Neutralizing the Systemic Toxicity of Co-Formulations of Chemotherapeuitcs Using Magneto-electric Silica Nanocarriers for Specific Therapeutic Action Against Metastatic Cancer Cells

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Background

We intend to target non-targetable cancer cells by tuning, the magnetic field induced force exerted by label-free magnetic nanomaterials at the cell membrane interface to selectively permeabilize the more compliant cancer cell membranes but not the stiff cell membranes of healthy cells.

Systemic delivery of chemotherapeutics is toxic to all cells but more so to cancer cells due to the high metabolic rate of cancer cells. Targeted drug delivery using antibody tagged drug carriers reduce the dosage required to kill cancer cells and thereby mitigate the toxicity associated with systemic drug delivery. But the lack of unique targetable biomarkers on aggressive cancer cells such as triple (3º) negative breast cancer makes targeted drug delivery options a non-starter. But with the recent advances in biophysics, it is well documented that cancer cells have significantly different cell membrane stiffness in comparison to healthy cells. Cancer cell membranes are less stiff, more compliant and the cells as a whole are easily deformable.[1] The difference in membrane physical properties can be exploited by applying a force that is above the threshold required to permeabilize the cancer cells, but the force is still below the threshold required to permeabilize normal cells..

The cancer cell permeabilization and cancer cell-specific drug delivery have been validated *in vitro* in our studies.

1. Swaminathan et. Al. *Cancer Res* **71**, 5075-80 (2011)

Magnetoelectric Silica Nanocarriers (Mag-E-Si-Ns) as ON-Demand Drug Delivery Vehicles

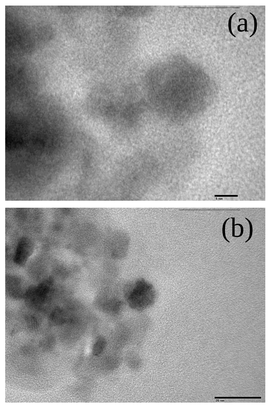


Figure 1. High-resolution transmission electron microscopy images of silica capped Cobalt-ferrite nanoparticles with a magnetic core and piezoelectric shell. The magnetoelectric silica drug nanocarriers were designed to electroporate cancer cell membranes specifically in the presence of an external magnetic field. Once it has permeated into the cell, an alternating magnetic field causes the electrostatic bonds to the drug molecules to stretch and break, thus releasing the drug inside of the cell. Scale bars = 5nm in (a) and 20nm in (b).

