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Cancer Imaging and Therapy with Metal Nanoparticles

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Nanotechnology offers unique opportunities for cancer detection, therapy and the ability to monitor therapeutic interventions. This potential has to be analyzed in context of challenges that need to be overcome in translation of nanoparticles to clinical applications including specific delivery in tissues and clearance from the body. Here, we will present a case study of plasmonic nanoparticles in cancer imaging and therapy.

It has been convincingly shown that nanotechnology can provide unique solutions to revolutionize diagnosis and treatment of many devastating diseases such as cancer.[1] [2-5] One specific area of great interest is development of nanoparticles for molecular specific imaging, therapy and combined imaging/therapy.[6] Many inorganic nanoparticles exhibit unique size-dependant properties, and therefore, provide a bright signal for enhanced imaging contrast and sensitive detection of molecular specific biomarkers of cancer and other pathologies. The most noticeable examples include superparamagnetic nanoparticles for MRI [5], luminescent quantum dots [7, 8], and plasmonic nanoparticles [9, 10] [11-13]. Multiplexing different types of nanoparticles and targeting molecules provides a common platform for multiple imaging applications with a high degree of flexibility[14-16].

Plasmonic nanoparticles such as gold and silver may be used for combined imaging and photothermal therapy of cancerous cells[12, 15, 17]. Furthermore, it was demonstrated that plasmonic nanoparticles can be combined with another inorganic material, for example iron oxide for MRI, to form hybrid nanomaterials that provide easily detectable signals in more than one imaging modality.[18-20] In addition, molecular targeted nanoparticles exhibit significantly increased avidity, and they can be simultaneously decorated with different types of biomolecules which determine their delivery, targeting specificity and molecular therapeutic properties.[14, 21, 22] Therefore, plasmonic nanoparticles can provide solutions to one of the major challenges of modern medicine - efficient delivery of therapeutics and molecular specific treatment of pathology with real-time imaging for guidance and monitoring.

One of the major problems in selective detection, monitoring and treatment of cancer is the fact that cancer biomarkers are not unique to cancer cells. Identical molecules are present in normal cells as well; however, their expression is often elevated in cancer [23]. Therefore, selectivity toward cancerous cells can be achieved through development of molecular probes that dramatically change properties in the presence of an over-expressed biomarker.

Particles of the noble metals exhibit optical resonances in the visible range which are attributed to oscillations in the free-electron/ion-core plasma of the material, and which are therefore known as *plasmon* resonances. Closely-spaced plasmonic nanoparticles exhibit strong color change, quadratic increase in cross-section per particle, and modified polarization of the scattered light [24]. These properties can be used to develop imaging modalities that increase contrast between isolated and closely-spaced nanoparticles.

We have been developing contrast agents based on gold nanoparticles for imaging of epidermal growth factor receptor (EGFR) [9, 19, 25-27], metallo-proteases 2 and 9 [25], oncoproteins associated with HPV 16 induced carcinogenesis [25], and actin [10, 28]. We explore non-linear phenomena which are exhibited by plasmonic nanoparticles when they form closely spaced assemblies in the presence of a target molecule such as overexpressed cancer biomarker, e.g. EGFR. The assembly formation by nanoparticles is mediated by targeting molecules such as antibodies that interact with the biomarker of interest. Our experiments with living cells showed that EGFR mediated assembly and plasmon coupling of gold bioconjugates results in a dramatic spectral shift of more than 100 nm in plasmon resonance frequency of the nanoparticles giving a very bright red signal [26]. In addition, coherent scattering effects result in a quadratic increase in the total scattering cross-section of closely spaced nanoparticles. These effects allow optimization of excitation wavelength for highly sensitive detection of labeled cells, even in the presence of single unbound gold bioconjugates. The effect of plasmon resonance coupling for molecular specific imaging has been demonstrated for dark-field reflectance and confocal reflectance microscopy [9, 19, 26]; optoacoustic imaging [29]; two-photon luminescence; and photothermal therapy [15]. We have also shown that plasmon coupling can be used

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for imaging of EGFR activation and trafficking as formation of EGFR dimers and further intracellular uptake in early and late endosomes is associated with progressive color changes from green to red, respectively, with each stage of EGFR cycle being associated with a distinct color of EGFR bound nanoparticles.

Recently, we have demonstrated the use of bi-modal MRI/optical nanoparticles for combined MRI/optical molecular imaging and photothermal treatment of cancer [15]. This opens up exciting opportunities for new strategies for combined detection, diagnosis, treatment and monitoring of carcinogenesis in future clinical practice. We have also reported the first multi-functional imaging platform using plasmonic nanoparticles that incorporates both cytosolic delivery and targeting moieties on the same entity for imaging of intracellular targets such as actin [10, 21]. These nanosensors can be adapted to target various intracellular processes.

A major roadblock in translation of inorganic nanoparticles to clinical practice for systemic targeting of cancer cells is their non-biodegradable nature. In addition, sizes of coated nanoparticles that are used in biological applications are too large for body clearance in desirable time frames. The accumulation and potential long-term toxicity of nanoparticles is a major concern. Recently, it was demonstrated that particles with hydrodynamic diameters less than 5.5 nm are efficiently eliminated from the body by urinary excretion [8]. However, plasmonic nanoparticles with resonances in the NIR region are at least 50 nm in size, and often > 100 nm, severely limiting their body clearance rates.

Here, we will present hybrid polymer/inorganic nanoclusters smaller than *ca.* 100 nm. These nanoclusters consist of individual primary gold particles with sizes less than 5 nm. The cluster assembly is facilitated by interactions between the small capping ligands on the primary nanoparticles. Tight packing of the primary particles in the nanoclusters results in strong NIR extinction. As a result the clusters produce strong optical contrast in cells, as determined by dark-field reflectance and hyperspectral microscopy. The nanoclusters are stable at physiological pH and deaggregate in pH environment that mimics lysosomes down to essentially primary nanoparticles with *ca.* 4 nm gold core diameter. Furthermore, the nanoclusters deaggregate in live cells over time. Therefore, this novel plasmonic nanocomposite material provides a simple route to design and synthesis of strong NIR absorbing nanoparticles which can degrade to easily clearable components under physiological conditions, thus allowing safe translation into clinical practice.

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