UST Global Internship 2017 Research Report

AUGUST 23, 2017

MR/optical imaging probes and their applications for in vivo image-tracking of immune cells

1. Synthesizing contrast agents for MRI

2. Labeling of MNPs and optical dyes in cells and their characterization

3. Supplementary experiments and comments/thoughts

1. Synthesis of Magnetic Nanoparticles (MNPs)

Synthesized iron-oleate complex using thermal decomposition method

Metal chloride
+
Na-oleate

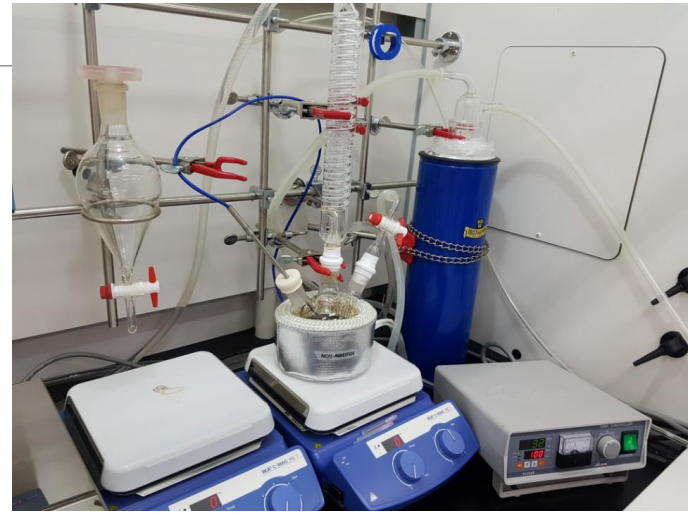
→

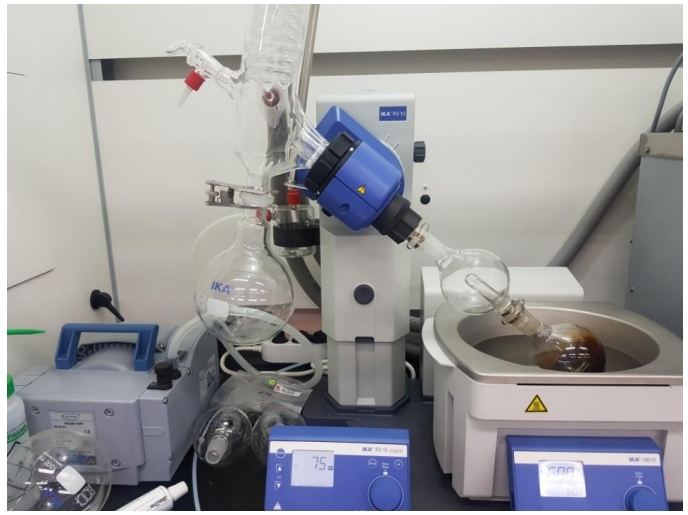
Metal-oleate complex
+
NaCl

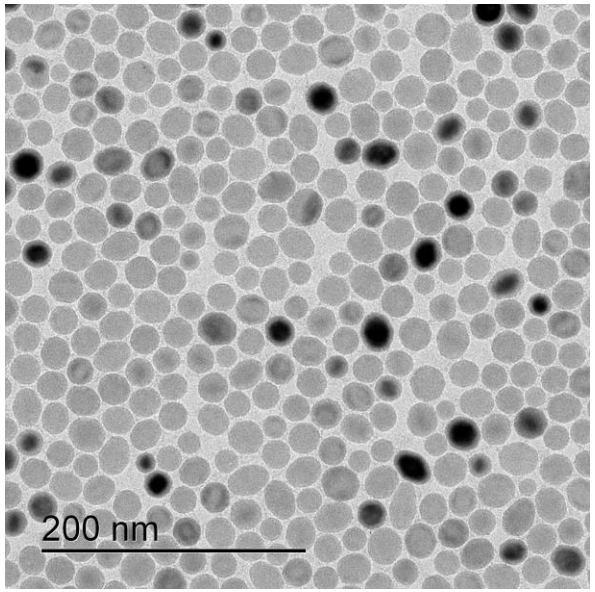
The Fe-oleate complex is then coated with methoxy-PEG

