



SOCIETY OF PHYSICS STUDENTS

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SPS Chapter Research Award Interim Report

Project Title	Hybrid Photo-Magnetic Actuation for Target Specific Killing of Damaged Cells
Name of School	Southeast Missouri State University
SPS Chapter Number	6538
Total Amount Awarded	\$1,955
Project Leader	Varun Sadaphal

Abstract

At Southeast Missouri State University, in this study, we plan to explore an innovative actuation strategy for smart nano-structures to target and kill damaged/cancerous cells. The proposed therapeutic strategy has the potential to eliminate the residual cancer cells, thus may be used as adjuvant therapy for better results.

Statement of Activity

Interim Assessment

The rise in bio-magnetic and bio-medical research being conducted in universities and several government labs across the world provides great encouragement to develop an instrument which is capable of performing characterization studies on various types of nanoparticle systems and is an effective stage to conduct in-vitro cellular hyperthermia experiments. The proposed instrument will allow for dynamic actuation of nanoparticle solutions and simultaneously observe the actuation effects on biological cells. Currently, there are efforts to cure metastatic tumors through the targeted delivery of functionalized nanoparticles. This can be achieved through precise delivery of drug in a highly-localized area, or by inducing cell apoptosis in malignant cells via external excitation of targeted nanoparticles. The excitation of nanoparticles can cause localized heating that can cause cellular hyperthermia in harmful cells while leaving benign cells unharmed. There are multiple ways to make this technique successful. In case of targeted drug-delivery, PEG co-polymer coated nanoparticles can be loaded with therapeutic drugs. These loaded particles can release drugs via external excitation of nanoparticle due to thermo-responsive behavior of PEG which tends to shrink with increase in temperature and in turn release drugs at the intended tumor site. Similarly, cellular hyperthermia can be achieved via these nanoparticles by coating them with anti-bodies or aptamers. These coated particles interact with the surface proteins and DNA of a malignant cell and cause the particles to move in that direction, i.e., target them [1].

Using more than a single nanoparticle system in an actuation instrument can produce multiple effects, and these synchronous mechanisms can be used for novel applications. In the proposed system, FeNPs are actuated through a high frequency oscillating magnetic field and this produces a sharp temperature increase. The laser actuation of AuNPs causes them to produce a comparatively small temperature change, but along with this they produce oxidative species [2]. These oxidative species, when exposed to cells, can result in a phenomenon called 'oxidative stress' which can aid in further degradation of the targeted cell. Achieving a system configuration that can simultaneously actuate these nanoparticles has the potential to generate multiple effects on biological material. Applying this hybrid actuation in a flowing solution allows for the simulation of a body environment and will allow for large volumes of nanoparticle solutions to be actuated in a given time period.

Up until now, our group has been able to complete the following tasks, and these tasks have enabled us to move forward with dynamic actuation for next stage of experimentation:

- Laser Adjustments
- Photo-Magnetic Actuator Coil and Electronics Design
- Physico-Chemical Characterization of the Nano-carriers via Static Actuation

Physics and Engineering Physics seniors and juniors are involved and performing various activities related to this project. Mr. Varun Sadaphal, Mr. Colten Peterson, Mr. Dylan Wolk, and Mr. Heath Parkinson – all majoring Physics and Engineering Physics will work in a team during the project period. All of them are SPS members. Mr. Sean Thomas (Chemistry major) is also in the group and provides help with nano-particle synthesis and characterization measurements.

This project has initiated a great interest in STEM majors of our university. Many students are now willing to get trained to perform these tasks. This will help in a continued development of this project in the future. For the current participants, it has opened new pathways which involve graduate school admissions, conference visits and publishing papers in scientific journals to share our research with the scientific community.

