Multimodal Qdot based nanoprobe for real time noninvasive bioimaging

NSF NIRT Grant 0506560

PIs: **Brij M. Moudgil[1], Glenn A. Walter[2], Edward W. Scott[3] and Swadeshmukul Santra[4]**

1 Materials Science and Engineering & Particle Engineering Research Center,
2. Physiology and Functional Genomics, 3. Molecular Genetics and Microbiology,
University of Florida & 4. NanoScience Technology Center, Chemistry and
Biomolecular Science Center, University of Central Florida.

Introduction and Objectives: In a number of tissues stem cells are known to be recruited from their respective niche's to repopulate or fuse with existent cells. However, the exact cellular signal and conditions that favor cell targeting and fusion are currently not well understood and is an area of intensive research. Tracking cell movement in real time in biological processes requires use of highly sensitive and photostable probes. Conventional organic fluorescent dyes and green fluorescent protein based labels are not suitable for long time monitoring since they lack photo-stability, which limits the time for which they can be tracked in biological processes. There is substantial research and development interest in using fluorescent nanoparticles, e.g. dye-doped nanoparticles, fluorescent polymer microspheres, gold nanoparticles, quantum dots (QD), for bioimaging applications[1] such as cell labeling, pathogen detection, early diagnosis of tumors etc. QD stand out amongst nanoparticle based labels because of their small size (<10nm i.e. about 1000 times smaller than a typical cell); high photostability, making them suitable for real time, highly sensitive biological experiments, and are suitable for multiplexing (multiple labeling while using single excitation wavelength). Optical imaging however provides limited anatomical background information, and also suffers from artifacts due to tissue absorbance and scattering. Hence at present there is a considerable research effort to develop multifunctional nanoparticles that can be detected simultaneously by more than one imaging technique. In this regard probes combining detection capability by optical and magnetic resonance imaging (MRI) are advantageous as they integrate the advantages of high sensitivity (from optical method of detection, e.g., fluorescence) with the potential of true three dimensional imaging of biological structures and processes at cellular resolution (MRI). The motivation of our NIRT project is to develop a multimodal QD-based nanoprobe that will be optically active to track cell delivery and will be further acted upon when a labeled stem cell fuses with tissue expressing β-galactose to generate MR contrast. Specifically, our main objectives are (1) To engineer surface functionalized Near Infrared (NIR)multimodal QD, (2) Fabricate QD conjugated Beacon which generates MR off/on contrast for signaling gene expression, (3) Check the efficacy of the Beacon using *in vitro* differentiation and toxicity assays and (4) *In vivo* tracking of stem cell migration and signaling of gene expression.

Approach: During the first year of the project, our focus has been towards the design and synthesis of the Multimodal QD- Beacon (MQB). The design of the MQB can be considered to be composed of two components: (i) Multimodal QD with NIR optical core and (ii) ability to act as a cellular beacon.

(i) Multimodal QD (MQD). We have previously demonstrated the synthesis of the CdS: Mn core/ ZnS shell QD [2] with 18-28% quantum yield and used them successfully for rapid and effective labeling of the rat brain tissue. Continuing with the same QD material we have

synthesized multimodal QD (MQD) by integrating a Gd (III) chelated to silica shell onto CdS: Mn/ZnS QD [3].

The optical core of ~3nm of diameter is covered by a modified silica shell which contains amino groups for bioconjugation and chelating groups for binding the Gd (III). Fig. 1 shows the bright yellow emission from the QD using the 366nm hand held multiband UV light source. We have found that this MQD can be used to label muscle derived stem cells (Fig 2a), J77 murine macrophages, hemopoietic stem cells, and neural stem cells [4]. We have determined that MQD can be used to label non-phagocytic cells either a using cationic transfection agent (poly-l-lysine) or electroporation. The number of Gd(III) ions (paramagnetic ions, responsible for MR contrast) per particle in the MQD as determined using inductively coupled plasma analysis was determined to be ~107 as compared to 6-70 for polymer based and 5-1331 for Dendrimer based contrast agents. The Proton longitudinal (T_1) and transverse relaxation times (T_2) were determined at 4.7 T, for a series of diluted MQD (from 0.36 to 0.0012 mM of Gd) and their efficacy as MRI contrast agent, commonly expressed by relaxivity (R_i, i=1, 2), were determined to be 20.5 and 151 $mM^{-1}s^{-1}$, respectively. When compared with commercially available contrast agents (Gadopentate dimeglumine, Gadoteridol, Gadodiamide etc.) under the same measurement conditions, MQD exhibit much higher R_1 and R_2 values. From the R_2/R_1 ratio of ~7.4 it is noted that these may be more effective as a T_2 contrast agent but still can be used to generate positive contrast in solution and within cells at 4.7T. Fig. 2b shows the positive contrast generated from the MQD as compared to QD (without any Gd) used as control.

