Specific detection of anti-Her2 PEGylated PrecisionMRX™ nanoparticles measured using superparamagnetic relaxometry

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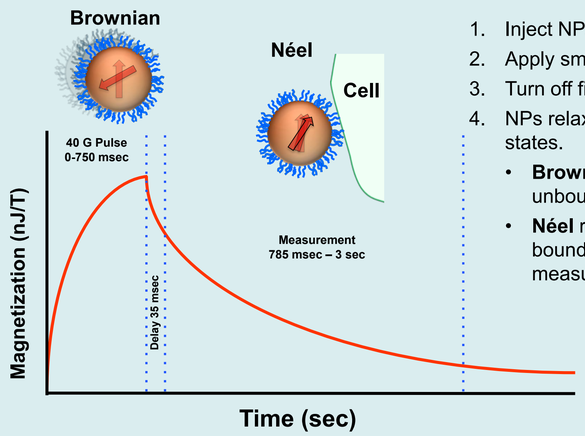
Introduction

Superparamagnetic relaxometry (SPMR) is a combination technology that utilizes superconducting quantum interference devices (SQUIDs) to measure the magnetization of superparamagnetic magnetite (Fe_3O_4) nanoparticles (NPs) in vivo. Our PrecisionMR® PEG coated Fe_3O_4 NPs are labelled with a tumor-targeting moiety (i.e., a monoclonal antibody) and intravenously injected. Subsequently, the NPs are magnetized by a low field magnetic pulse in the MRX™ instrument and only those particles that are bound to their target site are measured by the SQUID sensors. Unbound nanoparticles are not detected.

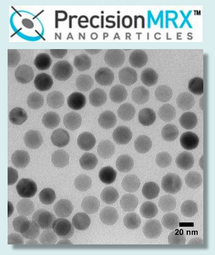
Objective

Demonstrate the utility of SPMR in detecting cancer using PEGylated PrecisionMRX® NPs that are covalently linked with anti-Her2 antibody (mAb) targeting Erb-B2 in vitro and in vivo while maintaining longer circulation time when compared with NPs without a PEG coating.

Néel Relaxation Background



1. Inject NPs
2. Apply small magnetizing pulse
3. Turn off field
4. NPs relax to their equilibrium states.
   - **Brownian** motion of unbound NPs (fast)
   - **Néel** relaxation of NPs bound to cells (slow and measurable)



SPMR only detects NPs bound to cells/tissues

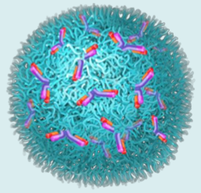
Methods

Nanoparticle surface functionalization and characterization

25 nm PrecisionMRX® NPs were functionalized with a carboxylate (COO^-), PEG, or PEG + anti-Her2 mAb surface. Bound and free mAb were determined via ELISA

Dynamic Light Scattering (DLS)

Surface	Diameter (nm)	PDI	Zeta Potential (mV)
COO^-	46	0.04	-40
PEG	70-100	0.10	-10
PEG + anti-Her2	85	0.10	0

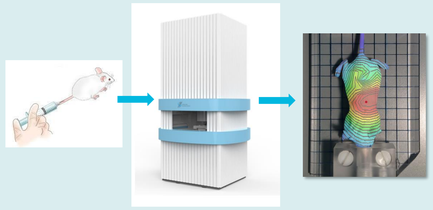


Specific binding in vitro

Used 50 ug of anti-Her2 mAb to competitively block binding of 300ng anti-Her2 conjugated NPs to Erb-B2 overexpressing (MCF-7/Her218) and negative (MCF-7) breast cancer cells. Cells were harvested, centrifuged, and pellets were subsequently measured on the MRX instrument.

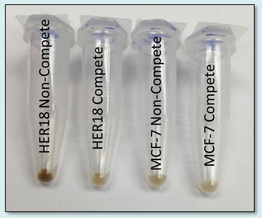
Specific binding in vivo

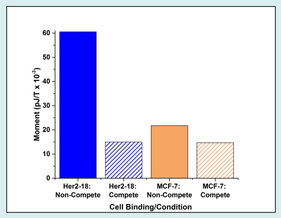
- COO-, PEGylated, or anti-Her2 conjugated PEG NPs were intravenously injected into BT474 (Erb-B2 (+)) or MCF-7 (Erb-B2 (-)) tumored mice
- MRX measurements were taken over timepoints, and a dipole map was generated for signal localization
- Mice were euthanized and organs were excised for ex-vivo MRX measurement



Results – In Vitro Specificity

In Vitro Competition Assay

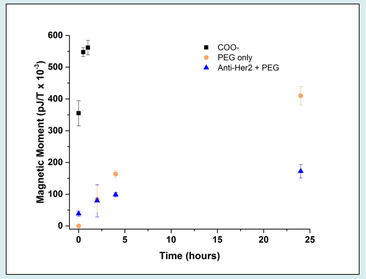




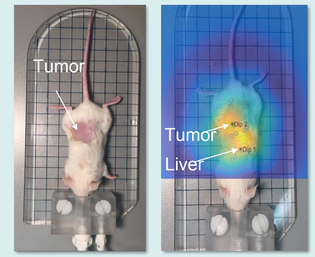
Minimal uptake was observed in Erb-B2 (-) cells as compared with overexpressing

Results – In Vivo Specificity

Non-tumored mice: in vivo signal over 24 hours

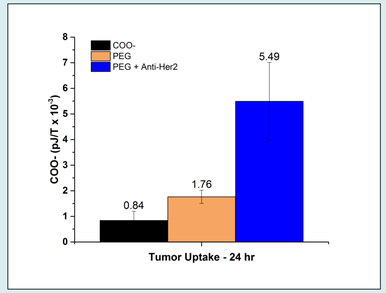


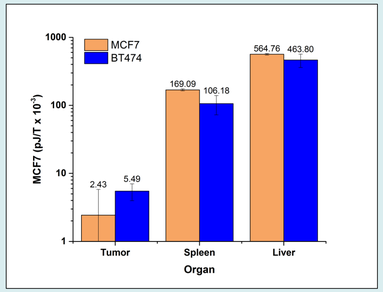
Tumored mice: in vivo dipole map



- **PEG and anti-Her2 PEG NPs also showed a much more gradual uptake than the COO- with the anti-Her2 NPS giving a signal at 24 hours of less than half the 24 hour signal for the PEG NPs**
- **The overlay of the dipole map with the whole mouse image indicates two dipole resolution**

Tumor delivery: ex vivo measurements





Preliminary data suggest targeted tumor delivery of anti-Her2 functionalized NPs over 24 hr. vs. PEG only NP (2.25X increase)

Conclusions and Future Work

- Developed a PEG coated, anti-Her2 targeted PrecisionMRX® NP that specifically binds Erb-B2 expressing breast cancer cells in vitro and in vivo while remaining in circulation longer than the unPEGylated (COO-) NPs
- Future work is focused on identifying a NP formulation that optimizes targeting and stealth for clinical application of MRX technology in detecting solid tumors.

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