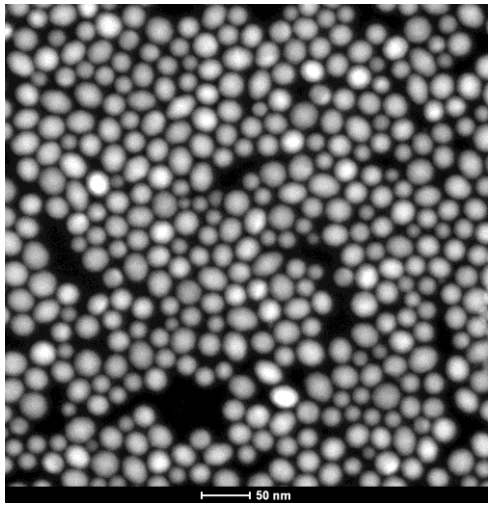
Materials used include: condenser, RT cooling (right), Argon gas, ultra tip-sonic, ultra-centrifuge, UV-spectrometer, etc.

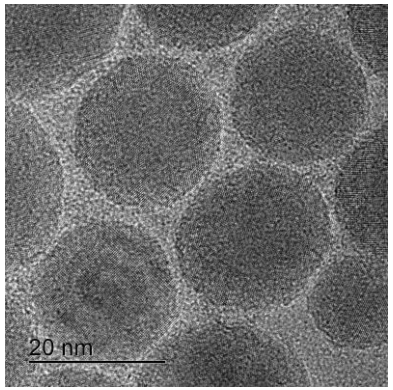
The MNP will be used as probes for cell labeling and tracking











TEM (Transmission Electron Microscopy)

TEM was used for observing the size and shape of the MNPs.

Darker shades indicate that thicker/multiple particles exist.

STEM (Scanning Transmission Electron Microscopy) shows particles in white color.

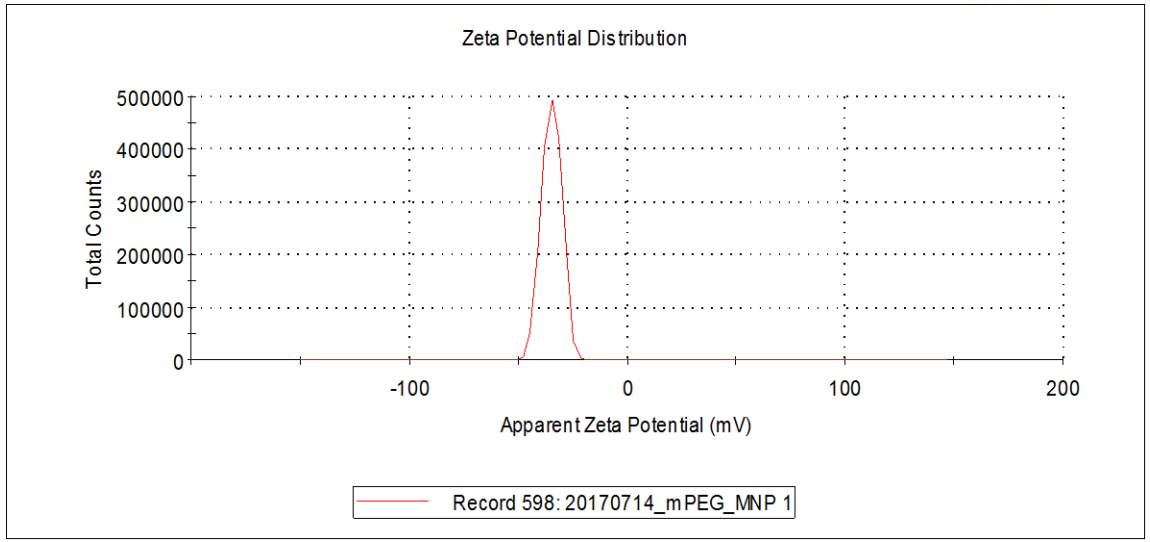
DLS (Dynamic Light Scattering)

