SOCIETY OF PHYSICS STUDENTS
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SPS Chapter Research Award Proposal

Project Proposal 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 Requested	$1,955

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.

Proposal Statement

Overview of Proposed Project

In this study, we aim to address some of the existing challenges arising from high intensity chemo-radiotherapies, which has demonstrated only modest improvement in the treatment of high-risk cancers. Moreover, undesirable drug specific and radiation therapy incurred side effects enhance the risk of developing into a second cancer at a later stage. An innovative technique that holds promise in the area of cancer diagnosis and therapeutics to perform precise drug delivery, multimodal therapy, and detection of circulating or residual cancer cells, all of which can play crucial roles in the treatment of high risk cancers, is the development of novel nanostructures coupled with smart actuation strategies. Oscillating magnetic field induced hyperthermia or photo-thermal destruction of cancer cells are among the most promising approaches among these, however, both fall short of addressing several concerns, including the use of high intensity magnetic or optical irradiation coupled with lower yield at clinically viable dose-level.

Thus, it is evident that innovative approach possessing novel therapeutic potential needs to be implemented in order to overcome the existing challenges. In this study, a safer and alternative multimodal therapeutic strategy involving simultaneous optical and alternating current (AC) magnetic field stimulation of a multifunctional nano-carrier system will be implemented to guide cancer cell destruction. This novel technique will permit the use of low-intensity photo-magnetic irradiation and reduced nanoparticle dose level during the treatment. The study is important, because if successful, our study will generate the proof of concept that smart nanostructure based photo-magnetic hybrid irradiation is a viable approach to remotely guide neuroblastoma cell destruction, which may be adopted in clinical management post modification to treat aggressive cancers.

A project such as this will help us gain important real-life working flavor, communication and dissemination of results to the scientific societies, train in time management and provide invaluable team-work experience by dividing tasks among each other with respect to our field of studies. This endeavor, due to its inter-disciplinary nature is not only limited to our department, rather students from other fields of science and technology can be major contributors towards the development of this project, which will possibly create a vivid learning environment for every student. Finally, this will lead to future work-force development and may initiate entrepreneurial endeavors in the STEM areas for the involved students, who will be in the forefront of the technological innovation nation-wide in the near future.

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 [1]. 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 [2]. In addition to these, there may be several drug specific side effects, for example, cisplatin and carboplatin can affect kidneys [3], doxorubicin is a cardio toxic agent [4], 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 [1].

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. In this study, three different types of nanostructures will be used to accomplish the objectives: (1) core-shell magnetic nanospheres (CSMNSs), (b) Polyvinylpyrollidone (PVP) capped gold nanoparticles (AuNPs), and (c) cisplatin loaded thermo-responsive nanoparticles (CPNPs). First two protagonists (i.e., the CSMNS and the AuNPs) will induce a coupled hyperthermia and oxidative stress under the hybrid photo-magnetic irradiation, whereas the CPNPs will cause sustained release of the imbibed cisplatin during the treatment. This will result in enhanced synergy between the cisplatin and photo-magnetic hyperthermia mediated cytotoxicity inducing mechanisms, and intensify the oxidative stress induced damage, all at a relatively lower irradiation and nanoparticle exposure level.

Expected Results

This study will enable high risk neuroblastoma cell exposure to varying combination of optical and magnetic field excitation in presence of specifically designed nano-carriers– thereby augmenting the positive outcomes of separate actuation strategies and the nano-carrier functionalities. Based on our previous observation with separate actuations, we anticipate significantly stronger heating response under the hybrid optical-AC magnetic field (magnetic field intensity ~ 60 Oe, frequency ~120 kHz, laser power 300 mW @ 520 nm), in the range of 8-10.5 K, even with a relatively lower nanoparticle dose level. Based on the data we get for our static volume results at different concentrations of the nanoparticles in a solution, we will calculate optimal ratios for iron and gold nanoparticles to be present in a flowing solution consisting of suspended tumor cells. We will establish an optimal flow rate that will allow for an 8 Celsius degree temperature change in the flowing solution. The flow system optimization process will allow for a large volume of solution to be actuated over a short time period. We anticipate complete ablation of the B35 neuroblastoma cells at a reasonable flow rate, thus providing the basis of constructing an approach to kill the residual cancer cells following available treatment approaches.

Description of Proposed Research - Methods, Design, and Procedures

Photo-Magnetic Actuator and Electronics Design

In order to develop a highly adjustable stage for placement of the coil, we will use PVC pipes to create a 1D stage where the two coils can be individually moved back and forth which change the magnetic flux through the center, and hence can be adjusted with size of any flow cell we choose to use in the future. For optical actuation, a 400 mW laser with a wavelength of 532 nanometers will be projected through a beam expander and this will produce a beam diameter of 1.5 cm when it reaches the solution in the photo-magnetic actuator. A major goal of this system is to maximize the energy transfer between the laser and gold nano-particles. To achieve this, the following tasks will be performed: (a) increasing the beam diameter of the laser after being projected through the beam expander, which will allow more of the solution to be heated by the laser and at a faster rate; (b) installing mirrors in the photo-magnetic actuator to reflect some of the scattered photons back through the solution, thus increasing the SAR of the nanoparticles; and (c) realigning the laser so the most intense part of the beam is aligned with the center of the solution.

