

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

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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 ($\cdot\text{OH}$), superoxide anion radical (O_2^-), hydrogen peroxide (H_2O_2), and energetically excited oxygen (singlet oxygen $^1\text{O}_2$). 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'-dichlorofluorescein diacetate (DCFDA) was embedded into Ormosil nanoparticle by post-loading technique. The DCFDA nanoprobe showed enhanced selectivity towards H₂O₂ 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* H₂O₂ production from stimulated macrophages could be quantitatively monitored by the DCFDA PEBBLE nanoprobe 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 nanoparticles were targeted to cancer by incorporating with an antibody and displayed sufficient photoacoustic contrast effect in a prostate cancer model *in vitro*.