DLS measures the intensity fluctuations of scattered light arising from Brownian Motion (random movement of particles)

Intensity signal from larger particles change more slowly

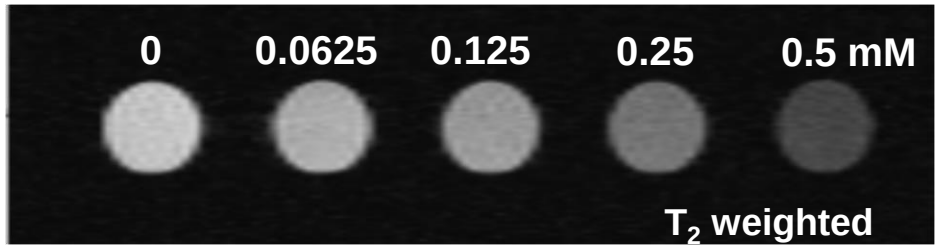
Measures zeta potential (charge) in aqueous media

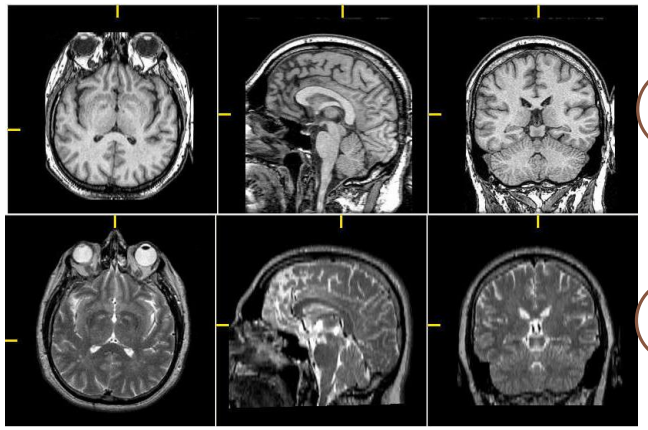
Displays number, volume, and intensity distributions of nanoparticles based on their hydrodynamic diameters, and correlograms for analysis



MR Imaging

MR Imaging uses strong magnetic fields to take images of organs in the body.
T1-weighted image (longitudinal relaxation) shows substances in brighter shades, while T2-weighted image (transverse relaxation) shows them in darker shades.

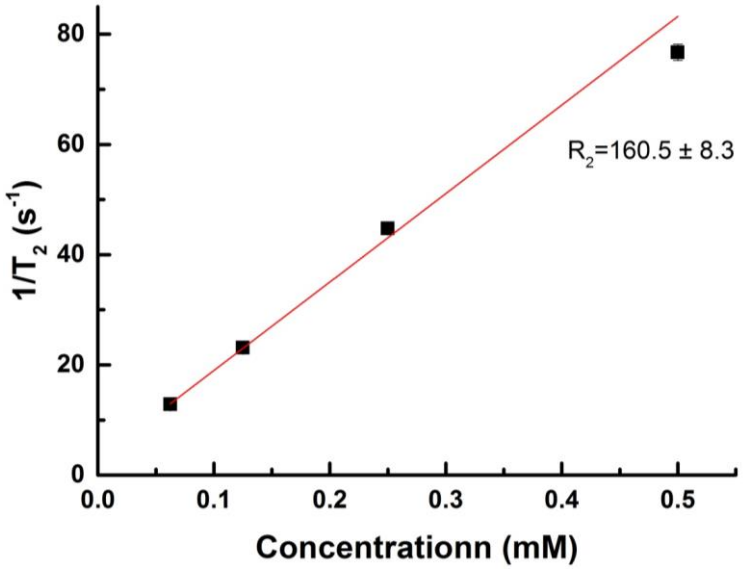




T1

T2

Before treating cells with magnetic nanoparticles, MR images of the particles (in PCR tubes) were taken to measure its contrast property.
r_2 = relaxivity [$mM^{-1}s^{-1}$]
$1/T_2 = R_2$ = relaxation rate
T_2 = relaxation time [s]



