# NDnano Summer Undergraduate Research
# 2016 Project Summary

**1. Student name**: Rachel Hanley

**2. Faculty mentor name:** Dr. Tiffanie Stewart

**3. Project title:** Penetrating Cancerous Multicellular Aggregates with Magnetoelectric Nanoparticles

**4. Briefly describe any new skills you acquired during your summer research:**

During my summer research I attained a number of skills that I know will stand to me when I go forward in both my education and career. Some of the key skills that I learned include nanoparticle fabrication and functionalization, and also a number of possible characterization techniques that can be used in order to achieve a more in depth understanding of the nanoparticle's properties. These skills include instrumentation techniques such as Atomic Force Microscopy (AFM), Scanning Electron Microscopy (SEM) which were both used to obtain the size and morphological properties of our magnetoelectric nanoparticles. Other instrumentation training that I received included X-ray Photoelectron Spectroscopy (XPS) and Fourier Transform Infrared Spectroscopy (FTIR) which were used to obtain information on the chemical properties of the nanoparticles. I have also become familiar with cell handling and cell culturing. I am now confident in my abilities to grow and culture cells in both monolayer and into 3D spheroids (or multicellular aggregates (MCAs). When investigating the penetration depth of the MENs into the multicellular aggregates I also received the opportunity to image the MCAs under the fluorescent microscope and also to cryosection the MCAs using a microtome. I was also involved in the production and participation of a Nanofilm submission to the National Nanotechnology Initiative in which a short 3 minute video was made describing the magnetoelectric nanoparticles (MENs) properties and how they can be used in the treatment of cancer.

**5. Briefly share a practical application/end use of your research:**

The magnetoelectric nanoparticles fabricated in this project can be used to specifically target cancerous cells leaving the healthy cells unaffected and have also been shown to penetrate deeply into cancerous multicellular aggregates that are proven to be more resistant to current cancer therapies. Through the application of an external DC magnetic field the MENs specifically target the cancer cells and provide an on-demand release of drugs following the application of an external AC magnetic field. The future of MENs in the treatment of cancer is promising, however, further research and in-vivo experimentation still needs to be carried out. Other applications of MENs are also being investigated including using them as a noninvasive treatment of Parkinson's and other neurodegenerative diseases and also to deliver anti-HIV drugs across the blood–brain barrier in the treatment of HIV/AIDs.

## 6. Project summary and Results:

Ovarian cancer has a relatively high mortality rate despite positive response at first treatment. A primary reason ovarian cancer is difficult to treat is that the mechanism of disease progression is unusual. Ovarian cancer derived from epithelial tissue that characteristically forms multicellular aggregates (MCAs). MCAs represent 3-dimensional, avascular metastatic spheroids that form invasive foci in the intraperitoneal cavity late in disease stages. MCAs are characteristically more resistant to current cancer therapies. It is speculated that ovarian cancer MCAs are highly neoplastic and aggressive clusters of cancer cells because they avoid anoikis when shed, and also have the ability to implant and invade in the mesothelial cell lining, which promotes metastasis. Furthermore, spheroids lacking vasculature present a challenge for drug delivery to the inner core cells of the aggregates.

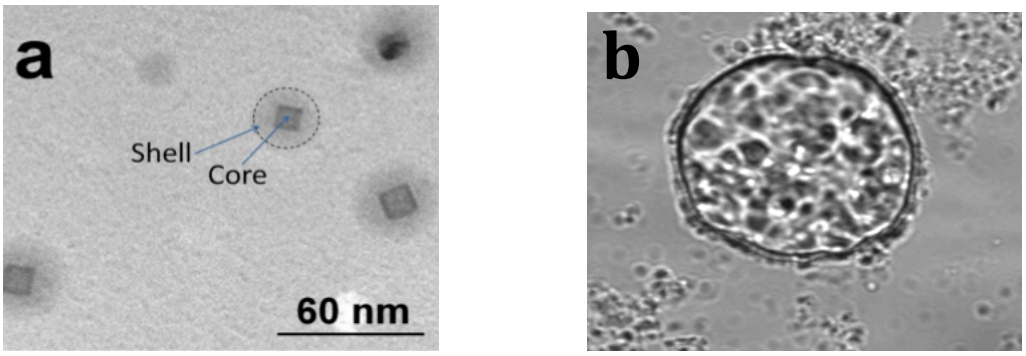


**Fig 1. A) Transmission Electron Microscopy (TEM) image showing the coreshell nanostructure of MENs (spinel $CoFe_2O_4$ core and perovskite $BaTiO_3$ shell). B) Multicellular aggregate of Ovarian Cancer DOV13 cell line.**

