DESIGN and SYNTHESIS of MULTIFUNCTIONAL NANOPARTICLES for MULTI-MODAL IMAGING and THERANOSTICS APPLICATIONS

By

Du Li

Institute for Biomedical Materials &Devices
School of Mathematical and Physical Sciences,
Faculty of Science

Supervisors:
Prof. Dayong Jin
Prof. Xiangyang Shi
Dr. Shihui Wen

This thesis is presented for the degree of Doctor of Philosophy
September 2020

CERTIFICATE OF ORIGINAL AUTHORSHIP

I, Du Li, declare that this thesis, submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the School of Mathematical and Physical Sciences, Faculty of Science at the University of Technology Sydney.

This thesis is wholly my own work unless otherwise reference or acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

I certify that the work in this thesis has not previously been submitted for a degree nor has it been submitted as part of the requirements for a degree at any other academic institution except as fully acknowledged within the text. This thesis is the result of a Collaborative Doctoral Research Degree program with Donghua University.

This research is supported by the Australian Government Research Training Program.

Signature: Production Note:
Signature removed prior to publication.

Date: 30/09/2020

ACKNOWLEDGEMENTS

Firstly, I would like to thank my principal supervisor Professor Dayong Jin at University of Technology Sydney. I am grateful for his support, comments and guidance though-out my Ph.D. study. He kept me going in the right direction when I could be so easily deviated and distracted. Particularly, I would like to thank Jin for his making me think about why I was doing what I was doing. With his guidance, I really enjoy challenging myself and unveiling the myth step by step in research.

Secondly, I would like to thank my co-supervisor Professor Xiangyang Shi at Donghua University. He gave me a myriad of valuable suggestions and walked me though the way of doing scientific research. He laid a solid foundation for me, especially in finding projects motivations, experiment details and manuscript writing.

Other people I would like to thank are:

i. My mentor Doctor Shihui Wen for his generous support though-out all these years as well as for comments on writing of these projects. He always had time to listen to my problems and so often supplied ideas for solutions.
ii. Professor Mingwu Shen, Doctor Rui Guo, Doctor Xueyan Cao, Doctor Jia Yang, Doctor Chen Peng, Doctor Yue Wang, Doctor Jiajia Zhou, Doctor Fan Wang for their ideas and suggestions.
iii. Doctor Hui Liu, Doctor Yu Luo, Doctor Benqing Zhou, Doctor Wenjie Sun, Doctor Fanfan Fu, Doctor Jiayan Liao, Mengya Kong, Wei Hu, Changchang Zhang, Zhijun Ouyang for their general support and companions.
iv. All my friends from Institute for Biomedical Materials &Devices (IBMD) at University of Technology Sydney and College of Chemistry, Chemical Engineering and Biotechnology at Donghua University, who made the whole Ph.D. extremely enjoyable.

Finally, I would like to thank my family, particularly my soul mate Shihui Wen and my parents for their invaluable support and encouragement. I would like to thank China Scholarship Council for its financial support to finish my cotutelle Ph.D. degree. Without support from these people, my Ph.D. degree would not have been possible.

Table of Contents

List of Publications

Published Papers:

