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TITLE: Nanoparticle-Based Contrast Enhancement for Discriminating Indolent From Aggressive Prostate Cancer

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14. ABSTRACT

This program aims to develop a new imaging technique that will enable earlier detection of clinically relevant prostate cancer and help doctors to distinguish between aggressive cancers that need immediate therapy and more slowly growing indolent tumors that can be passively followed through watchful waiting or active surveillance management. Specifically, we aim to image metallic and polymer nanoparticles as they congregate within cancer regions of the prostate. We are using electrical property sensing devices that we have developed for imaging the prostate. We hypothesize that as nanoparticles congregate within the prostate the electrical properties of the prostate will change. For example, if metal nanoparticles are injected into a man and bind to prostate cancer cells, the high electrical conductivity of the metal particles will cause the overall conductivity of the prostate in the region of these nanoparticle to change. We have been developing electrical property sensing techniques to image the prostate and aim to use this approach to image nanoparticle concentrations within the prostate. This program is specifically investigating how sensitive electrical property sensing devices are to changes in concentration of these nanoparticles in both bench-top experiments and in in vivo animal experiments. We expect that by the end of this exploration we will have demonstrated that electrical property sensing technologies are able to detect different levels of nanoparticle congregation within a prostate cancer model and expect that we will be well-positioned to move to more rigorous animal model validation and human studies.

15. SUBJECT TERMS

Nothing listed

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INTRODUCTION

This program aims to develop a new imaging technique that will enable earlier detection of clinically relevant prostate cancer and help doctors to distinguish between aggressive cancers that need immediate therapy and more slowly growing indolent tumors that can be passively followed through watchful waiting or active surveillance management. Specifically, we aim to image metallic and polymer nanoparticles as they congregate within cancer regions of the prostate. Researchers are developing proteins that can seek out and bind specifically to prostate cancer cells. These proteins can be bound to metallic or polymer nanoparticles and suspended in a liquid solution that can ultimately be injected directly into the prostate or into the blood stream of the patient. These hybrid nanoparticles (protein + core nanoparticle) will seek out and bind to prostate cancer cells. We are exploring novel techniques to image these nanoparticles that are extremely low cost, can be used directly in the doctor's office, can provide real-time feedback, are safe, and can be done repeatedly for those men put on more active surveillance type approaches requiring multiple visits to their doctor to check on their prostate cancer. This technique uses electrical property sensing devices that we have developed for imaging the prostate. We hypothesize that as nanoparticles congregate within the prostate the electrical properties of the prostate will change. For example, if metal nanoparticles are injected into a man and bind to prostate cancer cells, the high electrical conductivity of the metal particles will cause the overall conductivity of the prostate in the region of these nanoparticle to increase. Alternatively, if polymer-based nanoparticles are injected, we would expect the conductivity to decrease if there is prostate cancer cells that these particles bind to. We have been developing electrical property sensing techniques to image the prostate and aim to use this approach to image nanoparticle concentrations within the prostate. This program is specifically investigating how sensitive electrical property sensing devices are to changes in concentration of these nanoparticles in both bench-top experiments and in in vivo animal experiments. We expect that by the end of this exploration we will have demonstrated that electrical property sensing technologies are able to detect different levels of nanoparticle congregation within a prostate cancer model and expect that we will be well-positioned to move to more rigorous animal model validation and human studies.

KEYWORDS: prostate cancer; electrical impedance; nanoparticles

ACCOMPLISHMENTS:

What were the major goals of the project?

Aim 1: Bench-top evaluation of a nanoparticle contrast agent

Major Task 1: Evaluate nanoparticle contrast in a saline model
Milestones: Relationship between electrical properties and NP concentration will be established
Status: Completed

Major Task 2: Evaluate nanoparticle contrast in a gelatin model
Milestones: Relationship between electrical properties and NP concentration will be established for a semi-solid phantom; impedance recording procedure to be used for animal model studies will be confirmed
Status: In progress, anticipated completion by September 2016

Aim 2: In vivo animal model evaluation

Major Task 1: Submit documents for IACUC approval
Milestones: Obtain IACUC in vivo animal model
Status: Animal protocol approved by Dartmouth's IACUC on October 4, 2016. Protocol documents were submitted to the USAMRMC Animal Care and use Review Office (ACURO) on October 6, 2016 and are pending review. We received a notice from ACURO on October 11th indicating that their internal review is underway.