Updated Background for Proposed Project

Due to biological heterogeneity of high-risk tumors, different therapeutic strategies are pursued. While reduced intensity therapeutic approaches, for example, surgery alone or in combination with moderate intensity chemotherapy are usual line of treatment for less aggressive tumors, high intensity chemo-radiotherapies are usually favored for tumors with more aggressive features [3]. Although the use of high intensity chemo-radiotherapies have demonstrated only modest improvement in the treatment of high-risk tumors, undesirable side effects include mouth sores, nausea, hair loss, and most importantly, increased chance of infection [4]. In

addition to these, there may be several drug specific side effects, for example, cisplatin and carboplatin can affect kidneys [1], doxorubicin is a cardio toxic agent [2], cyclophosphamide can damage bladder as well as ovaries and testicles [5], which in future may affect fertility. Unfortunately, despite implementing all advanced treatment modalities, 50-60% patients in high risk groups have a relapse, and there is no known curative treatment available to date [3].

Nano-structured materials and smart surfaces carry excellent treatment potential for development of novel clinical solutions because they can be designed to target/detect specific cancer cells and be remotely tuned to release measured doses of therapeutic agents, which in turn may improve treatment efficacy, decrease therapy time, and decrease the quantities of the therapeutic agent necessary for effective treatment 10-50-fold [6-7]. In order to meet these goals cumulatively, “combinatorial therapeutics” approaches consisting of various nanostructures and advanced instrumentation are becoming one of the most exciting forefront fields, while in its infancy till now.

Therefore, we set ourselves the goal of enhancing the treatment efficacy by combining a group of smart nanostructures, each of which are capable of performing a specific task with a novel strategy that has been unexplored thus far – simultaneous photo-magnetic actuation of a flowing nanoparticle solution. In this study, three different types of nanostructures will be used to accomplish the objectives: (1) PEG co-polymer coated magnetite (Fe_3O_4) nanospheres, (b) Polyvinylpyrrolidone (PVP) capped gold nanoparticles (AuNPs), and (c) iron and gold nanoparticle embedded multi-walled carbon nanotubes (IA-CNTs). The first two protagonists (i.e., the Fe_3O_4 nanospheres and the AuNPs) will induce a coupled hyperthermia and oxidative stress under the hybrid photo-magnetic irradiation, whereas the IA-CNTs are capable of providing an increased ‘specific absorption rate’ under similar actuation parameters along with an additional deteriorative effect on cells due to presence of toxic-CNTs. This will result in enhanced synergy in photo-magnetic hyperthermia mediated cytotoxicity inducing mechanisms, and intensify the oxidative stress induced damage, all at a relatively lower irradiation and nanoparticle exposure level.

Description of Research - Methods, Design, and Procedures

Static Actuation Tests

Photo-actuation of gold nanoparticles was performed for several concentrations of gold nanoparticle solutions. The purpose of this set of experiments was to investigate the relationship between the amount of gold nanoparticles in a solution and the temperature increase of the solution when exposed to a laser. Based on static volume photo-actuation tests, we will determine the concentration and volume flow rate of the solution to run through the flow cell during flow characterization phase of the project.

Static magnetic actuation of FeNP solutions was performed to characterize ability of the coils to magnetically heat these particles. It was important to understand static magnetic actuation using the coils so that flowing magnetic actuation parameters could be established. In this set of experiments, the dependence of magnetic heating on current, resonance frequency, and FeNP concentration was investigated. The results of these experiments were meant to produce the necessary input parameter-actuation relationships needed for future flow characterization experiments.