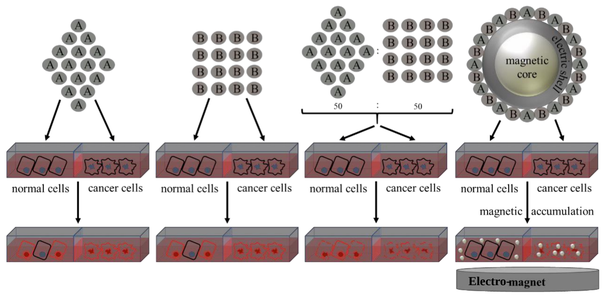
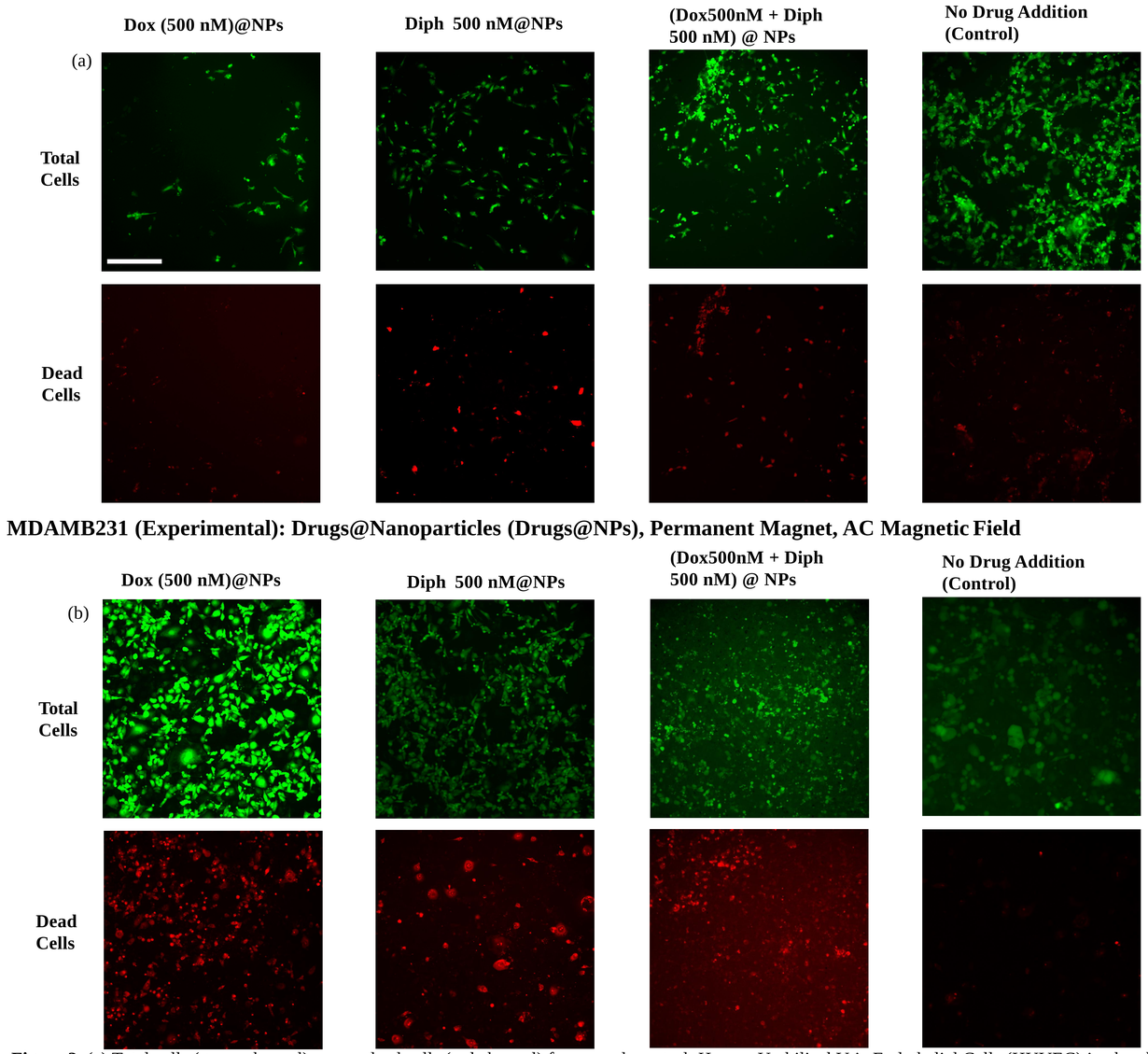


Figure 2. Schematic illustration of the workflow for the addition of free drugs and drugs conjugated to Mag-E-Si-Ns, to normal control cells and to cancer cells. "A" represents doxorubicin (Dox) and "B" represents diphyllins (Diph). These drugs were added as a 50:50 mixture in co-formulations. Two doses of the drug molecules tested were 20 nM and 500 nM. Magnetic fields were used to target Mag-E-Si-Ns to cancer cells and for drug release in cancer cells. Illustration is not to scale.

Cell Viability Assay to Assess Cancer Cell Killing Efficacy of Mag-E-Si-Ns Delivered Drug Therapeutics

HUVEC (Control): Drugs@Nanoparticles (Drugs@NPs), Permanent Magnet, AC Magnetic Field



MDAMB231 (Experimental): Drugs@Nanoparticles (Drugs@NPs), Permanent Magnet, AC Magnetic Field

Figure 3. (a) Total cells (green channel) versus dead cells (red channel) for normal, control Human Umbilical Vein Endothelial Cells (HUVEC) in chamber slides exposed to drugs (doxorubicin or diphyllins, or a combination of both) released from the surface of the Mag-E-Si-Ns drug carriers. Additionally, these slides were incubated and exposed to a permanent magnet for 24 hours, and then an alternating current magnetic field (A.C. mag field) for 10 hours. The live/dead assay was performed 48 hours after initial drug exposure. (b) Live versus dead cells for MDAMB231 cells, a triple negative breast cancer cell line, exposed to the drug-conjugated nanoparticles a permanent magnet for 24 hours, and an AC mag field for 10 hours. Cell survival was tested 48 hours after initial drug exposure.

Dose dependent cell death was seen when 500 nM each of doxorubicin or diphyllins or a combination of both were released from the Mag-E-Si-Ns. The cancer cell specificity of the Mag-E-Si-Ns is visually evident from the cell death of the cancer cell population when exposed to 500nM of doxorubicin or diphyllins or doxorubicin + diphyllins. Additionally, it can be seen that the control cell line, HUVEC, experienced less than 30% cell when exposed to the same concentrations (500 nM) of drugs. Annexin V staining did not show any apoptotic cells at the endpoint. Scale bar = 100 micrometer

Cancer Cell Specificity and Anti-Cancer Efficacy of Magnetoelectric Silica Drug Nanocarriers used for ON-Demand Drug Delivery

