To
my wife Eun Jung and my daughter Ella Jaehee
my parents and brothers
and
my parent-in-laws

Acknowledgements

I would like to begin by thanking my advisor, Professor Raoul Kopelman for all his support and guidance. His passion and energy for research always amazes me and all other members in our group. I have admired him and wished to be like him all the time. Especially, I want to deeply appreciate his generosity and trust on me. Dr. Kopelman has given them to me not only when I am doing well but also when I made mistakes and disappointed him. I could not have been here finalizing my degree without his encouragement.

I would like to thank my committee members, Professor Michael D. Morris, Professor Alnawaz Rehemtulla, Professor Alan J. Hunt, Professor Shuichi Takayama, and a previous member Professor Matthew O'Donnell, for their kindness and sympathy to my research. I am deeply grateful to their valuable advices and will keep them in mind for my career in the future.

I also deeply thank Dr. Yong-Eun Lee Koo in our group. As a manager and an intermediate mentor, she has been made a great contribution to all my research and has given me unforgettable understandings.

Special thanks to Dr. Shai Ashkenazi in Biomedical Engineering at the University of Michigan for letting me participate in his photoacoustics research. He led to open my eyes to look at a new aspect of my research and helped me to gain some promising outcomes. Collaboration with him was an important opportunity for

me to have confidence as a researcher. I would also like to be grateful to Professor Martin Philbert and Dr. Hao Xu in School of Public Health at the University of Michigan. They provided me all supports and cooperation for biological experiments and it made huge contribution to improve the quality of my research.

Thanks to the members of Kopelman group who helped me in research and in life. Dr. Fei Yan, Dr. Wenzhe Fan, Dr. Katherine Tyner, Dr. Maria Moreno and Dr. Hoe Jin Hah helped me greatly in my research by teaching me and sharing with me their knowledge and skills in PEBBLE synthesis, properties, and applications. I also thank to Dr. Rodney Agayan, Dr. Wei Tang, Dr. Matthew King for their advice. I also thank to all other Kopelman group members for their helps and friendship.

Last, I would deeply thank my family for their endless love, faith, and supports. My wife, Eun Jung sacrificed her wonderful career to be with me and gave me a priceless present, our daughter, Ella Jaehee. My parents and brothers have been always supportive for my life and study and been concerned about my health. My parent-in-laws have also given me a great generosity and understanding. I will never be able to thank enough to them for all their supports and sacrifices. I hold them in the highest regards.

Table of Contents

List of Figures

List of Tables

List of Appendices

Abstract

Development of Nanoparticle Based Tools for Reactive Oxygen Species and Related Biomedical Applications

Reactive oxygen species (ROS) are various oxygen derived intermediates produced from the reduction of molecular oxygen and highly reactive / cytotoxic byproducts of aerobic metabolisms in biology. ROS includes hydroxyl radicals (·OH), superoxide anion radical (O_2^-), hydrogen peroxide (H_2O_2), and energetically excited oxygen (singlet oxygen $^1O_2$). ROS are capable of oxidizing various biomolecules, interrupting their cellular functions, and consequently, inducing cell death. ROS play various roles in normal and pathogenic conditions in biology. However, our understandings about ROS still largely remain in qualitative stages because their exceptionally unstable nature makes the investigations of ROS highly challenging.

This work demonstrates how to utilize nanoparticle-encapsulation to ROS related research and applications with improved properties. Three independent nanoparticle based tools have been developed using PEBBLE (Photonic Explorer for Biomedical use with Biologically Localized Embedding) technology with organically modified silicate (Ormosil) matrix. First, singlet oxygen sensitive nanoparticle probes were synthesized by encapsulating a singlet oxygen molecular probe, 1,3-diphenylisobenzofuran (DPIBF), which is the most sensitive but not appropriate for biological uses, into protective Ormosil matrix. They exhibited improved singlet oxygen sensitivity over conventional

molecular probes. Based on this established sensitivity, the direct quantity of singlet oxygen generated from an *in vitro* photodynamic therapy (PDT) for cancer was able to be determined. Second, hydrogen peroxide detecting nanoparticle probes were also developed. The non-specific ROS detecting molecular probe, 2',7'-dichlorofluorescin diaceate (DCFDA) was embedded into Ormosil nanoparticle by post-loading technique. The DCFDA nanoprobes showed enhanced selectivity towards H_2O_2 by excluding the interferences from other ROS by screening effect of nanoparticle matrix based on the combination of size exclusion, lifetime exclusion, and hydrophobicity. An *in vitro* H_2O_2 production from stimulated macrophages could be quantitatively monitored by the DCFDA PEBBLE nanoprobes with low nM of resolution. Third, dual-functional nanoparticles containing near-infrared absorbing indocyanine green dye (ICG) were developed for photoacoustic imaging/diagnosis and photodynamic therapy for cancer. The ICG nanoparticles showed capability of generating singlet oxygen for PDT. Tissue mimicking phantoms containing these nanoparticles were built with diffusive agarose gels and they were successfully imaged by 2-D and 3-D photoacoustic imaging systems. ICG nanopartcies were targeted to cancer by incorporating with an antibody and displayed sufficient photoacoustic contrast effect in a prostate cancer model *in vitro*.