Figure 1: Bright Yellow emission from CdS:Mn/ZnS QD (right) under UV excitation with de-ionized water as control (left).

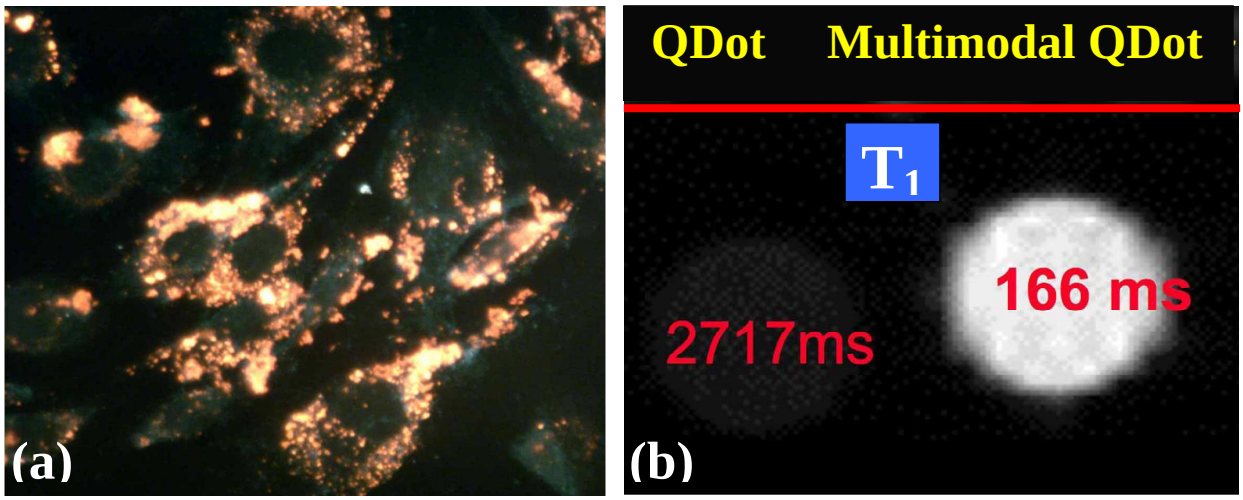

Figure 2. (a) Fluorescence emission from the MQD uptaken by the muscle derived stem cells and (b) Positive T_1 contrast from MQD (on right) as compared to QD (without Gd(III)) as control (left) at 4.7T.

From the above experiments, the concept of MQD is demonstrated. Preliminary results indicate that due to high tissue scattering and autofluorescence it is difficult to detect stem cells labeled with UV-visible QD when transplanted into deep tissue (>500µm). As a next step we are working on the synthesis of the NIR optical core e.g. of PbS, CdTe, which are expected to facilitate deep tissue imaging.

(ii) *Beacon* component: *In vivo* imaging of gene expression in real-time is a challenging task. Meade's group has demonstrated the turning "ON" of MRI contrast agents selectively upon enzymatic reaction [5]. Specifically, the access of water to the first coordination sphere of the chelated paramagnetic Gd (III) ion was blocked with a galactopyranose residue that can be cleaved by beta-galactosidase (β-gal) enzyme, the product of a common marker gene. Following the cleavage of the galactopyranose, the water exchange and relaxation is enhanced by Gd (III), thus behaving much like a MRI sensor. In our NIRT we have proposed to develop nanoparticle based MRI contrast agent by manipulating silica surface chemistry to attach macrocyclic ligands, charged with Gd (III) and containing galactopyranose residue to the silica surface (Fig. 3).

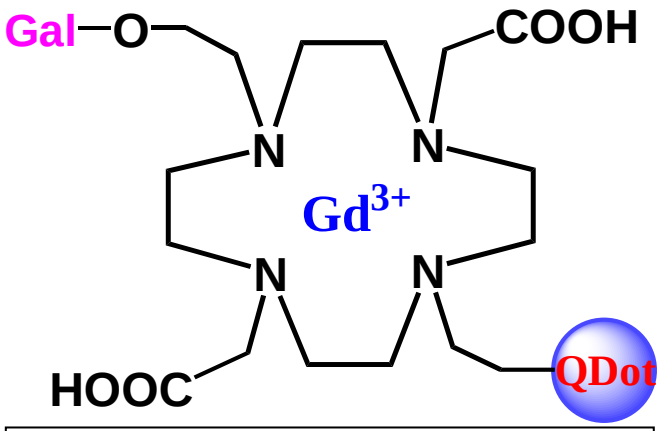

Figure 3: Schematic design of MQB, showing galactopyranose residue and QDot conjugated Cyclen ligand binding Gd (III)

We have carried out the synthesis the MQB by preparing the bromo derivative of galactose and using it to carry out the N-alkylation of cyclen amine. The N-alkylated cyclen was further reacted with bromo dervatized CdS: Mn/ZnS QD followed by charging with Gd(III). Currently we are conducting detailed characterization of MQB's and investigating its MRI and optical properties for their future application as beacons.