2. Labeling of MNP

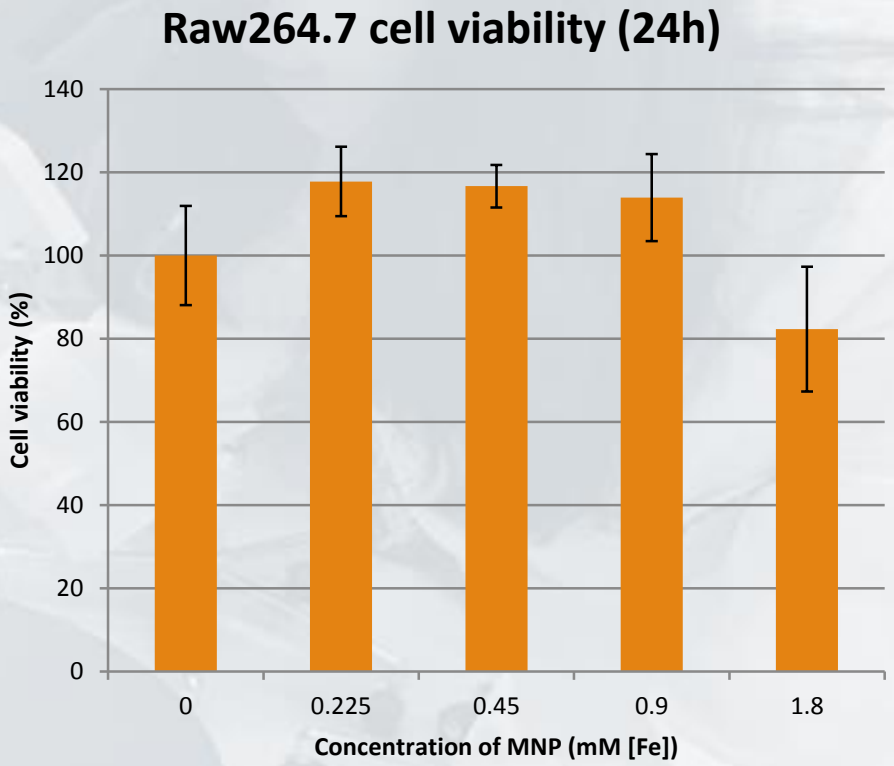
Preparation of cells (Raw264.7 cell culture)

MNP treatment on cells: 0, 0.25, 0.5, 1, 2% (0, 0.225, 0.45, 0.9, 1.8 mM[Fe])

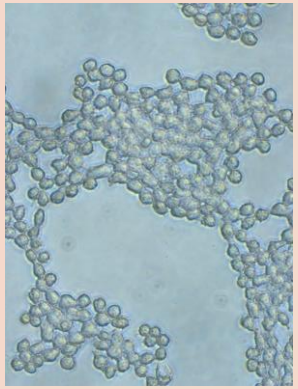
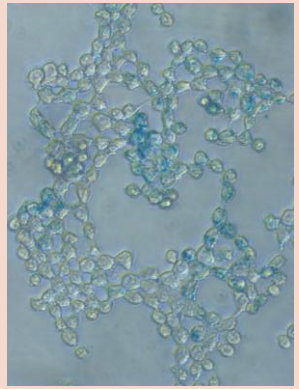
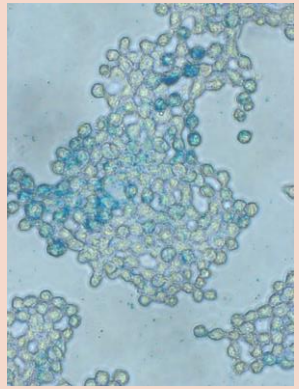
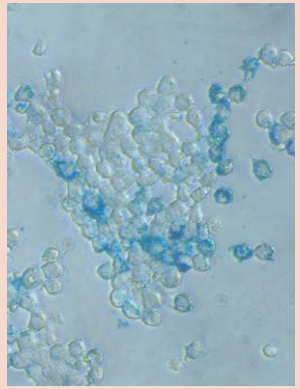
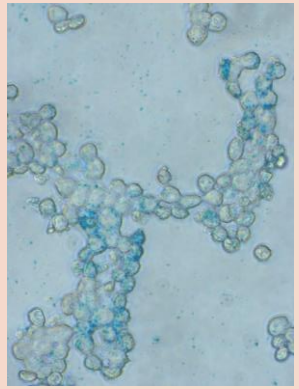
Cell viability check by MTT assay

