Modifying gold nanoparticle surfaces with peptides to enhance localization to the nucleus of MCF10a cells

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Introduction

- Cancer diagnostics and therapeutics require advanced probes capable of efficient targeting and biocompatibility.
- Gold nanoparticles satisfy these requirements by acting as antennas that amplify chemical signatures created by inelastic light scattering.

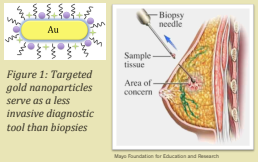


Figure 1: Targeted gold nanoparticles serve as a less invasive diagnostic tool than biopsies

- Inelastic (Raman) scattering of incident light measures molecular vibrations, which are chemically specific signatures that allows for the spectroscopic detection and identification of molecules.

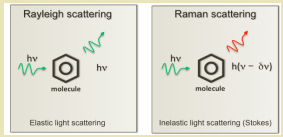


Figure 2: Elastic and inelastic scattering of light

- The coherent oscillation of conduction electrons (surface plasmon resonance) in gold nanoparticles causes the unique optical properties responsible for the amplification of Raman scattering.

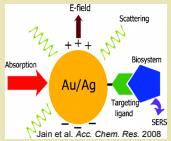


Figure 3: Gold absorbs light in the visible range, concentrating light at its surface

- Biocompatibility is maximized by displacing the cytotoxic surfactant, cetyl trimethylammonium bromide (CTAB) with thiolated PEG and through citrate exchange via polystyrene sulfonate (PSS).

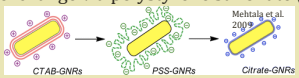


Figure 4: Citrate exchange on the surface of gold nanorods

- Nanoparticles are uptaken through receptor mediated endocytosis (RME) and localized to the nucleus via a nuclear localization sequence (NLS).

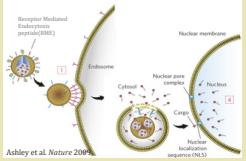
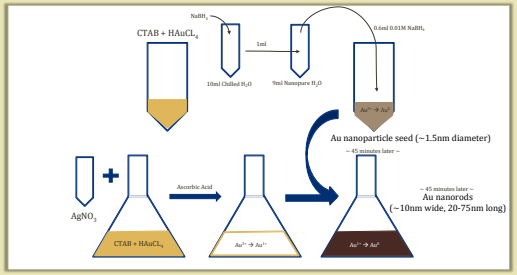


Figure 5: Receptor mediated endocytosis and nuclear localization of gold nanoparticle

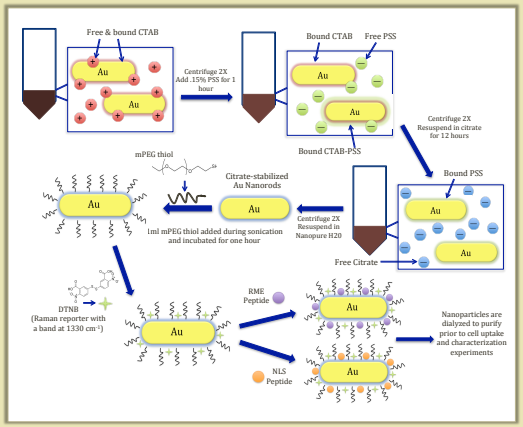
- The goal of this investigation was to develop a biocompatible, localized probe for assessing breast cancer with a non-invasive technique

Methods

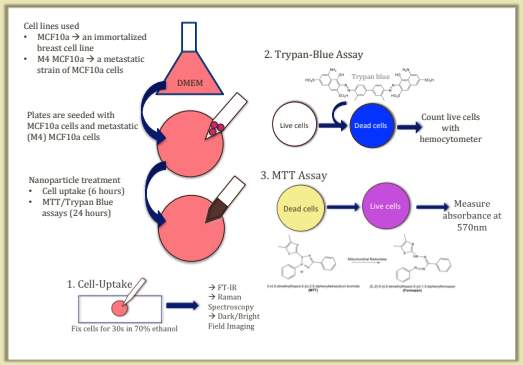
Nanoparticle Synthesis



Surface Modification



Cell Culture



Results

Nanoparticle characterization shows retention of shape and stability during citrate exchange

TEM Imaging

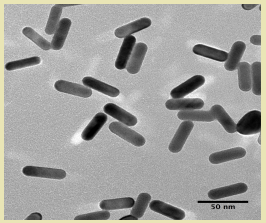


Figure 6: TEM imaging revealed that nanorod shape was maintained through citrate exchange with an aspect ratio of 3.25 +/- 0.58 with a length of 37.1nm.