1. **Du Li**, Shihui Wen*, Mengya Kong, Yongtao Liu, Wei Hu, Bingyang Shi, Xiangyang Shi*, and Dayong Jin*. Highly Doped Upconversion Nanoparticles for in Vivo Applications under Mild Excitation Power. ***Analytical Chemistry*** 92(16) 10913-10919.
2. **Du Li**, Shihui Wen, Xiangyang Shi*, Dendrimer-entrapped metal colloids as imaging agents, ***Wiley Interdisciplinary Reviews: Nanomedicine and Nanobiotechnology*** 7(5) (2015) 678-690.
3. **Du Li**, Yongxing Zhang, Shihui Wen, Yang Song, Yueqin Tang, Xiaoyue Zhu, Mingwu Shen, Serge Mignani, Jean-Pierre Majoral, Qinghua Zhao*, Xiangyang Shi*, Construction of polydopamine-coated gold nanostars for CT imaging and enhanced photothermal therapy of tumors: an innovative theranostic strategy, ***Journal of Materials Chemistry B*** 4(23) (2016) 4216-4226.
4. **Du Li**, Jia Yang, Shihui Wen, Mingwu Shen, Linfeng Zheng, Guixiang Zhang*, Xiangyang Shi*, Targeted CT/MR dual mode imaging of human hepatocellular carcinoma using lactobionic acid-modified polyethyleneimine-entrapped gold nanoparticles, ***Journal of Materials Chemistry B*** 5(13) (2017) 2395-2401.
5. **Du Li**, Shihui Wen, Wenjie Sun, Jiulong Zhang, Dayong Jin, Chen Peng*, Mingwu Shen*, Xiangyang Shi*, One-Step Loading of Gold and Gd2O3 Nanoparticles within PEGylated Polyethylenimine for Dual Mode Computed Tomography/Magnetic Resonance Imaging of Tumors, ***ACS* Applied *Bio Materials*** 1(2) (2018) 221-225.
6. Guangxiang Chen#, **Du Li#**, Jingchao Li, Xueyan Cao, Jianhua Wang*, Xiangyang Shi*, Rui Guo*, Targeted doxorubicin delivery to hepatocarcinoma cells by lactobionic acid-modified laponite nanodisks, ***New Journal of Chemistry*** 39(4) (2015) 2847-2855. (first co-authors)
7. Yongxing Zhang#, Shihui Wen#, Lingzhou Zhao#, **Du Li#**, Changcun Liu, Wenbin Jiang, Xiang Gao, Wentao Gu, Nan Ma, Jinhua Zhao*, Xiangyang Shi*, Qinghua Zhao*. Ultrastable polyethyleneimine-stabilized gold nanoparticles modified with polyethylene glycol for blood pool, lymph node and tumor CT imaging. ***Nanoscale*** 2016, 8, 5567–5577 (first co-authors)
8. Yu Zou#, **Du Li#**, Mingwu Shen, Xiangyang Shi, Polyethylenimine-Based Nanogels for Biomedical Applications, ***Macromolecular Bioscience*** 19(11) (2019) 1900272. (first co-authors)

9. Benqing Zhou, Linfeng Zheng, Chen Peng, **Du Li**, Jingchao Li, Shihui Wen, Mingwu Shen, Guixiang Zhang*, Xiangyang Shi*, Synthesis and Characterization of PEGylated Polyethylenimine-Entrapped Gold Nanoparticles for Blood Pool and Tumor CT Imaging, ***ACS Applied Materials & Interfaces*** 6(19) (2014) 17190-17199.
10. Jianzhi Zhu, Chen Peng, Wenjie Sun, Zhibo Yu, Benqing Zhou, **Du Li**, Yu Luo, Ling Ding, Mingwu Shen, Xiangyang Shi, Formation of iron oxide nanoparticle-loaded γ-polyglutamic acid nanogels for MR imaging of tumors, ***Journal of Materials Chemistry B*** 3(44) (2015) 8684-8693.
11. Shihui Wen, Lingzhou Zhao, Qinghua Zhao, **Du Li**, Changcun Liu, Zhibo Yu, Mingwu Shen, Jean-Pierre Majoral, Serge Mignani, Jinhua Zhao, Xiangyang Shi*, A promising dual mode SPECT/CT imaging platform based on 99mTc-labeled multifunctional dendrimer-entrapped gold nanoparticles, ***Journal of Materials Chemistry B*** 5(21) (2017) 3810-3815.
12. Lingzhou Zhao, Shihui Wen, Meilin Zhu, **Du Li**, Yan Xing, Mingwu Shen, Xiangyang Shi, Jinhua Zhao*, 99mTc-labelled multifunctional polyethylenimine-entrapped gold nanoparticles for dual mode SPECT and CT imaging, ***Artificial Cells, Nanomedicine, and Biotechnology*** (2018) 1-11.
13. Chaohao Chen, Fan Wang*, Shihui Wen, Qian Peter Su, Mike C.L. Wu, Yongtao Liu, Baoming Wang, **Du Li**, Xuchen Shan, Mehran Kianinia, Igor Aharonovich, Milos Toth, Shaun P. Jackson, Peng Xi, Dayong Jin*, Multi-photon near-infrared emission saturation nanoscopy using upconversion nanoparticles, ***Nature Communications*** 9(1) (2018) 3290.
14. Jianhong Wang, **Du Li**, Yu Fan, Menghan Shi, Yunxia yang, Le Wang, Yitian Peng, Mingwu Shen, Xiangyang Shi, Core-shell tecto dendrimers formed via host-guest supramolecular assembly as a pH-responsive intelligent carrier for enhanced anticancer drug delivery, ***Nanoscale*** 11(46) (2019) 22343-22350.
15. Tingting Xiao, **Du Li**, Xiangyang Shi, Mingwu Shen*. PAMAM Dendrimer-Based Nanodevices for Nuclear Medicine Applications, ***Macromolecular Bioscience*** 20(2) (2020) 1900282.
16. Yu Zou, **Du Li**, Yue Wang, Zhijun Ouyang, Yucheng Peng, Helena Tomás, Jindong Xia, João Rodrigues, Mingwu Shen, Xiangyang Shi, Polyethylenimine Nanogels Incorporated with Ultrasmall Iron Oxide Nanoparticles and Doxorubicin for MR Imaging-Guided Chemotherapy of Tumors, ***Bioconjugate Chemistry*** 31(3) (2020) 907-915.
17. Ni Zhang, Yue Wang, Changchang Zhang, Yu Fan, **Du Li**, Xueyan Cao, Jindong Xia, Xiangyang Shi, Rui Guo, LDH-stabilized ultrasmall iron oxide nanoparticles as a platform for hyaluronidase-promoted MR imaging and chemotherapy of tumors, ***Theranostics*** 10(6) (2020) 2791-2802.