Treating higher concentration of MNP to the cells may cause damage and lower cell viability

The goal is to find the optimal amount of MNP that can guarantee both good cell viability and good signal emission for cell tracking.

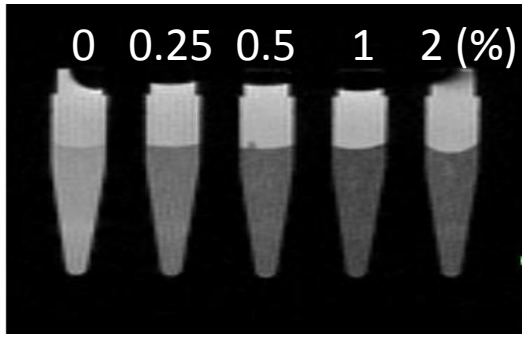


Prussian blue staining & MR Imaging

% MNP treated/ Concentration (mM[Fe])	0 / 0	0.25 / 0.225	0.5 / 0.45	1 / 0.9	2 / 1.8
Microscope image					

Cells were treated with MNPs and fixed on a 96-well plate. The uptake of the particles, which contain Fe, into the cells were shown in blue.

An image of treated cells were also taken with MRI, which is shown on the right.



3. Supplementary Experiments

- **Cell culture (Raw264.7, DC2.4, etc.)**

  Various types of cell lines were needed for the experiments in the lab

- **UV-spectrophotometer**

  Measures light absorption/scattering of particles

- **Calcein-AM release assay**

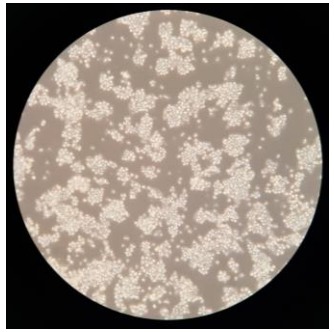
  Measures how many cancer cells have been killed by natural killer cells, using calcein-AM labeling

- **Western blotting**

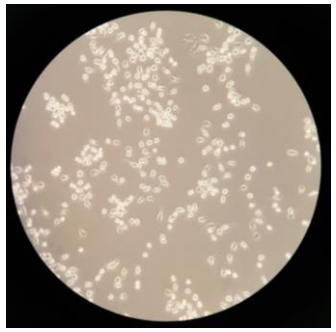
  This method was used for detecting specific proteins in a sample of tissue extract.

- **Nude mouse dissection**

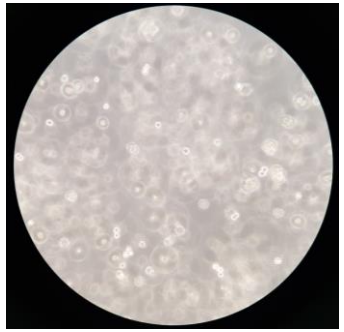
  Shadowing nude mouse blood and organ extraction