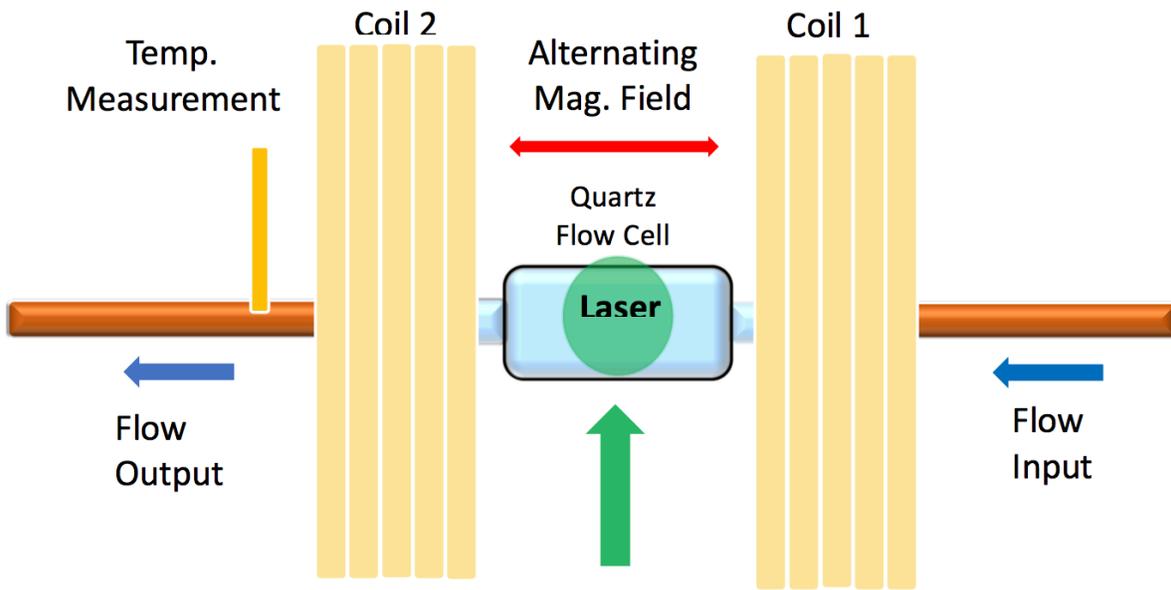
Dynamic Actuation Tests

After static actuation experiments, flow characterization parameters were established for dynamic actuation. The major goal of the dynamic actuation experiment is to characterize the instruments actuation performance for laser and magnetic actuation. Ultimately, hybrid dynamic photo-magnetic actuation will be characterized to show that this process is feasible and significant. After flow characterization is completed, live cell actuation experiments will be designed based on the results of the characterization experiments.

To perform dynamic experiments, nanoparticle solutions were injected into the flow tubing using a syringe pump. All solutions were pushed to a preset starting point before any actuation systems were enabled and experiments began. For flow characterization experiments, a flow rate of 0.2 ml/min will be as well as a current of 1.6 A_{rms}. Three milliliters of solution were used for each experiment. Temperature measurements were taken as the flowing solutions passed over the tip of the fiber optic sensor located at the exit point of the

system. The solution then flows into a disposal beaker. After each experiment, rubbing alcohol was used to clean the flow system from nanoparticles.

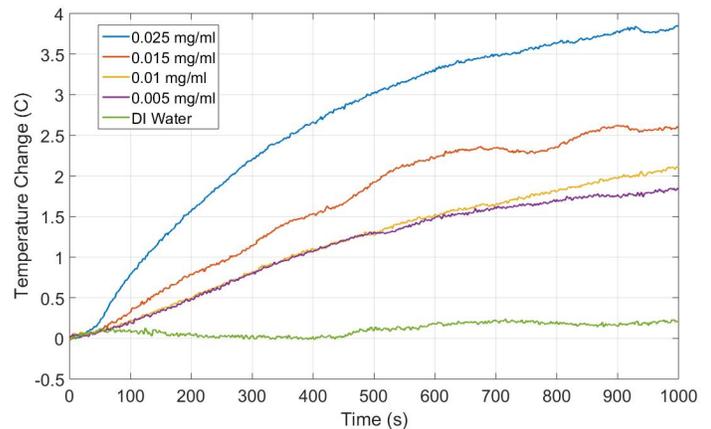
The dynamic actuation concept is depicted in the figure below:



Initial Results

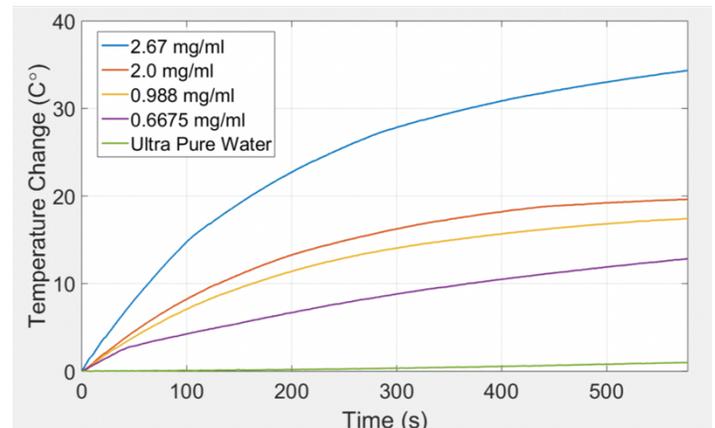
Static Laser Actuation: Concentration Dependence

To characterize the process of laser actuation, a static volume experiment was devised. A 1 ml quartz cuvette was obtained due to its optical transparency. The path length of the cuvette is 3 mm, and this allows for light energy to excite more gold nanoparticles without being significantly attenuated. The width of the cuvette is 11-mm, and this allows the 13-mm diameter laser to irradiate the entire cuvette width. The following gold-NP/water volumetric ratios were chosen for this experiment: 1/9, 2/8, 4/6, 5/5. Respectively, the concentrations of these solutions were 0.005 mg/ml, 0.01 mg/ml, 0.015 mg/ml, and 0.025 mg/ml.



Static Magnetic Actuation: Concentration Dependent

Three diluted iron-nanoparticle solutions were produced. The full concentration used was 2.67 mg/ml. The other two solutions were made by reducing the concentration to 75% (2 mg/ml), 37% (0.988 mg/ml) and 25% (0.6675 mg/ml) respectively. The solutions were actuated for 20 minutes each at 1.2 Arms and a resonance frequency of 152 kHz was used. Ultra-pure water was also exposed to static magnetic actuation as a control.



Statement of Next Steps

Plan for Carrying Out Remainder of Project (including Timeline)

The proposed project is being performed in the Nano-Bio-Engineering laboratory at Southeast Missouri State University. The Nano-Bio lab is equipped to perform wet chemistry, photo-magnetic energy transfer modulated temperature regulation measurements, cell and tissue culture, UV-Vis spectroscopy, atomic force and fluorescence microscopy, dynamic light scattering, and flow cytometry.

The flow cell design is under way and is expected to be completed by 30th June 2017. The dynamic photo-magnetic actuation tests will then be conducted and analyzed based on the nanoparticle concentration and the magnetic field strength which they were exposed to in these experimental runs. These tests should be completed by 30th August 2017. Cell culture of B35 neuroblastoma will then begin and simultaneously be exposed to different concentrations of our synthesized nanoparticles and analyzed for its biocompatibility. Biocompatibility tests should be done by 31th September 2017. Cell destruction experiments will be performed after this when a suspended mammalian cell solution containing the nanoparticles will be exposed to hybrid photo-magnetic fields under set parameters which were calibrated in our initial instrument testing. Everything mentioned above is scheduled to be completed by 30th November 2017. This sets us up for preparation of our final report after assimilating all the results and analyzing them.

Timeline: Below is a Gantt chart outlining the task structure and time schedule.

[Legend: **Green** → Completed; **Blue** → In-progress]

Activity ↓	Timeframe →		Month 1-6		Month 7-12	
	H1	H2	H1	H2	H1	H2
Laser Adjustments						
Photo-Magnetic Actuator Coil and Electronics Design						
Physico-Chemical Characterization of the Nano-carriers						
Interim Report Preparation (Due 31 st may, 2017)						
Flow Cell Design and Photo-Magnetic Actuation						
Specific Absorption Rate (SAR) measurement						
Cell Culture and Biocompatibility Assessment						
B35 Neuroblastoma Cell Destruction - Hyperthermia						
Final Report preparation (Due 31 st December, 2017)						

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