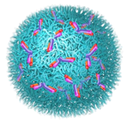
Targeted Detection of Ovarian Cancer Using Functionalized Iron Oxide Nanoparticles

*Eric Smith-Nguyen, Marie Zhang**
Imagion Biosystems, Inc., San Diego, CA, USA
**Corresponding email: marie.zhang@imagionbio.com*



Poster 3590

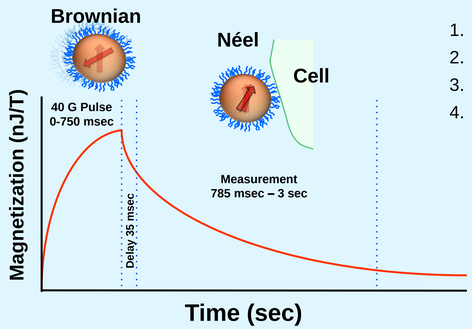
Introduction

Superparamagnetic Iron oxide nanoparticles (SPIONs) have been used for a variety of *in vivo* and *ex vivo* applications in the biomedical sciences. SuperParaMagnetic Relaxometry (SPMR) is a highly sensitive detection technology that can differentiate the magnetic signature of SPIONs bound to tumor cells from unbound nanoparticles. PrecisonMRX® NPs are extensively characterized SPIONs composed of 25 nm cores that are currently used in a variety of applications including Magnetic Particle Imaging, MRI contrast, and magnetic hyperthermia.

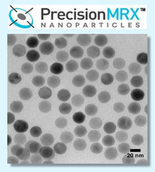
Objective

In previous studies, we have demonstrated that when conjugated with anti-HER2 antibody such as Trastuzumab, PrecisonMRX® NPs exhibited great specificity and selectivity towards HER2+ve tumor cells in vitro and in vivo. In current studies, we expanded our nanoparticles applications to other cancers, such as ovarian cancer. Here we present the proof-of-concept studies of anti-CA125 and anti-GPC-1 antibody conjugated NPs for targeted detection of ovarian cancer by SPMR.

Superparamagnetic Relaxometry



1. Inject NPs
2. Apply small magnetizing pulse
3. Turn off field
4. NPs relax to their equilibrium states.



MRX

Nanoparticles that reach and bind to the target cells (Néel relaxation) are measurable by superconducting quantum interference device (SQUID) magnetometers (MRX instrument developed in house), while unbound nanoparticles (Brownian motion) such as those freely circulating in the bloodstream are not detected and bone and normal tissue do not produce any magnetic signal.

CA125 Ovarian Target

The CA125 is a tissue-specific antigen expressed in ovarian cancer. It is associated with greater than 80% of epithelial ovarian neoplasms. OC125, a murine monoclonal antibody, reacts with glycosylation-dependent antigens present exclusively in the cleaved portion of the molecule.

Methods

OC125 Antibody NP Functionalization and Characterization

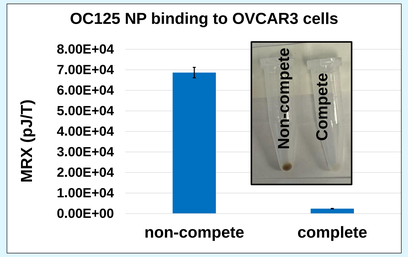
PrecisionMRX® NPs were encapsulated by a layer of polymer and then functionalized with carboxylate (COO^-) surface. PEG + OC125 antibody were subsequently conjugated onto the polymer surface. Size of resulting NPs were measured by DLS. Bound and free mAb were determined via ELISA.

Surface	Diameter	PDI	# of Ab/NP	% of free Ab
PEG + OC125 Ab	70-80 nm	0.1-0.2	~ 1	<20%

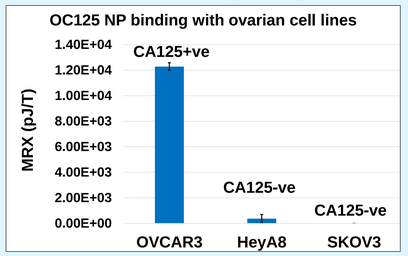
- A variety of ovarian cell lines (1x10^6 cells cultured on 6-well plates), such as OVCAR3 (CA125+ve, passage <12 as OVCAR3 lost significant CA125 expression after >15 passages), HeyA8 and SKOV3 (both CA125-ve), were incubated with 100μg of OC125 NPs overnight.
- Cells were washed, harvested, centrifuged, and pellets were subsequently measured on the MRX instrument.
- Cell competition study was done by pre-incubating cells with free OC125 antibody.

Results

Cell Competition Assay



Cell Selectivity Assay



- **OC125 Nanoparticles can clearly distinguish CA125+ve and CA 125-ve expressing cell lines. Furthermore, signal can be competed out by pre-incubating with free antibody, demonstrating specificity and selectivity.**

*Miltuximab is provided by GlyTherix, Ltd

GPC-1 Ovarian Target

Antigen glypican-1 (GPC1) is a proteoglycan located on cell surface composed of a membrane-associated protein core anchored to the cytoplasmic membrane. GPC1 may play a functional role in the control of cell division and growth regulation. The expression of GPC1 has been found to be elevated in many cancer cells, including ovarian. Miltuximab (GlyTherix, Ltd) is a humanized IgG antibody targeting GPC-1*.

Methods

GPC-1 Antibody NP Functionalization and Characterization

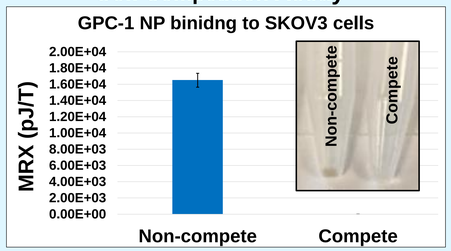
PrecisionMRX® NPs were encapsulated by a layer of polymer and then functionalized with carboxylate (COO^-) surface. PEG + GPC-1 antibody were subsequently conjugated onto the polymer surface. Size of resulting NPs were measured by DLS. Bound and free mAb were determined via ELISA.

Surface	Diameter	PDI	# of Ab/NP	% of free Ab
PEG + GPC-1 Ab	~70 nm	<0.1	4-5	<5%

- GPC-1 positive ovarian cell line SKOV3 (1x10^6 cells cultured on 6-well plates) were incubated with 100μg of GPC-1 NPs overnight.
- Cells were washed, harvested, centrifuged, and pellets were subsequently measured on the MRX instrument.
- Cell competition study was done by pre-incubating cells with free GPC-1 antibody.

Results

Cell Competition Assay



- **GPC-1 Nanoparticles can clearly bind to GPC-1 expressing cell line, SKOV3. Furthermore, signal can be competed out by pre-incubating with free antibody, demonstrating specificity.**

Conclusions and Future Work

Together, these results suggested that our antibody conjugated nanoparticles can provide targeted and specific delivery to ovarian cancer cells and generate measurable signal using our MRX detection instrument. These proof-of-concept studies lay out groundwork for developing methods of future targeted ovarian cancer detection.