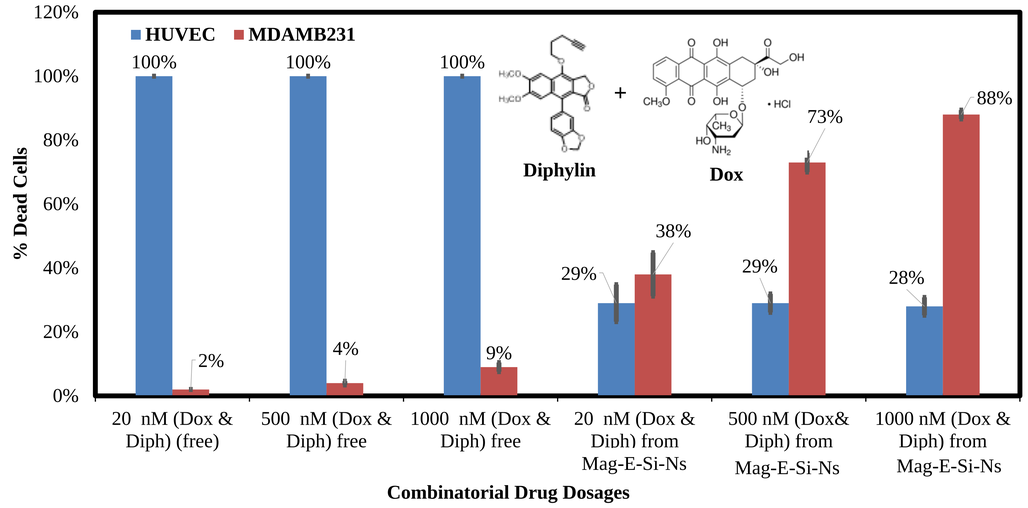


Figure 4: LIVE/DEAD cell viability assay of MDAMB231 and HUVECs demonstrate the enhanced anticancer efficacy of Dox released form magnetically directed [Dox+Diph]@Mag-E-Si-Ns. Combined formulations of [Dox+Diph] delivered on Mag-E-Si-Ns were 10x to 19x fold times more effective in killing cancer cells than free drug formulations of the same dosage of [Dox+Diph].

Enhanced Anti –Cancer Efficacy of Magnetoelectric Silica Nanocarriers is Dependent on External Magnetic Field

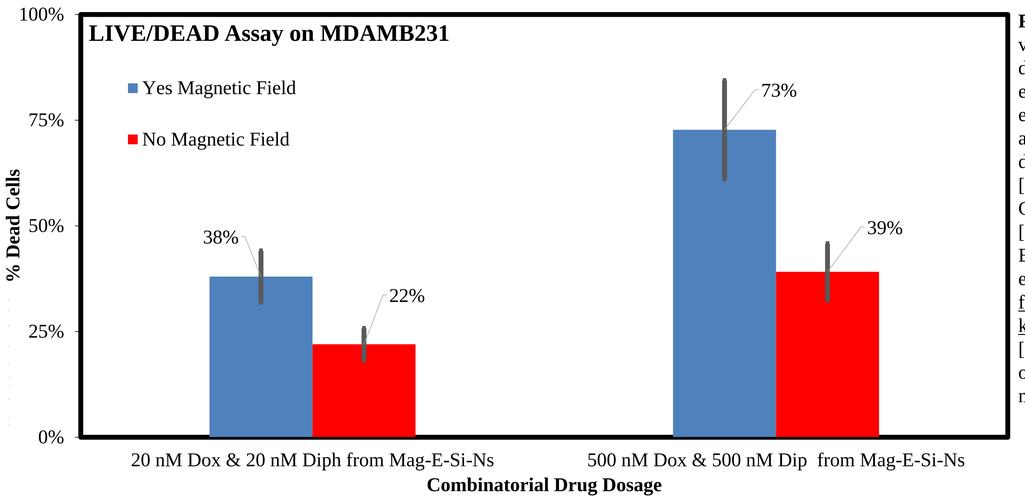


Figure 5: LIVE/DEAD cell viability assay of MDAMB231 demonstrated the need for the external magnetic field to enhance cancer cell permeation and maximize intracellular drug delivery from [Dox+Diph]@ Mag-E-Si-Ns. Combined dosages of [Dox+Diph] delivered on Mag-E-Si-Ns in the presence of an external magnetic field were 2x fold times more effective in killing cancer cells than [Dox+Diph] dosages delivered on Mag-E-Si-Ns in zero magnetic field.