Major Task 2: Conduct and evaluate in vivo animal model studies of nanoparticle contrast
Milestones: First in vivo evaluation of using nanoparticles as an electrical property contrast agent in a prostate tumor animal model; Validation that nanoparticles concentrations are detectable with electrical property sensing; initial optimal electrical property parameters for use in detecting different NP concentrations within prostate in an in vivo system
Status: To be completed over next 6 months; anticipated completion by Jan 2017

What was accomplished under these goals?

1) Major Activities (Saline Model) – Our major focus of the 1st year of this program was to develop an electrode array for saline tests and to begin evaluation of using nanoparticles as a contrast agent for electrical impedance measurements. It took longer than expected to identify the appropriate Research Associate to carry out all of the work proposed here. Half way through the first year of this program, we identified Katsiaryna Kekalo, Ph.D., an expert in nanoparticles and animal studies, to work on this project. She has helped to 1) optimize the electrode array used for saline tank experiments, 2) identified the appropriate nanoparticles to evaluate, 3) optimize our testing procedure, 4) carried out our initial saline cell experiments to evaluate the potential electrical impedance contrast the NPs might provide, and 5) assist with drafting our IACUC protocol.

Multiple test cells were designed and fabricated in order to optimize the repeatability of our measurements. The final cell is show in Figure 1. This test cell was used to measure saline over multiple days and times to ensure repeatability. Once we demonstrated robust and repeatable liquid impedance measurements we evaluated gold nanoparticles to determine how much potential contrast was achievable. Figures 2 & 3 show example sets of curves demonstrating how the impedance (resistance and reactance) varied with different concentrations of NPs. Note that the resistance decreases with increased NP concentration (e.g. from 7200 Ω to 7600 Ω at 100 Hz). Very minimal variation in reactance was observed.

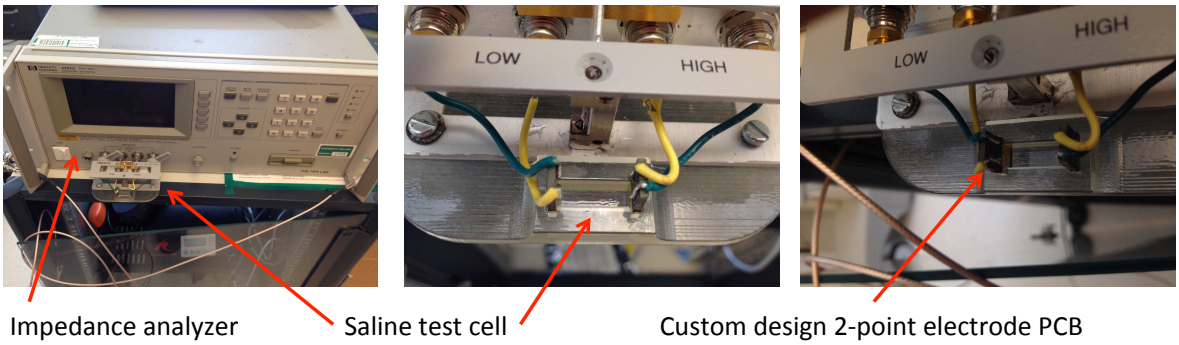

Figure 1: Test cell designed and fabricated for evaluating potential NP contrast enhancement in liquid phantoms. Cell dimensions are 7 mm x 7 mm x 12 mm.

