Multifunction nanocomposites based on fluorescent carbon and magnetic nanoparticles: An effective MR/fluorescence imaging probe

Rishi Awasthi[1], Sachidanand Srivastava[2], Namdeo S Gajbhiye[3], Deepak Tripathi[4], Mohit Kumar Rai[4], Vikas Agarwal[4], Vinita Agrawal[5], and Rakesh Kumar Gupta[1]
[1]Radiodiagnosis, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, India, Lucknow, Uttar Pradesh, India, [2]Chemistry, Indian Institute of Technology, Kanpur, Lucknow, Uttar Pradesh, India, [3]Chemistry, Indian Institute of Technology, Kanpur, Kanpur, Uttar Pradesh, India, [4]Immunology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, India, Lucknow, Uttar Pradesh, India, [5]Pathology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, India, Lucknow, Uttar Pradesh, India

Introduction: Recently, nanostructure based multimodal imaging probe has become more critical with the increasing demand to diagnose, monitor, and treat diseases more precisely and accurately[1]. Among all multimodal probe, MR/fluorescence technique is the most developed and successfully implemented in biomedical research and clinical practice. Owing to its biocompatible and non-toxic nature and high magnetic susceptibility effect on proton relaxation rates, superparamagnetic iron oxides (Fe_3O_4 and γ-Fe_2O_3) of nanosize range have been used as T2/T2* MR contrast agents from last several years[2]. On the other hand, recently, widespread interest has been developed in fluorescent carbonaceous nanomaterials of different morphologies including nanodots, nanoflanks, nanosheets, and nanotubes. In this context, present study aimed to develop a simple and novel chemical approach to prepare MNPs doped carbogenic nanocomposite (IO-CNC) for MR and fluorescence imaging.

Materials and Methods: Recently, we have synthesized a highly fluorescent carbogenic nanomaterial by partial thermal decomposition of organic precursors and found that fluorescent properties are similar to conventional semiconductor quantum dots[3]. We now report the synthesis of IO-CNC by partial thermal decomposition of organic precursors in the presence of Fe_3O_4 (6 nm) MNPs. Fe_3O_4 and carbogenic nanomaterial were chosen as the magnetic and fluorescent component for the MR/fluorescent probe, respectively. Relaxometric measurements were conducted to see the MR contrast behavior of nanocomposite. MTT assay was conducted to look for cytotoxicity effect of nanocomposite. In addition, *in vivo* MRI experiments were performed to see the behavior of contrast in various tissues on a 3.0 T GE MRI scanner. *In vitro* and *in vivo* fluorescence imaging was conducted to see the efficiency of IO-CNC as a fluorescence biomarker (Figure 1).