([1] - [5] are closely related to my Ph.D. program)

Book Chapters:

1. Zhijun Ouyang, **Du Li**, Xiangyang Shi*. Dendrimer-based tumor-targeting systems.
2. Changchang Zhang, **Du Li**, Xiangyang Shi*. Hybrid Nanogels for Photoacoustic Imaging and Photothermal Therapy.

Conferences:

1. **Du Li**, XiangyangShi, Lactobionic Acid-Targeted Polyethyleneimine-Entrapped Gold Nanoparticles for Dual Mode CT/MR Imaging of Liver Cancer Cells, Suzhou, 2014（Poster）
2. **Du Li**, Fanfan Fu, XiangyangShi. Lactobionic Acid-Targeted Polyethyleneimine-Entrapped Gold Nanoparticles for Dual Mode CT/MR Imaging of Liver Cancer Cells. Abstract for the Sino-German Symposium on Colloid and Interface Materials. 2014.（Poster）
3. **Du Li**, Xiangyang Shi, Chelator-Free Gadolinium-Doped Polyethyleneimine-Entrapped Gold Nanoparticles for Dual Mode CT/MR Imaging. The 2015 International Symposium for Advance Materials Research (ISAMR 2015), August 16-20, 2015, Taiwan, China. (Poster）
4. **Du Li**, Xiangyang Shi, Polydopamine-Coated Gold Nanostars for CT Imaging and Enhanced Photothermal Therapy of Tumors. Chinese Materials Conference 2015. (Poster）
5. **Du Li**, Xiangyang Shi, Dayong Jin, Polydopamine-coated gold nanostars for CT imaging and enhanced photothermal therapy of tumors, SPIE BioPhotonics Australia, SPIE, 2016, p. 1. (Poster)

ABSTRACT

Tumor imaging can localize neoplastic lesions in the body. Since early detection of tumors will improve early treatment and prognosis, continuing to enhance current multi-modal tumor imaging approaches towards higher sensitivity, resolution, and speed is critically needed in guiding the personalized therapy. Contrast agents are often essential in significantly improving the imaging quality to achieve better limits of detection for cancer cells. Clinically used contrast agents are generally based on small molecules, which suffers from severe disadvantages such as short half-decay time and non-specificity. With the rapid development of nanotechnology, nanoscale contrast agents have been extensively synthesized and applied for early detection of tumors with prolonged residence time in the bloodstream and passive targeting abilities. Nanoparticle-based contrast agents have relatively large surface-to-volume ratios to enhance surface binding and the amount of payloads for nanomedicine delivery. Their compositions, morphology and surface properties are tunable, which renders them with unique properties compensating the drawbacks of molecular contrast agents. Nanoscale contrast agents can incorporate tumor targeting agents to promote the specific targeting abilities with improved imaging contrast and detection sensitivity. Moreover, multifunctional nanoparticles can be made with robust and well-defined surfaces to incorporate multimodal imaging, targeting biomolecules, and drug delivery as both diagnostic agents and theranostic vehicles.

