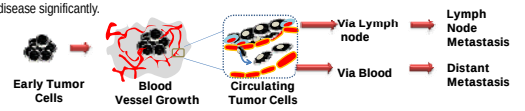
Photoacoustic Detection of Circulating Prostate, Breast and Pancreatic Cancer Cells using targeted Gold Nanoparticles: Implications of Green Nanotechnology in Molecular Imaging

Ravi Shukla[1], Sagar Gupta[2], Nripen Chanda[1], Ajit Zambre[1], John Viator[2], Priyabrata Mukharjee[3], Debabrata Mukhopadhyay[3], Raghuraman Kannan[1], Kattesh V. Katti[1]
Departments of [1]Radiology and [2]Biological Engineering, University of Missouri-Columbia, MO; [3]Mayo Clinic, Rochester, MN

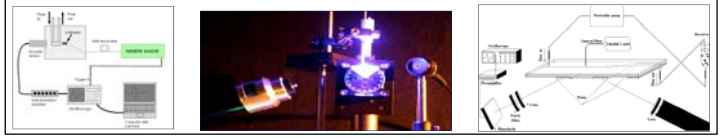
Circulating Tumor Cells

Circulating tumor cells are hallmarks of metastasis cancer. The presence of circulating tumor cells in blood stream correlates with the severity of disease. It has been postulated that circulating tumor cells (CTC's) can be detected in human blood. CTC's are those cells that separate from the primary tumor and travel to distant regions of the body to form secondary tumors. (See Figure) A blood test for CTC's has the potential to aid 'clinical decision making' in the treatment of cancer. The presence of CTC's has been positively correlated to disease state and may be used to monitor disease progression. Many approaches are being tested for CTC detection. Technical barriers for these procedures include non-repeatability, numerous opportunities for human error, as well as lengthy preparations and analysis, thus precluding their implementation as viable clinical tools. Current detection of metastatic cancer is done by waiting until tumors are large enough to be detected by traditional imaging. In many cases` these tumors, though detectable, are untreatable. However, CTC's are disease-specific factors that can be used as a variable to detect metastasis and to guide therapy methods. Although extremely rare, detection of CTCs represent a preferred alternative to invasive biopsies and bone marrow procedures traditionally used for the detection, characterization, and monitoring of tumor cells. The ability to identify, isolate, and molecularly characterize CTCs could influence the treatment of metastatic disease significantly.



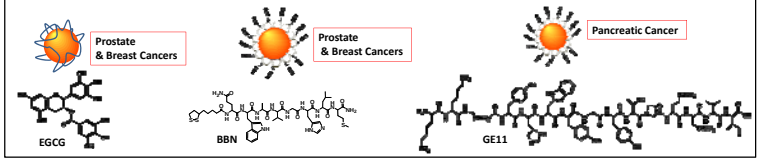
Photoacoustics for CTC Detection

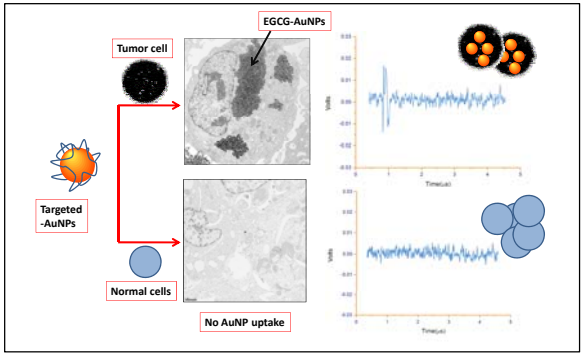
Photoacoustics, conceptually described as a laser induced ultrasound, can be generated in cells by a number of mechanisms. In this case, we rely on thermoelastic expansion, in which a 5 ns laser pulse is selectively absorbed by a chromophore. The chromophore undergoes rapid heating and expansion, thus creating a high frequency pressure wave. For example in melanoma cells, laser pulses were absorbed by melanosomes, in turn creating a photoacoustic response. Photoacoustic detection of tumor cells is attractive technique for potential applications in diagnosis of CTC's. However, the sensitivity of photoacoustic imaging of tumor cells depends on their photon absorption characteristics. The photoacoustic apparatus for detecting circulating tumor cells is shown in Figure. A frequency tripled Nd:YAG laser pumped an optical parametric oscillator that was tunable from 410-2400nm. The pulse energy was approximately 5 mJ. Exact pulse energy was monitored with an energy meter. The laser pulse duration was 5 ns and was coupled into a 1000 µm optical fiber. The transducer was made from a piezolelectric material and was used to measure the photoacoustic response over the laser wavelength range of 470 to 570nm. In this set up, the optical fiber was directed to the targets that rested within a well made of acrylamide resting on a piezoelectric transducer. The acrylamide well was used to hold the cellular material and to ensure proper acoustic coupling to the transducer. The transducer was made with a polyvinylidene difluoride film. It's sensitivity was approximately 85mV/bar.

