Magneto-Fluorescent Carbon Coated Superparamagnetic Iron Oxide Nanoarchitectures (SPIONs) for Multimodal Imaging and Cancer Theranostics

A thesis submitted for the award of the degree of

Doctor of Philosophy

By

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Preface

The present thesis entitled "Magneto-fluorescent carbon coated superparamagnetic iron oxide nanoarchitectures (SPIONs) for multimodal imaging and cancer theranostics" is submitted in candidacy for the award of a PhD degree from Indian Institute of Technology (IIT) Mandi. The work presented in this thesis was carried out during the period of August 2015 to June 2020 at School of Engineering, IIT Mandi under the guidance of Dr. Jaspreet Kaur Randhawa.

The research work was fully funded by Ministry of Human Resource Development (MHRD), Government of India. The central hypothesis of the work was to design novel magneto-fluorescent nanoarchitectures for multimodal imaging in cancer theranostics for biomedical applications. The work is dully supported with peer reviewed published articles and patent.

IIT Mandi, established in 2009, has rapidly risen among the premier institutes in India. It is located in the Kamand valley on the banks of Uhl, a tributary of the river Beas. Kamand is approximately 14 kms from Mandi town and has an average elevation of 1044 meters from sea level. There is great variation in the climatic conditions of Himachal due to extreme variation in elevation. The climate varies from hot and sub humid tropical in the southern tracts to cold, alpine and glacial in the northern and eastern mountain ranges with more elevation.

Unusually, it is worth mentioning the Global Pandemic of Coronavirus (COVID-19) declared on March 11, 2020 by WHO, which made this journey little sinister and ominous particularly during inclusive Lockdown.

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Thesis Certificate

This is to certify that the thesis entitled "**Magneto-fluorescent carbon coated superparamagnetic iron oxide nanoarchitectures (SPIONs) for multimodal imaging and cancer theranostics**" submitted by **Mr. Ashish Tiwari** to the Indian Institute of Technology, Mandi for the award of the degree of **Doctor of Philosophy (PhD)** is an original record of research work carried out by him under my supervision. The contents of this thesis, in full or in parts, have not been submitted to any other Institute or University for the award of any degree or diploma. In keeping with the general practice of reporting scientific observation, due acknowledgements have been made wherever the work described is based on the findings of other investigators.

Date:

Place: Mandi

Dr. Jaspreet Kaur Randhawa

Assistant Professor
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Declaration by the Research Scholar

This is to certify that the thesis entitled "**Magneto-fluorescent carbon coated superparamagnetic iron oxide nanoarchitectures (SPIONs) for multimodal imaging and cancer theranostics**", submitted by me to the Indian Institute of Technology Mandi for the award of the degree of **Doctor of Philosophy (PhD)** is an original record of research work carried out by me under the supervision of **Dr. Jaspreet Kaur Randhawa**. The contents of this thesis, in full or in parts, have not been submitted to any other Institute or University for the award of any degree or diploma. In keeping with the general practice of reporting scientific observation, due acknowledgements have been made wherever the work described is based on the findings of other investigators.

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Last but not the least, I thank to one and all, who have been associated throughout my journey to complete this dissertation, for their love, affection and unconditional livelihood to make this dream come true.

Abstract

Cancer has been, and still remains, one of the most chronic disease to treat. As a result of severe adverse effects experienced from current cancer treatment and clinical trial studies, there has been a consistent growing interest in the development of an efficient cancer theranostics system that can effectively cure the cancer, but render healthy tissue unharmed. A prior objective of the present thesis was to develop such cancer nanotheranostics systems and evaluate their therapeutic efficacy in real time cancer theranostics with full proof of concept strategies.