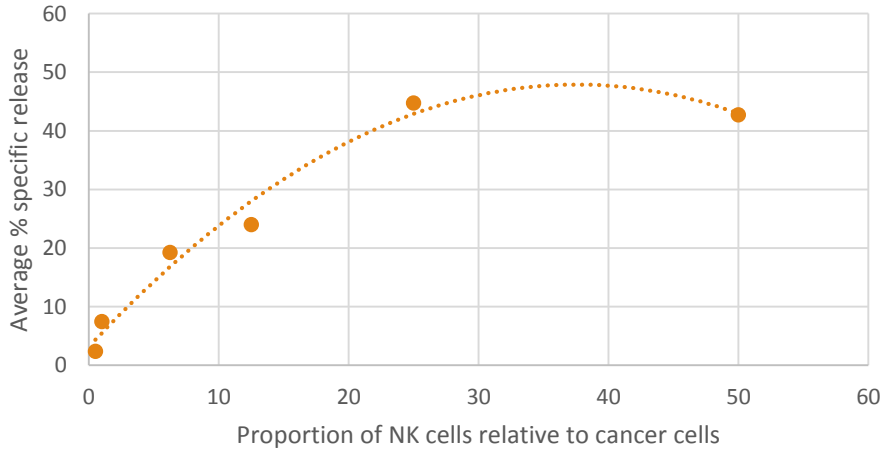
Raw264.7
(macrophage)



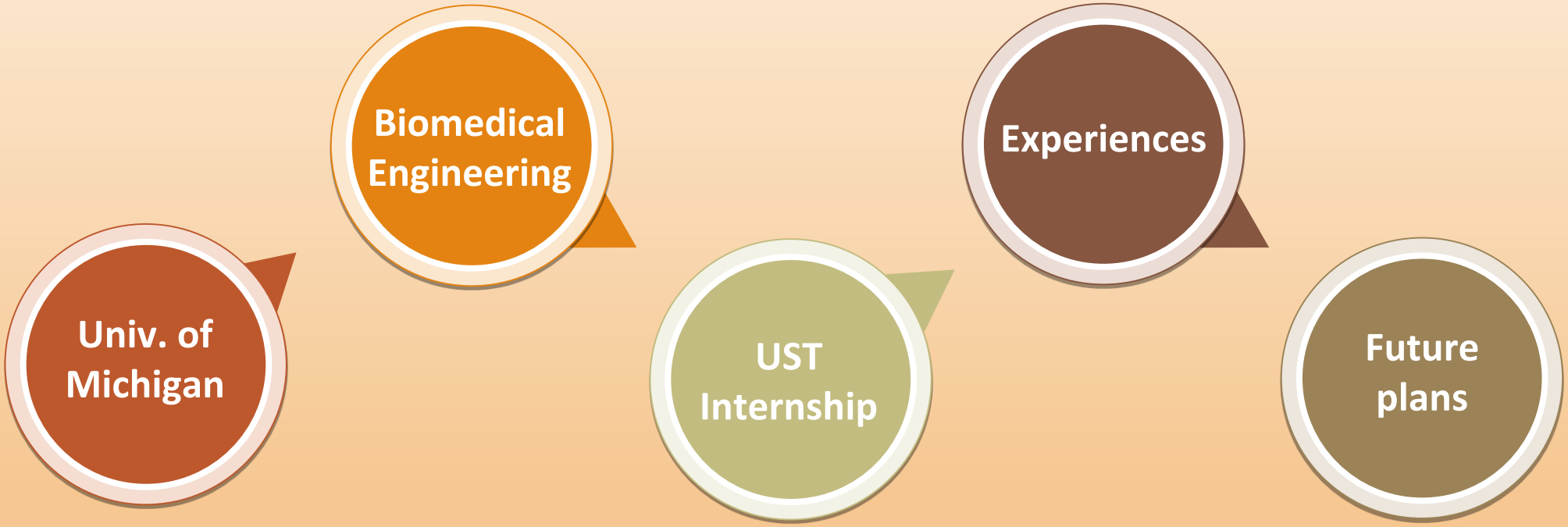
DC2.4
(dendritic cell)



K562
(leukemia cell)



Comments, Questions?



Thank you!!