A fiber optic temperature sensor will be utilized to record nanoparticle solution temperature as a function of time. The sensor will be placed where the solution flows out of the actuator, because the solution at that point is nearing the end of its activation period.

Photo-magnetic Actuation and Flow-Cell Related Experiments

A quartz cuvette of volume 5 mL will be obtained in order to conduct variable static volume experiments. The cuvette can be placed in the PMA at variable heights relative to the laser beam axis so that maximum irradiance can be achieved. During actuation, the temperature of the nanoparticle solution will be measured and the time of exposure will be recorded. A temperature increase of 5-8°C represents a successful

actuation cycle, because this temperature increase is required to induce cell lysis. To conduct flowing fluid heating, a quartz flow-cell of volume 0.4 mL will be obtained and connected to a length of insulated flow tubing. One end of the flow tubing will be connected to an injector that is controlled by a microfluidic pump. The diameter of the tubing will depend on the dimensions of the flow cell input channel. The injector will be kept at 37°C (body temperature) so that eventually suspended cancer cells can be injected into the system. A maximum flow rate should be established so that the most solution can be actuated during a given time. This can be obtained by increasing the flow rate until the temperature change at the base of the flow chamber is less than desired. Refer 'Figure 1' for detailed schematic diagram.

B35 Neuroblastoma Cell Culture and Coupled Hyperthermia Experiments

B 35 rat neuroblastoma cells (ATCC, Manassas, VA) will be routinely cultured at 37°C in 5% CO_2 and 85% relative humidity by using Dulbecco's modified Eagle's medium (DMEM, Invitrogen, Carlsbad, CA) derived complete media which contains 90% DMEM, and 10% fetal bovine serum (FBS). For the experiments, about 10,000 cells/sq.cm will be seeded in TPP tissue culture tube flasks (10 sq.cm growth surface area) containing 2ml of DMEM complete media and will be allowed to grow for 48 hours or more until 70% confluence is observed. All the experiments will be performed in triplicates. After 48 hours of cell growth and attachment, the cells will be washed with serum free DMEM and will be exposed to the NPs (various concentrations of MNPs and/or AuNPs), which will be colloidally suspended in the culture media. Immediately after the addition of NPs (MNPs and/or AuNPs), the cells will be exposed to AC magnetic field exposure / optical irradiation / hybrid optical-AC magnetic field exposure [Magnetic field intensity 60 Oe, frequency 120 kHz, laser power 300 mW] for 15min. Following irradiation, the cells will be placed in the incubator for 3hr and 45min as part of the treatment. After 4 hours of NP exposure and irradiation, the cells will be washed with serum free DMEM and will be cultured back into 2ml complete DMEM media until the beginning of the next exposure cycle. The treatment will be repeated thrice for every 24hr. At the end of the final exposure, live cell imaging will be performed to assess cell proliferation.

Plan for Carrying Out Proposed Project

Physics and Engineering Physics seniors and juniors will be mainly involved and perform 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) will also join the group and provide help with nano-particle synthesis and characterization measurements. All the group members have previously participated in Nano-bio-engineering workshop and acquired excellent skills in the areas of nanoparticle synthesis and characterization, magnetic heating measurements, mammalian cell culture, light and fluorescence microscopy, flow cytometry and other associated techniques, to name a few. Specific task structure and the time schedule is provided in the following section.

The proposed project will be performed in the Nano-Bio-Engineering laboratory in 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 laboratory director and the faculty advisor Dr. Santaneel Ghosh's expertise lies in the area of synergistic actuation strategies that combine and augment the positive outcomes of opto-magnetic stimulation and smart nanovector modulation of intracellular pathways. He has authored or coauthored 30+ peer-reviewed articles and have given a number of invited talks/seminars at international conferences/symposia in the areas of nano-scale device fabrication, advanced healthcare materials, novel microfluidics devices, drug delivery in targeted cells and cell signal modulation.