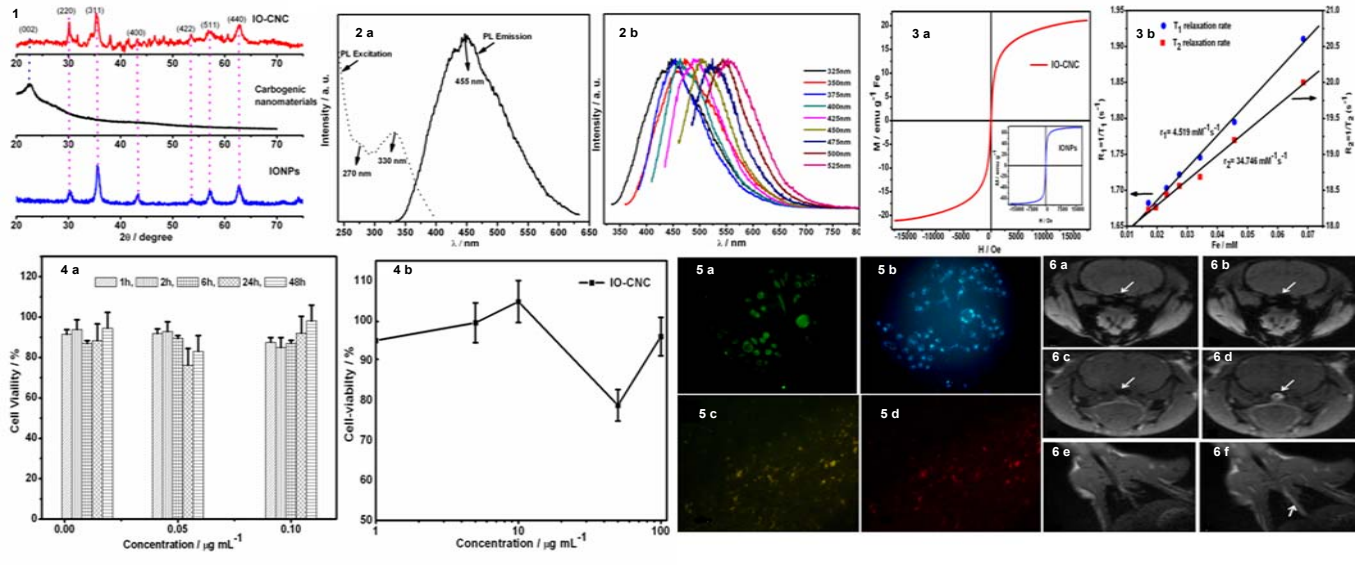

Figure: 1) *Powder XRD patterns of Fe_3O_4 MNPs (blue), carbogenic nanomaterials (black), and IO-CNCs (red);* ***2)*** *(a) PL emission (solid line) and excitation (dashes) spectrum of IO-CNC, (b) Wavelength dependent PL emission spectra of IO-CNC;* ***3)*** *(a) Magnetic hysteresis (M–H) curve of IO-CNC (inset shows M-H curve of IONPs), (b) Relaxometry experiments: relaxation rates R1=1/T1 and R2=1/T2 (s^{-1}) of water protons in presence of IO-CNC as a function of Fe (mM) concentration (Fe concentration was decided with atomic absorption spectrometer);* ***4)*** *MTT assay results after incubation of (a) IO-CNC with mouse macrophage cells (RAW 264.7) at different concentrations (0.01, 0.05, and 0.1 μg mL^{-1}) for 1, 2, 6, 24 and 48 h, (b) Cell viability of IO-CNC was evaluated at different concentration (1, 5, 10, 50, and 100 μg mL-1) for 2h incubation period.* ***5)*** *(a) Fluorescence images of RAW 267.7 Cells incubated for 2 h with IO-CNC (5.0 μg mL^{-1}), (b)To confirm the internalization of nanoparticles, cells were stained with Hoechst fluorescent dye, (c) Fluorescence microscopic images of rat spleen taken by using green window (450-480 nm) and (d) red window (525-595 nm) ×40X.* ***6)*** *(a) Showing a T2* image of rat brain in axial plane which showed a strong T2* effect (arrow) (b) after the IOCNC injection, Similarly (d) images taken after the administration showed a significant enhancement in the vasculature (arrow) on T1-weighted axial image as compared to (c) the precontrast one, (e) On SPGR images taken in coronal plane, the blood vessel (arrow) became well prominent(f) after the injection of synthesized IOCNCs.*

Results and Discussion:

IO-CNC has been synthesized by thermal decomposition of organic precursors in presence of Fe_3O_4 (6 nm) MNPs. IO-CNC showed wavelength tunable fluorescence properties with high quantum yield (QY). Also, magnetic studies confirm superparamagnetic nature of IO-CNC at room temperature. IO-CNC shows MR contrast behavior by affecting the proton relaxation phenomena. The measured longitudinal (r1) and transverse (r2) relaxivities value are 4.52 $mM^{-1}s^{-1}$ and 34.75 $mM^{-1}s^{-1}$, respectively. No apparent cytotoxicity was observed and nanocomposite showed biocompatible nature. *In vivo* MR studies show both T1 and T2* contrast behavior of nanocomposite. Fluorescence imaging shows selective uptake of IO-CNC by macrophages in spleen. IO-CNC shows excellent wavelength tunable fluorescence properties with high QY as well as superparamagnetic nature at room temperature. MR studies show its contrast behavior with relaxivity ratio r2/r1~ 7.69. The cytotoxicity studies against macrophage cell line show its non-cytotoxic nature, which reasons its utility to be developed as a potential molecular and cellular imaging probe. The results of *in vivo* MR and fluorescence imaging strongly suggest that these multimodal imaging probes can be used as both T1 and T2 contrast agent as well as equally effective fluorescent biomarker. In order to obtain better control over MR and fluorescence properties, one can vary the atomic ratio of magnetic nanoparticles to organic precursors. This novel and facile approach opens up a new route to design carbonaceous based multifunctional nanocomposites for various biomedical applications including various types of multimodal imaging as well as effective drug delivery systems and targeting agents.

***References*:**

1- A. Louie, *Chem. Rev*. 2010, *110*, 3146-3195; 2-S. Srivastava, Rishi Awasthi, N. S. Gajbhiye, V. Agarwal , A. Singh, A. Yadav, R. K. Gupta, *J. Colloid. Interface. Sci.* 2011, *359*, 104–111; 3- S. Srivastava, N. S. Gajbhiye, *Chem. Phys. Chem.* DOI: 10.1002/cphc.201100188