The central hypothesis of this thesis was to enable multimodal imaging ability in magnetic nanoparticles by associating fluorescence in to their structures and insitu tuning of the magneto-fluorescent properties. Multifunctional magneto-fluorescent nanoarchitectures were developed in an easy and facile single step synthesis method avoiding multistep process and any kind of post synthesis modifications. A full proof of the property tuned synthesis protocol is described and proven through the characterization results. Advanced with the development of three different magneto-fluorescent nanoarchitectures, we evaluated their potential in MR imaging, fluorescence imaging, single particle imaging and tracking respectively. In addition, we also explored the equivalent value for the use of magneto-fluorescent nanoarchitectures in stimuli responsive drug delivery, magnetic hyperthermia, neuroengineering, protein sensing and magnetic field induced fluorescence engineering applications. This thesis successfully achieved all the above biomedical applications and significantly addresses the challenges as stated above and stand potentially in achieving the high throughput results in real time cancer theranostics.

In summary, magneto-fluorescent carbon coated superparamagnetic iron oxide (SPIONs) nanoarchitectures especially designed to practically confronting property oriented applications, persistent with physio-chemical and biological experimental studies, have been established as a promising proof of concept for real time multimodal imaging, neuroengineering and cancer theranostics in biomedical applications.

List of Patent and Publications

Patent

1. Ashish Tiwari et al. Single Step Synthesis of Multimodal Magneto-Fluorescent Core-Shell Superparamagnetic Iron Oxide Nanoparticles and Fluorescent Carbon Nanodots.

Patent application number: 202011021910

Published Articles (Thesis)

1. **Ashish Tiwari**, Navneet C. Verma, Anup Singh, Chayan K. Nandi, and Jaspreet K. Randhawa. "Carbon Coated Core–Shell Multifunctional Fluorescent SPIONs." Nanoscale 10, (2018): 10389-10394.

2. **Ashish Tiwari**, Ashutosh Singh, Ayan Debnath, Ankur Kaul, Neha Garg, Rashi Mathur, Anup Singh, and Jaspreet K. Randhawa. "Multifunctional Magneto-Fluorescent Nanocarriers for Dual Mode Imaging and Targeted Drug Delivery." ACS Applied Nano Materials 2, (2019): 3060-3072.

3. **Ashish Tiwari**, Navneet C. Verma, Jaspreet K. Randhawa, and Chayan K. Nandi. "Real-Time Observation of Magnetic Field-Induced Fluorescence Engineering in SPIONs." The Journal of Physical Chemistry C 123, (2019): 27759-27764.

4. **Ashish Tiwari**, Navneet Chandra Verma, Sibel Turkkan, Ayan Debnath, Anup Singh, Gerald Draeger, Chayan Kanti Nandi, and Jaspreet Kaur Randhawa. "Graphitic Carbon Coated Magnetite Nanoparticles for Dual-Mode Imaging and Hyperthermia." ACS Applied Nano Materials 3, (2020): 896-904.

5. **Ashish Tiwari**, Raj Kumar, Orit Shefi and Jaspreet Kaur Randhawa, "Fluorescently Mantled Carbon Coated Core-Shell SPIONs for Neuroengineering Applications." ACS Applied Bio Materials 2020.

6. Ashish Tiwari, Prachi Bhatia and Jaspreet Kaur Randhawa, "Systematic Spectroscopic Investigation of Structural Changes and Corona Formation of Bovine Serum Albumin over Magneto-Fluorescent Nanoparticles." RSC-Dalton Transactions 2020.

Abbreviations

AA	Ascorbic acid
AIEE	Aggregation induced emission enhancement
AMF	Alternating magnetic field
AMF	Applied magnetic field
AES	Auger electron spectroscopy
AFM	Atomic force microscopy
ATR	Attenuated total reflection
BALB/c	An albino, immunodeficient inbred strain of the house mouse
BET	Brunauer–Emmett–Teller
BJH	Barrett-Joyner-Halenda
BSA	Bovine Serum Albumin
C	Celsius
CCD	Charge coupled device
CHI	Chitosan
CLEM	Correlative light and electron microscopy
CLSM	Confocal laser scanning microscopy
CNs	Carbon nanostructures
CPCSEA	Committee for Purpose of Control and Supervision of Experiments on Animals
CT	Computed tomography
CY	Cyanine dyes
D	Drag coefficient
DI	Deionized
DLC	Drug loading capacity
DLS	Dynamic light scattering
DMEM	Dulbecco's modified eagle medium
DMSO	Dimethyl sulfoxide
DR	Drug release