This thesis reports the rational design, synthesis and tumor imaging applications of various nanoscale contrast agents, including gold nanoparticles (AuNPs) for Computed Tomography (CT) imaging, gadolinium ions modified nanoparticles for Magnetic Resonance (MR) imaging, upconversion nanoparticles for optical imaging. Hyperbranched polyethyleneimine (PEI) polymeric carriers have been used as the template for the fabrication of contrast agents, which offers several advantages, including abundant terminal groups for surface modification, improved solubility and stability, biocompatibility, and prolonged circulation. Particularly, I have demonstrated the dual mode CT/MR tumor imaging abilities of Au/gadolinium and Au/Gd_2O_3 nanoparticles-based nanoparticles. Polydopamine-coated Au nanostars have been fabricated for CT imaging and enhanced photothermal therapy of tumors. Furthermore, I have demonstrated the fabrication of photon up-converting nanoparticle probes with improved brightness to realize tumor imaging using low irradiance of near infrared excitation. These findings provide new ideas and directions for the development of new multimodal imaging contrast agents and multifunctional therapeutic agents.

KEY WORDS: Tumor imaging, CT imaging, MR imaging, Polyethyleneimine, gold nanoparticles, gold nanostars, Gd_2O_3, photothermal therapy, upconversion nanoparticles

List of Acronyms (in alphabetic order)

AA	Ascorbic Acid
ASGPR	Asialoglycoprotein Receptor
Au PENPs	PEI-entrapped gold Nanoparticles
Au NPs	gold Nanoparticles
CT	Computed Tomography
DA	Dopamine
DOX	Doxorubicin
DMEM	Dulbecco's Modified Eagle Medium
DLS	Dynamic Light Scattering
EPR	Enhanced Permeation and Retention
EGFR	Epidermal Growth Factor Receptor
FBS	Fetal Bovine Serum
FI	Fluorescein Isothiocyanate
$^{[18F]}$FDG	Fluorodeoxyglucose
FA	Folic Acid
FWHM	Full-Width at Half-Maximum
Gd_2O_3	Gadolinium Oxide
GNR	Gold Nanorod
H&E	Hematoxylin and Eosin
HCC	Hepatocellular Carcinoma
HU	Hounsfield Units
HA	Hyaluronic Acid
ICP-OES	Inductively Coupled Plasma-Optical Emission Spectroscopy
LA	Lactobionic Acid
LSPR	Localized Surface Plasmon Resonance
MA	Mercaptoacetic Acid
MOF	Metal Organic Framework
MPI	Magnetic Resonance Imaging

MR	Magnetic Resonance
MSOT	Multispectral Photoacoustic Tomography
MWCO	Molecular Weight Cut-off
$NaBH_4$	Sodium Borohydride
NHS	N-hydroxysuccinimide
NIR	Near Infrared
NPs	Nanoparticles
OA	Oleic Acid
OA^-	Oleate Anions
OAH	Oleic Acid Molecular
ODE	1-Octadecene
OCT	Optical Coherence Tomography
PBS	Phosphate Buffered Saline
pD	Polydopamine
PEG	Polyethylene Glycol
PEI	Polyethyleneimine
PEI-SH	Thiolated Polyethyleneimine
PDT	Photodynamic Therapy
PET	Positron Emission Tomographic
PTT	Photothermal Therapy
QDs	Quantum Dots
SPECT	Single Photon Emission Computed Tomography
SPR	Surface Plasmon Resonance
TEM	Transition Electron Microscopy
TUNEL	TdT-mediated dUTP Nick-End Labeling
UCNPs	Upconversion Nanoparticles
UV	Ultraviolet
XPS	X-ray Photoelectron Spectroscopy
XRD	X-ray Powder Diffraction