Vacuolar-ATPase Pump Inhibitor Diphyllin Inhibits ATP usage

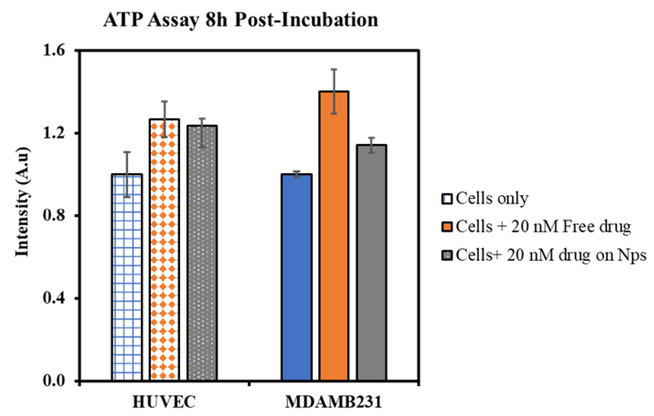
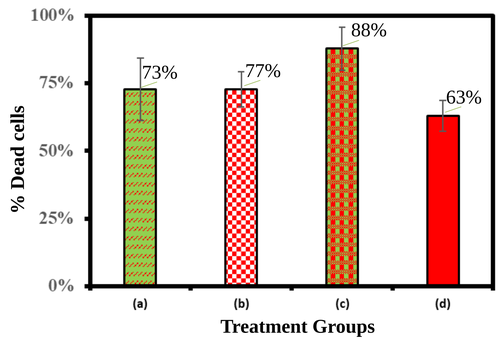


Figure 6: ATP assay was used to determine that release of Diphyllin from Mag-E-Si-Ns resulted in reduced utilization of ATP by cancer cells. This confirmed that Diphyllins released from Mag-E-Si-Ns do retain their ability to inhibit vacuolar-ATPase (V-ATPase) proton pumps by hampering ATP uptake by the proton pumps. V-ATPAse pumps are crucial for degrading the collagen surrounding cancer cells thus allowing them to metastasize. Diphyllins by inhibiting the V-ATPase pumps have the potential to be an anti-metastatic chemotherapeutic.

Synergistic Therapeutic Effect of Dox with Diph at Dose> 1µM



- (a) = **500 nM (Dox & Diph) (on Mag-E-Si-Ns + magnetic direction)**
- (b) = **500 nM Dox (on Mag-E-Si-Ns + magnetic direction)**
- (c) = **1000 nM (Dox & Diph) (on Mag-E-Si-Ns + magnetic direction)**
- (d) = **1000 nM Dox (on Mag-E-Si-Ns + magnetic direction)**

Figure 7: [Dox+Diph] released from Mag-E-Si-Ns had a synergistic anti-cancer effect at dosages>500 nM. A dosage of 1000 nM of [Dox+Diph] released from Mag-E-Si-Ns killed 25% more MDAMB231 cells as opposed to 1000 nM of Dox alone released from Mag-E-Si-Ns.

Diphyllin Inhibits Metastatic Capability of Triple Negative MDAMB231 Cells in Trans-well Migration Assay

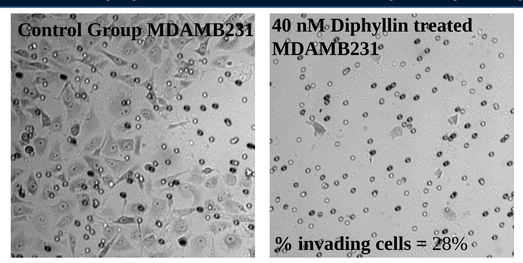


Figure 8: Trans-well migration assays from corning were used to assess the anti-metastatic capability of 40 nM Diphyllin released from Mag-E-Si-Ns, 4h post magnetic field exposure. Even at this low dose diphyllin reduced MDAMB231 invasion by an astounding 72% in a 16 hour time period. This correlates well with our ATP assay data in figure 7 where we noted that diphyllins released from Mag-E-Si-Ns inhibited ATP utilization by cancer cells.

Conclusion: This leads us to hypothesize that Diphyllins released from Mag-E-Si-Ns can be used complementarily at a low dose(<40 nM) to prevent tumor metastases during chemotherapeutic tumor debulking. Mag-E-Si-Ns bound Diphyllins and Doxorubicin in combination at a high dose (>500 nM)can synergistically kill cancer cells as well as prevent cancer cell metastasis. Our next step is to confirm our hypothesis in a mouse *in vivo* model.

Acknowledgements

The authors would like to thank BIPH Discovery Fund, ACS-IRG, Indiana CTSI for the the financial support for this project. We would like to thank the Woodward Family for the summer 2018 fellowship of Margo Waters. We would also like to thank Prof. Holly Goodson, Drs. Ian Lightcap and Yueying Liu of the Harper Cancer Research Institute for the technical assistance to make this project possible.