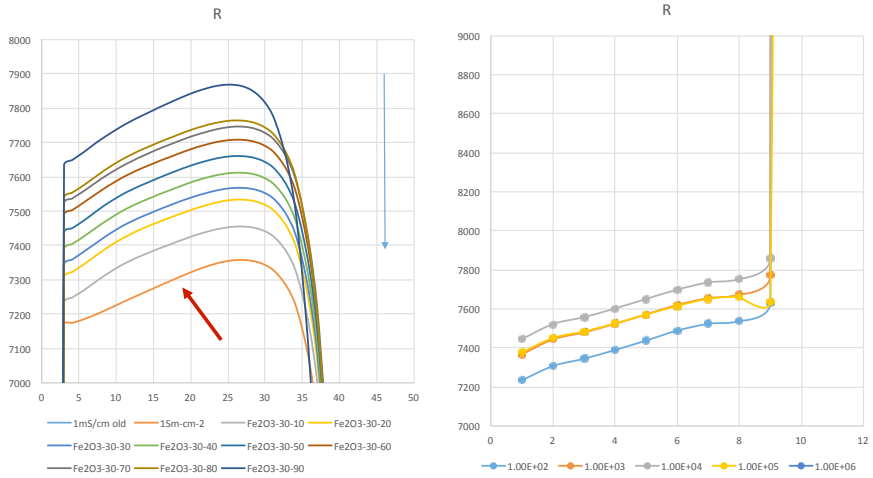

Figure 2: Resistance (R) as a function of signal frequency (left) and concentration (right). Note that the resistance decreases with higher concentration of NPs.

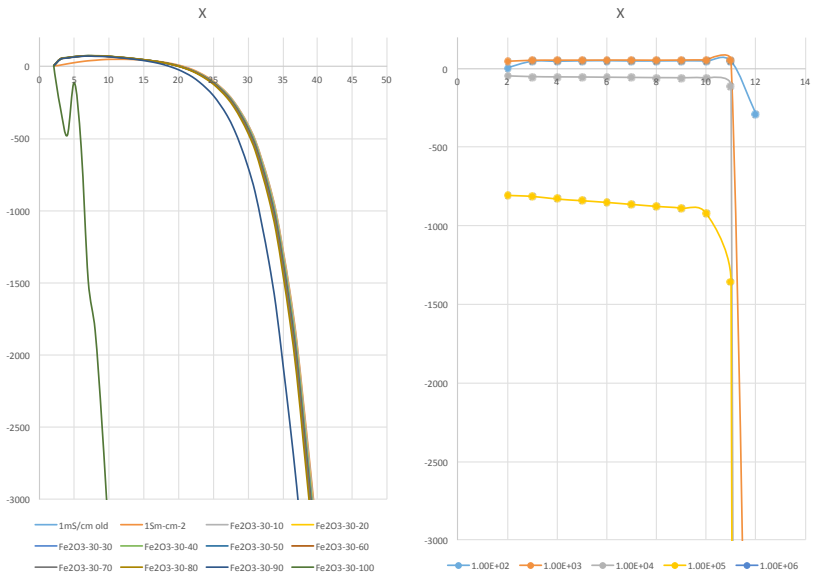

Figure 3: Reactance (X) as a function of signal frequency (left) and concentration (right). Note only minor reactance changes with increase concentration of NPs.

Current Conclusion and Hypothesis: The contrast observed was less than anticipated for both resistance and reactance. We believe this may be an artifact of the saline masking any influence of the small concentrations of nanoparticle. We hypothesize that the animal models may in fact show more contrast, especially if time is given for the saline solution (media in which the NPs are injected) is given time to washout. We expect that the NPs left behind in the cancer cells will provide more contrast (i.e. cancer + NP vs benign) then when no NPs are present (i.e. cancer vs benign).

Major Activities (Gel Model) – Since the contrast in saline was less than expected (primarily due to the high ionic pathway for current flow, we evaluated the potential NP contrast in gelatin models. The ions within these gels are more constrained (due to the semisolid nature of the material) and more closely emulate tissue. In addition, when the polystyrene nanoparticles (PNP) are embedded within the gelatin molds the NP are fixed in place and do not "settle out" or move as may be the case in a liquid phantom. Within the saline experiments we were able to dilute or concentrate a solution so that we could use the same NPs for multiple liquid phantoms of different concentrations. For the gel experiments, each gel we construct has a fixed NP concentration and the embedded particles can not be used again to create a different concentration. Due to the cost of NPs we elected to evaluate the potential contrast in gelatins for a fixed concentration, but for different particle sizes. We evaluated 3 sizes of polystyrene particles (1 μm, 5 μm and 10 μm); specifically, we used polystyrene microsphere procured from Phosphorex (Hopkinton, MA) – part numbers Polystyrene 112 (1 μm), Polystyrene 127 (5 μm), Polystyrene 118 (10 μm). Larger diameters were used in this study (than those proposed) in order to ensure that contrast was observed (we chose these larger particles because the contrast observed in our saline solution experiments was less than expected). In addition, these particles can be used in animal model studies and the 1 and 5 μm diameter particles are less than the average capillary diameter. Impedance data (resistance and reactance) were recorded from 2 cm x 2 cm x 2 cm gelatin cubes (8 cc). The gelatin's were constructed using 10:1 ratio of liquid to gel. Specifically, we used 10 ml of liquid and 1 g of gelatin. The liquids used include 1) 10 ml of deionized (DI) water, 2) 5 ml of DI water + 5 ml