EC	Echo time
EDC	N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride
EDTA	Ethylene diamine tetraacetic acid
EM	Electron microscope
EMR	Electromagnetic radiation
EMCCD	Electron multiplying charge-coupled device
EPR	Enhanced permeation and retention
eV	Electron volt
FA	Folic acid
FBS	Fetal bovine serum
FC	Field cooling
FDA	Food and Drug Administration
FESEM	Field Emission Scanning Electron Microscopy
FFT	Fast Fourier transform
FCNDs	Fluorescent carbon nanodots
FTIR	Fourier transform infrared spectroscopy
FU	Fluorouracil
FOV	Field of view
FPS	Frames per second
FWHM	Full width half maximum
HAADF	High-angle annular dark-field imaging
HRMS	High resolution mass spectrometry
HRTEM	High resolution transmission electron microscopy
Hz	Hertz
ICDD	International center for diffraction data
ID/gm	Injected dose per gram
ITLC	Instant thin layer chromatography
ILP	Intrinsic loss power

IV	Intravenous injection
JCPDS	Joint committee on powder diffraction standards
K	Kelvin
KA/m	Kiloampere per metre
kDa	Kilodalton
kV	Kilovolt
kW	Kilowatt
kHz	Kilohertz
LFG	linear filed gradients
mA	Milliampere
mM	Millimolar
MF	Magnetic field
MFE	Magnetic field effect
MFCSNPs	Multifunctional carbon coated core shell SPIONs
Mg/ml	Milligram per milliliter
$MgCl_2$	Magnesium chloride
MH	Magnetic hyperthermia
MHz	Megahertz
ml	Millilitre
mm	Millimetre
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
MRI	Magnetic resonance imaging\
Ms	Millisecond
mΩ	Milliohm
MV	Methyl viologen
NA	Numerical aperture
NaCl	Sodium chloride
$NaHCO_3$	Sodium hydrogen carbonate

NaOH	Sodium hydroxide
NBCF	National Breast Cancer Foundation
NCCS	National Centre for Cell Science
NHS	N-hydroxysuccinimide
nm	Nanometer
nM	Nanomolar
NMR	Nuclear magnetic resonance
PALM	Photoactivated localization microscopy
PBS	Phosphate buffered saline
PDT	Photodynamic therapy
PEG	Polyethylene glycol
PET	Positron emission tomography
PH	Potential of Hydrogen
PMT	Photomultiplier tube
PSF	Point spread function
PTT	Photothermal therapy
QDs	Quantum dots
QY	Quantum yield
R_2	Relaxivity
RB	Rhodamine dye
RBC	Red blood cells
RF	Radiofrequency pulsed
ROI	Region of interest
SAED	Selected area electron diffraction
SAR	Specific absorption rate
SDS	Sodium Dodecyl Sulfate
SEM	Scanning electron microscopy
SLP	Specific loss power

SMLM	Single molecule localization based methods
SPFI	Single particle fluorescence imaging
SPIONs	Superparamagnetic iron oxide nanoparticles
SPT	Single particle tracking
SQUID	Superconducting quantum interference device
STED	Stimulated emission depletion
STEM	Scanning transmission electron microscopy
STORM	Stochastic optical reconstruction microscopy
T	Tesla
Tc	Technetium-99m
TDD	Targeted drug delivery
TE	Echo time
TR	Repetition time
TEM	Transmission electron microscopy
TGA	Thermogravimetric analysis
TIRF	Total internal refraction fluorescence
TX	Trolox
U	Dipole-dipole interaction energy
USPSTF	United States Preventive Services Task Force
UV-Vis	Ultraviolet visible
VSM	Vibrating sample magnetometer
W/g	Watt/gram
XPS	X-ray photoelectron spectroscopy
XRD	X-ray diffraction
µL	Microliter
µm	Micrometre
µCi	Micro curie
ZFC	Zero field cooling

Table of Content