The aim of this research was to demonstrate magnetoelectric nanoparticles (MENs) capability of penetrating multicellular aggregates (MCAs) (Figure 1b) of different cancerous cell types and also to show the effect of a magnetic field on the depth of MENs penetration into these 3D MCAs. These MENs have unique intrinsic electrochemical and magnetic properties and in this research project have been shown to penetrate into 3-D MCAs with the potential of providing an on-demand release of drug. Through the application of a relatively weak magnetic field, MENs ($CoFe_2O_4$@$BaTiO_3$ nanostructures) create an external electric field that changes the nanoporosity of a cell membrane in a process known as nanoelectroporation. MENs take advantage of the fact that cancerous cells have a substantially lower electric potential and therefore display a lower threshold for nanoelectroporation compared to healthy cells. This allows MENs to specifically target and penetrate into cancerous cells while leaving the healthy cells unaffected. MENs penetrate spheroids by attracting their iron-based core towards the inner MCA, and simultaneously inducing nanoelectroporation via the electric field generated on the outer shell.

In the early stages of the project the magnetoelectric nanoparticles (figure 1a) were fabricated and fluorescently tagged with Texas red dye and paclitaxol (as these dyes proved to fluoresce the brightest under the fluorescent microscope). The MENs were fabricated using a two-step hydrothermal process: first the cobalt iron oxide core ($CoFe_2O_4$) was made followed by the barium titanate shell ($BaTiO_3$) and these were then mixed together and placed in a furnace for 6 hours. In this project, both ovarian cancer MCAs (DOV13 cell line) and glioblastoma MCAs were grown using the hanging drop method as seen in figure 2. The MCAs were then treated with the functionalized fluorescently tagged MENs, and an external DC magnetic field of 1500 Oe was applied. The MCAs were then imaged using an inverted fluorescent microscope every hour throughout the day to see how far the MENs penetrated into the cancerous MCAs. It was found that penetration occurred after 1 hour and the fluorescent intensity increased as a result of increased concentration of MENs collecting inside the MCA over time. This thus proves that the MENs can effectively penetrate into the MCAs where an on-demand release of drugs is possible. Other methods used in this project to determine the MENs penetration depth included imaging fixed MCAs on a glass slide and cryosectioning MCAs that were embedded in a collagen matrix using a microtome, however, imaging the live cells in their hanging drops proved to be the most efficient and least time-consuming method. From this research it can be concluded that MENs may be an effective drug delivery system to treat MCAs that are resistant to current cancer therapies.

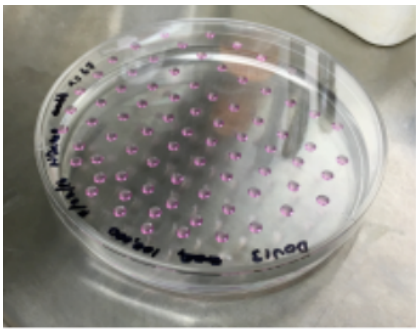

**Fig 2. Hanging drop method used to grow cancerous multicellular aggregates (MCAs).**

Publications (papers/posters/presentations):
Nanofilm Submission: "Magnetoelectric Nanoparticles (MENs) to Treat Cancer" https://youtu.be/e5gkWh1ywKM

Poster Session 07/27/16
Co-author
Title: "Magnetoelectric Nanoparticles to Specifically Target Cancer Cells in vitro"

Presentation Session 08/04/16
Title: "Penetrating Cancerous Multicellular Aggregates with Magnetoelectric Nanoparticles"