spring meeting. I will give talks at the 2007 IEEE Atlanta meeting and the END2CANCER conference later this year on the X-PDT topic. We have recently published a paper on X-PDT in *Materials Horizon* (DOI: 10.1039/C7MH00442G). We are invited to write a review article on X-PDT by *Bioconjugate Chemistry*, which is due this December.

4. Impact

- **What was the impact on the development of the principal discipline(s) of the project?**

  PDT has been extensively investigated in pre-clinical studies but their clinical applications have been limited by the shallow penetration of light. X-PDT as a new PDT derivative overcomes the shallow penetration problem by using X-ray as the energy source. The advance will greatly expand the applications of PDT for treatment of not only melanoma but a wide range of other diseases.

- **What was the impact on other disciplines?**

Our studies showed that X-PDT is essentially a RT and PDT combination therapy. In that sense, X-PDT is also a radiosensitizing technology. This may open doors for developments of new radiosenzitizers. The ZnPc nanocrystal mentioned above is a start of the campaign.

- **What was the impact on technology transfer?**

  Nothing to Report

- **What was the impact on society beyond science and technology?**

  This project has provided many research opportunities for undergraduate students and high school students.

5. Changes/Problems

Like mentioned above, we have some personnel changes and that has caused some delay. But we have hired good people. We expect to finish all the proposed studies within the one-year non-cost extension period.

6. Products

- **Publications, conference papers, and presentations**.
  1. Chen H, Sun X, Wang GD, Nagata K, Hao Z, Wang A, **Xie J**, Shen B, LiGa5O8:Cr-based theranostic nanoparticles for imaging-guided X-ray induced photodynamic therapy of deep-seated tumors, *Mater. Horiz.*, **2017**, in press. *acknowledgement of federal support (***yes***).*
  2. Cowger TA, Yang Y, Rink DE, Todd T, Chen H, Shen Y, Yan Y, **Xie J**, Protein-Adsorbed Magnetic-Nanoparticle-Mediated Assay for Rapid Detection of Bacterial Antibiotic Resistance, *Bioconjug Chem*, **2017**; 28(4):890-896. *acknowledgement of federal support (***yes***).*
  3. Chen H, Weizhong Z, Guizhi Z, **Xie, J**, Xiaoyuan C, Rethinking Cancer Nanotheranostics, *Nature Reviews Materials*, **2017**; 2:1-18 *acknowledgement of federal support (***yes***).*
  4. Zhen Z, Tang W, Wang M, Zhou S, Wang H, Wu Z, Zhonglin H, Li Z, Liu L, **Xie J**, Protein Nanocage Mediated Fibroblast-Activation Protein Targeted Photoimmunotherapy To Enhance Cytotoxic T Cell Infiltration and Tumor Control, *Nano Letters*, **2017**; 17(2):862-869 *acknowledgement of federal support (***yes***).*

- **Other publications, conference papers, and presentations.**

1. "X-ray Induced Photodynamic Therapy for Cancer Treatment", SPIE Conference, San Francisco, CA, January, 2017.
2. "Surface-modified nanoparticles for cancer imaging and therapy", Peking University, Beijing, China, July, 2017.
3. "X-ray Induced Photodynamic Therapy for Cancer Treatment", Jilin University, Jilin, China, July, 2017.
4. "Nanoparticle-mediated X-ray photodynamic therapy for cancer treatment", IEEE 2017, Atlanta, October, 2017.
5. "Nanoparticle-enhanced radiation therapy", END2CANCER conference, 2017, Oklahoma City, December, 2017.

7. Participants & Other Collaborating Organizations

What individuals have worked on the project?

Name:	*Dr. Jin Xie*
Project Role:	*P.I.*
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	*2*
Contribution to Project:	Led the project and designed the experiments
Funding Support:	
Name:	Dr. Wen Jiang
Project Role:	PostDoc
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	6
Contribution to Project:	Prepared the nanosensitizers
Funding Support:	
Name:	Dr. Zipeng Zhen
Project Role:	PostDoc
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	3
Contribution to Project:	Helped the animal studies
Funding Support:	
Name:	Benjamin Cline
Project Role:	Graduate Student
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	6
Contribution to Project:	In vitro X-PDT studies
Funding Support:	
Name:	Daye Lee

Project Role:	Graduate Student
Researcher Identifier (e.g. ORCID ID):	
Nearest person month worked:	6
Contribution to Project:	Peptide conjugation and cell uptake studies
Funding Support:	NSF

- **Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?**

  Nothing to Report

- **What other organizations were involved as partners?**

Organization Name: UNC

Location of Organization: Chapel Hill, NC

Partner's contribution to the project: Collaboration

Organization Name: Augusta University

Location of Organization: Augusta, GA

Partner's contribution to the project: Collaboration

8. Appendices

Nothing to Report