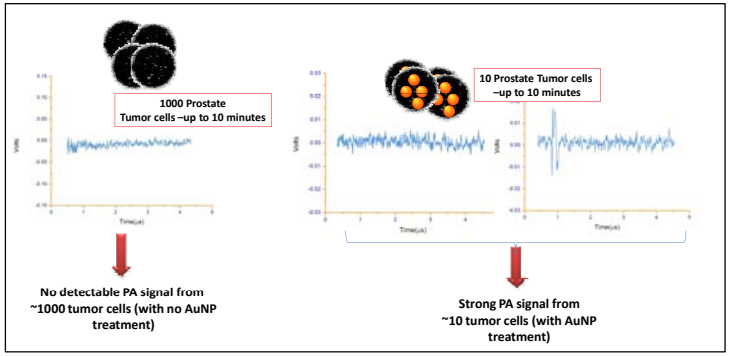


Targeted Gold Nanoparticles as PA Enhancers

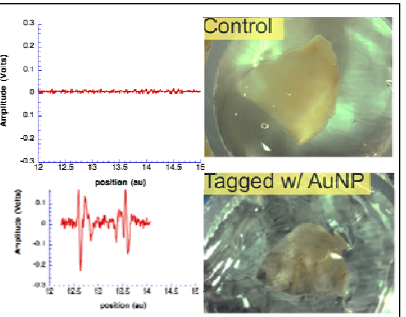
The sensitivity of photoacoustic imaging of tumor cells can be enhanced using gold nanoparticle (AuNP) as photoacoustic contrast agents. AuNPs embedded tumor cells offer significant advantages for diagnostic PA of single cells. As the PA absorptivity is directly proportional to the number of nanoparticles embedded within tumor cells, the propensity of nanoparticles to internalize within tumor cells will dictate the sensitivity for single cell detection. We have been developing biocompatible gold nanoparticles to use them as probes as part of our ongoing effort toward the application of X ray CT Imaging, Ultra Sound (US) and photoacoustic imaging of circulating prostate, breast, and pancreatic tumor cells. We, herein report our recent results which have shown that targeted gold nanoparticles internalize selectively within cancer cells providing threshold concentrations required for photo acoustic signals. In this context, we have identified epigallocatechin gallate (EGCG), gastrin releasing peptide (GRP), epidermal growth factor receptors (EGFR) as targeting vectors for PA detection. These targeting vectors are conjugated with AuNPs showed selective cellular internalization within prostate (PC-3), breast (T47D), and pancreatic (PANC-1) cancer cells providing threshold concentrations required for photoacuostic imaging (PA). We have developed a highly accurate method for the detection of circulating tumor cells utilizing targeted gold nanoparticles as photoacoustic probes.







In order to assess the sensitivity and applicability of PA in detecting tumor cells, we performed the following experiment: Targeted AuNPs were injected intravenously to mice bearing pancreatic tumor xenografts. In a separate set of mice, non-targeted AuNPs (AuNP-PEG) were injected. Mice were euthanized and pancreatic tumor tissue excised after 36 hours post injection period. Tumor tissue in both the sets were collected in cuvettes and were embedded in acoustic coupling gel. The photoacoustic excitation was detected using a Visualsonics Vevo 770 system (RMV-708 Transducer) coupled to a laser operating at 530 nm. In figure, the dark AuNP can be seen embedded within the pancreatic tissue sample. The control experiment no PA signal while the tissue with embedded AuNPs has a significant signal. This demonstrates both the sensitivity of the method and offers some intriguing prospects for future work developing photoacoustic imaging methods.



Conclusion:

The success in the detection of CTCs would depend on the ability of any technique to selectively detect cancer cells which are far fewer than normal cells in the body. Our results, as described above, clearly demonstrate that targeted gold nanoconjugates serve the dual roles: (i) selective internalization within prostate, breast or pancreatic cancer cells; (ii) more importantly the quantum of endocytosis of nanoparticles results in photoacoustic signals even with as few as 10 tumor cells. These results provide strong rationale toward the application of photoacoustic techniques for the accurate detection of CTCs and that carefully engineered gold nanoparticles can be used as photoacoustic probes for the detection of prostate, breast, and pancreatic cancers cells.

This work has been supported by grants from the National Institutes of Health/National Cancer Institute under the Cancer Nanotechnology Platform program (grant number: 5R01CA119412-01), NIH - 1R21CA128460-01, and University of Missouri-Research Board - Program C8761 RB 06-030