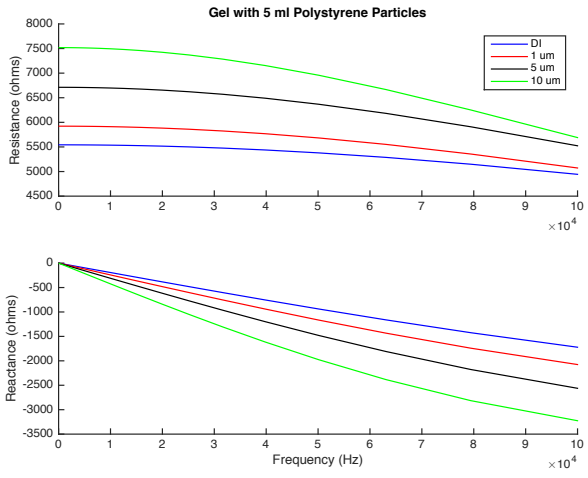

Figure 4. Impedance spectra for gelatin phantoms with different polystyrene particle sizes include 1 μm, 5 μm, and 10 μm. DI = deionized watere.

of 1 μm Polystyrene 112 particles, 3) 5 ml of DI water + 5 ml of 5 μm Polystyrene 127 particles, 2) 5 ml of DI water + 5 ml of 10 μm Polystyrene 118 particles. Figure 4 shows the resistance and reactance spectrum (from 100 Hz to 100 kHz) for the 4 gel models evaluated. The resistance contrast at low frequency (100 Hz) exceeded that of higher frequencies. Specifically, the observed contrast between Gel 1 and Gel 2 (1 μm PNP), Gel 1 and Gel 3 (5 μm PNP), Gel 1 and Gel 4 (10 μm PNP) were 7% (5543 Ω vs. 5922 Ω), 21% (5543 Ω vs. 6712 Ω), and 35% (5543 Ω vs. 7519 Ω), respectively. This contrast far exceeds that observed in our saline experiments. In addition, the 21% contrast provided by the 5 μm PNPs is easily detectable given the resolution of most impedance acquisition system.

Current Conclusion and Hypothesis: The gel experiments demonstrated that polymer particles embedded within a semisolid media will increase the impedance of the material. Intuitively, this makes sense since polymers represent high impedance materials. While the larger microspheres used here represents nanoparticles that are 100's of nanometers in diameter, the size is still clinically relevant as capillaries are typically > 5 μm in diameter (which should enable NP delivery even when injected systemically instead of intratumorally). The gel experiments suggest that polymer microspheres, provide contrasts exceeding 7% when 1 μm particles are used and greater than 20% when 5 μm particles are used. We hypothesize that using 1 or 5 μm PNPs will provide enhanced impedance contrast within an animal model and will be the particles that we use in our animal model.

2) Specific Objectives – Our specific objectives this first year were to develop a test cell for evaluating the potential contrast NPs might provide for electrical impedance sensing. The specific objective of the remaining time is focused on animal models studies to evaluate this potential contrast.
3) Significant Results and Key Outcomes:
   - We have developed and validated a test cell for making repeatable impedance measurements of liquid phantoms
   - We observed increase resistance with inclusion of metallic NPs in saline. The absolute change in resistance was ~400 Ω; however, this only represented an increase of ~6% with respect to the baseline impedance.
   - We demonstrated significant impedance contrast when polymer microspheres are embedded in a gelatin, tissue-simulating phantom.

What opportunities for training and professional development has the project provided?
While this program was not intended to provide training and professional development opportunities, we have provide an opportunity for Dr. Kekalo to expand her knowledge in nanoparticles (i.e. early exploration of the electrical properties of NPs) and to gain new knowledge in the measurement of electrical properties of media.

How were the results disseminated to communities of interest?
Nothing to Report.

