Modifying Gold Nanoparticle Surfaces to Improve Biocompatibility and Enhance Localization to the Nucleus of MCF10a cells

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Introduction: The last decade has seen an increase in the use of nanoparticles to combat the problems of biocompatibility and efficiency in cancer diagnostics. In particular, the use of targeted nanoparticle probes would be less invasive than biopsies and easily imaged in-situ using a visible light laser rather than a harmful UV laser. Inelastic (Raman) scattering of incident visible light measures molecular vibrations, which are chemically specific signatures that allow for the spectroscopic detection and identification of specific molecules. Unfortunately only one in every ten million photons are inelastically scattered, resulting in a weak Raman signal. Gold nanorods can amplify this signal since they exhibit a plasmon resonance peak at a wavelength close to the laser used for imaging, enabling them to act as an antenna and concentrate electromagnetic energy at the surface through a phenomenon known as surface enhanced Raman spectroscopy (SERS). Unfortunately, the problem of striking the delicate balance between efficacy and toxicity still remains unresolved. The synthesis of gold nanorods involves the use of a cytotoxic cationic surfactant (CTAB) that has historically been displaced by non-ionic polyethylene glycol (PEG). However, without the positive surface charge, cellular uptake is reported by previous studies to decrease by 94%. This investigation will strive to improve biocompatibility by replacing the cationic surfactant with an anionic stabilizing agent, citrate and enhance targeting and localization to the nucleus via conjugation of a receptor mediated endocytosis (RME) peptide and a nuclear localization sequence (NLS) peptide.

Materials and Methods: Nanorods were synthesized via a stabilizing solution involving cetyl trimethyl ammonium bromide (CTAB) and gold chloride, which was reduced by ascorbic acid in the presence of a solid gold seed and silver nitrate to direct gold crystal growth into rods. The rods grown were approximately 37nm in length with an aspect ratio of 3.25 ± 0.58. Excess CTAB was removed through centrifugation after which the rods were resuspended in polystyrene sulfonate (PSS). The PSS-CTAB complex on the surface of the nanorods was ripped off through two successive centrifugation steps and the rods were stabilized in citrate. The surface was further coated in PEG, a Raman reporter molecule (Dithionitrobenzoic acid (DTNB)), and the two targeting peptides, RME and NLS. MCF10a cells, immortalized breast cells, were incubated with nanorods for 24 hours and cell viability was assessed as percent absorbance at 570nm in an MTT assay and as a percentage of live cells in a Trypan-blue assay. Another batch of cells were incubated with nanorods for 6 hours, fixed with 70% ethanol on a low-emissivity slide and imaged using darkfield and Raman spectroscopy to assess cellular uptake.

Results and Discussion: UV-Vis results confirmed nanorods remained stabilized throughout the citrate exchange process with a plasmon resonance peak at ~700nm. The nanoparticles also retained their rod shape in citrate as evidenced by TEM imaging and the surface chemistry of the particles was confirmed through FT-IR spectroscopy and surface charge measurements (zeta potential). MTT and Trypan-blue assay results showed that the viability of citrate-stabilized particles was close to 100% and was considerably higher than CTAB and CTAB-stabilized particles. Imaging of cellular uptake experiments indicated successful localization to the nucleus of NLS-coated nanorods and to a lesser degree, RME&NLS coated nanorods. The RME-only coated nanorods did not show any cellular uptake.

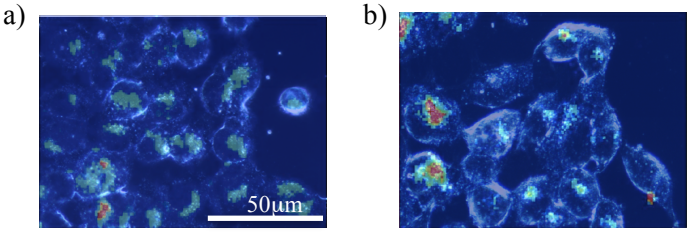

Figure 1: Intensity maps overlaid onto darkfield images highlight the 1330cm^{-1} shift of the Raman reporter molecule on the nanoaparticle surface to demonstrate areas within the cells where nanorods have concentrated. a) Cells treated with NLS-coated nanorods b) Cells treated with RME&NLS coated nanorods

Conclusion: The citrate exchange process successfully improved biocompatibility without compromising cellular uptake as evidenced by the cell viability assays. In addition, the presence of the NLS peptide enhanced nuclear targeting. Conversely, the presence of the RME peptide had no effect on nuclear localization and in the case of the RME&NLS-coated nanorods, may have even prevented some NLS from binding, leading to the observed decrease in uptake compared to the NLS-only coated nanorods. Future studies will focus on using a more specific receptor-ligand interaction for RME targeting as well as assessment of nanorod toxicity in living systems.