Normalized UV-Vis

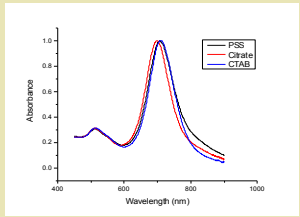


Figure 7: Normalized UV-Vis data from citrate exchange shows that nanorods are stabilized with a transverse band at 512nm and a longitudinal band at 710nm

FT-IR Point Spectra

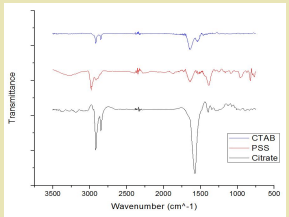


Figure 8: FT-IR data from each stage of the citrate exchange matches the literature values determined for CTAB, PSS and citrate, confirming the chemical composition on the nanorod surface

Zeta Potential

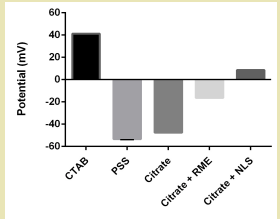


Figure 9: Zeta potential data from citrate exchange confirms that CTAB-coating is positive, PSS & citrate coating is negative and with the addition of the positively charged peptides (RME & NLS), surface charge neutralizes

Cell viability assays show improved biocompatibility

MTT Assay

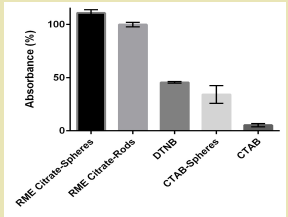


Figure 10: MTT assay absorbance measurements at 570nm demonstrate that CTAB treated cells had ~20% viability while citrate-coated particle treated cells had ~100% viability, indicating that citrate coated rods were less toxic to MCF10a cells

Trypan-Blue Assay

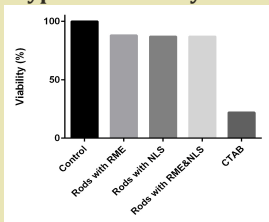


Figure 11: Cells treated with citrate-stabilized nanorods regardless of peptide addition, had high levels of viability whereas CTAB was toxic to the cells.

Cell-uptake imaging and spectroscopy show nuclear localization of nanorods

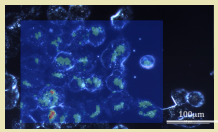


Figure 14: Overlaid image of Darkfield imaging of MCF10a cells that have been treated with NLS-conjugated nanoparticles and an intensity map image based on Raman spectra. Red areas indicate regions in the cell of concentrated nanorods

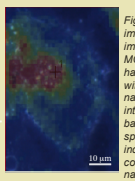


Figure 15: Overlaid image of a darkfield image of a single MCF10a cell that has been treated with NLS-conjugated nanoparticles and an intensity map image based on Raman spectra. Red areas indicate regions of concentrated nanorods in the cell.

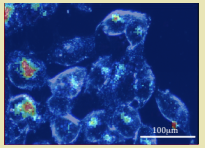


Figure 16: Overlaid image of a darkfield MCF10a cells that have been treated with both NLS & RME-conjugated nanoparticles and an intensity map image demonstrates the regions in the cell with gold nanoparticles.

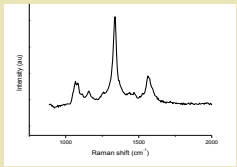


Figure 14: Raman spectra of gold nanoparticles with Raman reporter (DTNB) which has a Raman shift at 1330cm^{-1} due to symmetric NO_2 stretching as shown by the peak in the Raman spectra above.

Conclusions and Future Work

- Nanoparticles were successfully removed of CTAB and stabilized with citrate as evidenced by the UV-Vis, Zeta-potential and FT-IR spectra results.
- The MTT assay confirms a reduction in cytotoxicity of gold nanoparticles to MCF10a cells following citrate exchange
- Darkfield and Raman imaging confirm the cellular uptake of NLS conjugated nanoparticles
- In the future, conjugation of both peptides to the surface will be investigated

Acknowledgements

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