Future research plan: Experiments are underway to synthesize Near-IR QD probes, improvise on the design of the MQB to allow optimum coordination for Gd (III) which results in an efficient MR contrast and suitably modify the surface properties for a good dispersion of the MQB in the physiological working conditions. Subsequently, β-galactosidase activation of MQB will be used to selectively generate MR image contrast following stem cell differentiation into endothelial cells.

Education and outreach: The interdisciplinary nature of the research team NIRT team is reflected in the participation of 4 graduate students from 4 Departments and 2 Colleges (UF and UCF), besides postdocs and faculty. The team's effort for outreach was directed at several levels e.g. faculty, graduate and undergraduate. NIRT faculty have organized and participated in a monthly molecular imaging working group in collaboration with groups with similar research interests from chemistry, engineering and medicine departments at both UF and UCF. In addition NIRT team has co-organized and participated in the Particle Engineering Research Center (PERC) Industrial Advisory Board (IAB) meetings, where the research investigations were shared with a large number of Industrial members as well as the participating scientific

community form UF. As an innovative step NIRT team co-organized 'The First Annual Young Researcher's Forum' as a special session of the September 2006 PERC's IAB meeting. Over 40 young researchers representing over ten departments and six institutions, from Japan, Hong Kong, India, and across the United States converged in Gainesville for the purpose of establishing partnerships between young researcher's, and for identifying barriers to as well as guidelines for establishing successful collaborative research projects. With the aim of creating interest and promoting awareness amongst the UF and non-UF graduate students, NIRT team co-organized the "Particle Science Summer School in Winter", in April 2006 at PERC with "Engineered Particulate Systems and Particle Characterization for Biomedical Applications" as primary theme. At the graduate level, predoctoral students from the college of medicine and engineering have been supported by NIRT related activities. In addition as a part of an collaborative international master's program with the Karolinsky institute in Sweden, four students have either completed or are in the process of completing master's work related to NIRT support activities. The NIRT team collaborates heavily with the NSF supported National High Magnetic Field Lab to test nanoparticles at magnetic fields strengths above 4.7T and to provide outreach on the development of nanoparticles for high magnetic field detection. In less than a year 5 undergraduate students sponsored by The South East Alliance for Graduate Education and the Professoriate (SEAGEP) a member of the NSF Alliance for Graduate Education and the Professoriate family of programs, have been actively involved in the research activities directly related to NIRT project.

References

For further information about this project email Brij M Moudgil <**bmoudgil@erc.ufl.edu**>**, Swadeshmukul Santra <ssantra@mail.ucf.edu> , Glenn A Walter < glennw@phys.med.ufl.edu> and /or Edward W Scott <escott@ufl.edu>**

[1] P. Sharma, S. Brown, G Walter, S Santra and B Moudgil, " Nanoparticles for Bioimaging" Advances in Colloid and Interface Science, 123-126, 471-485, **2006.**

[2] S. Santra, H. Yang, J. Stanley, P. Holloway, B. Moudgil, G. Walter, R. Mericle, "Rapid and effective labeling of brain tissue using TAT-conjugated CdS:Mn/ZnS quantum dots". Chemical Communications (Cambridge, United Kingdom) 25, 3144-3146, **2005**

[3] H. Yang, S. Santra, G. Walter, and P. Holloway, "Gd(III)-Functionalized Fluorescent Quantum Dots as Multimodal Imaging Probes", Advanced Materials, 18, 2890-2894, **2006**

[4] S. C. Grant, T. Zheng, G. P. Marshall II, H. Yang, P. Sharma, H. Cornnell, S. Santra, P. A. Holloway, B. M. Moudgil, E. W. Scott, E. D. Laywell, G. A. Walter, A. S. Edison, D. A. Steindler, M. D. Weiss, 'MR Microscopy of Multipotent Astrocytic Stem Cells Labeled with Multimodal Qdots Applied to a Neonatal Murine Model of Hypoxic Ischemic Encephalopathy' International Society for Magnetic Resonance in Medicine Meeting, May **2006,** Seattle, WA. p. 1880.

[5] A. Y. Louie, M.M. Huber, E.T. Ahrens, U. Rothbacher, R. Moats, R.E. Jacobs, S.E. Fraser, T.J. Meade, 'In vivo visualization of gene expression using magnetic resonance imaging" Nature Biotechnology, 18, 321-325, **2000**