What do you plan to do during the next reporting period to accomplish the goals?
During the remainder of this program we will completed the following tasks:

1. *Complete animal model investigation of NP-based contrast enhancement of electrical properties* – We will follow the protocol described in our research narrative to evaluate if there is increased cancer to benign tissue contrast when NPs are injected into prostate tumors.

IMPACT:
What was the impact on the development of the principal discipline(s) of the project?
Based on the saline experiments, the increased contrast with metal NPs is limited to ~6%. Despite this, we expect that there may be more contrast enhancement in an in vivo setting because the impedance change will not be masked by the high conductive saline solution. We have found that in gelatin experiments, where the ionic conductivity is not as high as in a liquid phantom, the impedance contrast is larger. If we find this enhanced contrast to be true within an animal model, NPs may provide a novel approach to better detecting prostate cancers using electrical impedance imaging. An additional novel aspect of using polystyrene microspheres, is that these devices can be loaded with cytotoxic drugs. Once present at the tumor site the spheres can be energized (e.g. through thermal, optical, mechanical, magnetic, etc) methods in order to stimulate the microsphere to deliver their payload to the tumor. Impedance sensing of these microspheres may

enable clinicians to verify that the microspheres are aggregating within a tumor and therefore identify the optimal time to energize the microspheres for drug delivery.

What was the impact on other disciplines?
If we find that the NPs do provide enhanced tumor to benign contrast, this approach may be valid for other soft tissue cancers (i.e. breast, kidney, liver, etc). In addition, other application that use electrical impedance sensing for pathological assessment may benefit. For example, studies of blood flow or capillary recruitment using electrical impedance sensing may leverage additional contrast if NPs are injected arterially.

What was the impact on technology transfer?
Nothing to Report.

What was the impact on society beyond science and technology?
Nothing to Report.

CHANGES/PROBLEMS:
Changes in approach and reasons for change
Nothing to Report

Actual or anticipated problems or delays and actions or plans to resolve them
It took longer than expected to identify the appropriate Research Associate to carry out all of the work proposed here. Half way through the first year of this program, we identified Katsiaryna Kekalo, Ph.D., an expert in nanoparticles and animal studies, to work on this project. She has been actively working on this program and we are now on schedule to complete the tasks by the end of this no cost extension year.

Changes that had a significant impact on expenditures
Nothing to Report

Significant Changes In Use Or Care Of Human Subjects, Vertebrate Animals, Biohazards, And/Or Select Agents:

Significant changes in use or care of human subjects
Nothing to Report

Significant changes in use or care of vertebrate animals.
Animal protocol approved by Dartmouth's IACUC on October 4, 2016. *Protocol documents were submitted to the USAMRMC Animal Care and use Review Office (ACURO) on October 6, 2016 and are pending review. We received a notice from ACURO on October 11th indicating that their internal review is underway.*

Significant changes in use of biohazards and/or select agents
Nothing to Report

PRODUCTS:
Publications, conference papers, and presentations
Journal publications.
Nothing to Report
Books or other non-periodical, one-time publications.
Nothing to Report
Other publications, conference papers, and presentations.
Nothing to Report

Website(s) or other Internet site(s)
Nothing to Report

Technologies or techniques

We have constructed a robust cell for making liquid impedance measurements. This design and validation of it will be described in a journal paper that will be submitted during the remaining time in this program.

Inventions, patent applications, and/or licenses
Nothing to Report

Other Products
Nothing to Report

PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS
What individuals have worked on the project?

Name:	Ryan Halter (PI)
Project Role:	Assistant Professor
Researcher Identifier :	Not available
Nearest person month worked:	1
Contribution to Project:	Prof. Halter helped with cell design, training Dr. Kekalo to perform measurements, IACUC protocol writing, and project management
Funding Support:	N/A

Name:	Katsiaryna Kekalo
Project Role:	Research Scientist
Researcher Identifier :	Not available
Nearest person month worked:	2
Contribution to Project:	Dr. Kekalo helped with cell design, performed saline measurements, IACUC protocol writing, and data analysis
Funding Support:	N/A

Name:	Alex Hartov
Project Role:	Research Professor
Researcher Identifier :	Not available
Nearest person month worked:	1
Contribution to Project:	Dr. Hartov helped with cell design and programming our data acquisition device
Funding Support:	N/A

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?
Nothing to Report.