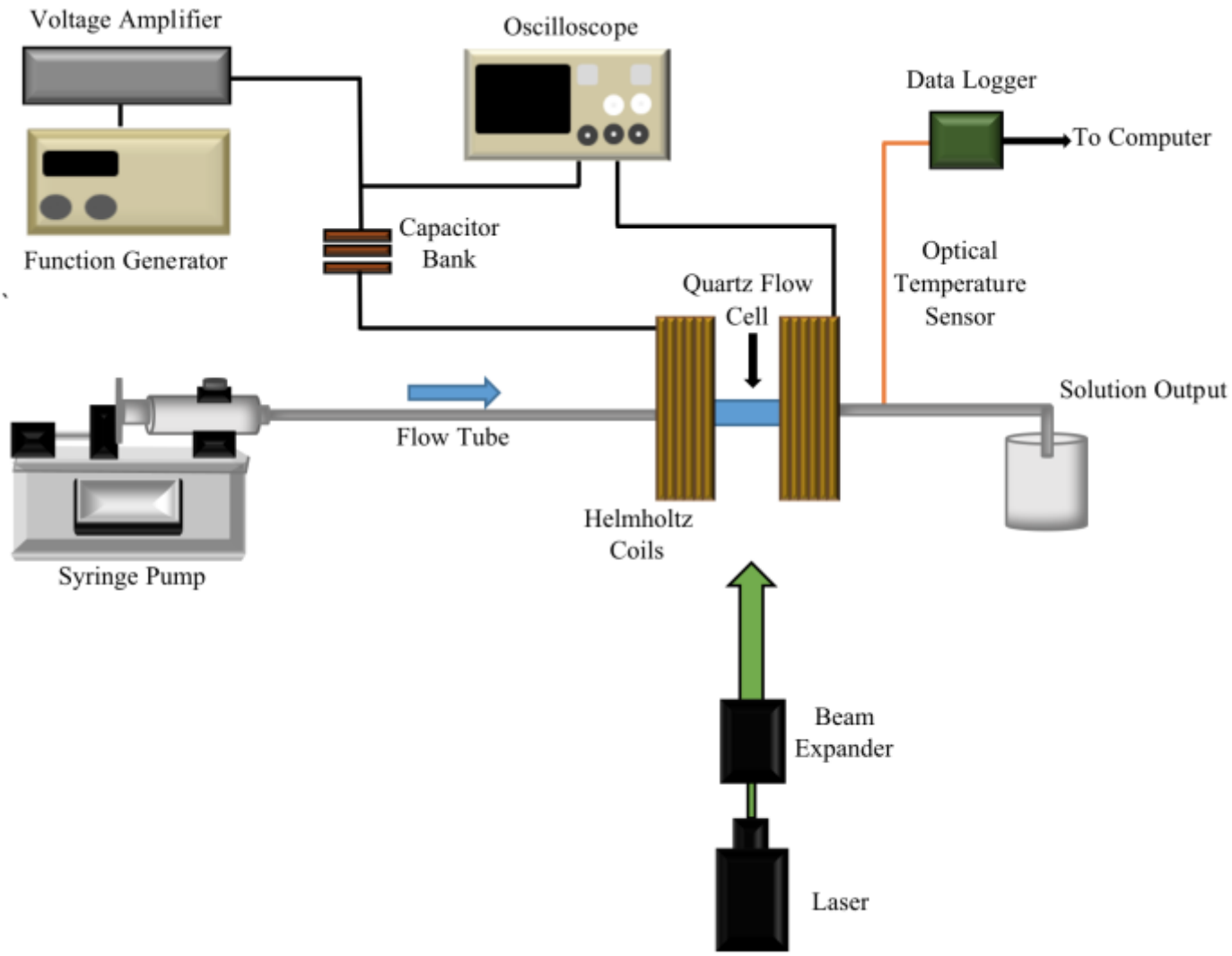

Figure 1. Detailed schematic diagram for photo-magnetic actuation using flow cell.

Project Timeline

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

Timeframe →	**Month 1-6**		**Month 7-12**	
Activity ↓	H1	H2	H1	H-2
Laser Adjustments				
Photo-Magnetic Actuator Coil and Electronics Design				
Physico-Chemical Characterization of the Nano-carriers				
Interim Report Preparation (Due 31st 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 31st December, 2017)				

Budget Justification

Litz Wire – Litz wire is designed to reduce skin effect and proximity effect losses in conductors used at frequencies up to about 1 MHz. It consists of many thin copper strands, individually insulated and woven together. In order for us to operate our equipment at kilohertz frequency, we will need these specialized litz wire which are designed to have minimal resistance at high frequency with the ability to pass 1-2 Amperes of current. Litz wire also minimizes the amount of joule heat that is generated during the actuation process. Our coil design requires a length of 500 feet because in order to generate a magnetic field flux density of 8-10mT, we will need 12 layers in each coil with 15-20 turns in each layer.

Quartz Flow Cuvette – Quartz allows high transmittance of light at visible wavelengths, especially at our operating 532nm green laser for photo actuation. Hence, using a flow cell made out of quartz can significantly improve the energy transfer from the laser to the gold nanoparticles in the flowing solution.

Teflon Sheet – Teflon is a highly insulating polymer of tetrafluoroethylene. The Teflon sheet will be utilized as insulation between adjacent layers in each Helmholtz coil. This will prevent high voltage breakdowns that are likely to occur as high current passes through the litz wire.

Consumables – In order to properly maintain our cell lines, we will require consumables like pipettes, TPP Tissue Culture Treated flasks, Tissue Culture Plates, DMEM, FBS to prepare the medium for cells to grow and proliferate.

Bibliography

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