

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

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Table of Contents

Dedication.....	ii
Acknowledgements.....	iii
List of Figures.....	vi
List of Tables.....	xiii
List of Appendices.....	xiv
Abstract.....	xv
Chapter	
1. Introduction.....	1
Reference.....	11
2. PEBBLE Nanoprobes for Singlet Oxygen Detection and Application to <i>in vitro</i> Photodynamic Therapy.....	13
Introduction.....	13
Experimental.....	17
Results and Discussion.....	29
Acknowledgments.....	56
References.....	57
3. Development of Ormosil PEBBLE Nanoprobes for Improved Hydrogen Peroxide (H ₂ O ₂) Selectivity and Application to Macrophage Activation Studies.....	60
Introduction.....	60
Experimental.....	65
Results and Discussion.....	73
Acknowledgments.....	95
References.....	96
4. Nano-PEBBLEs as Photoacoustic Imaging Agents with Simultaneous Near-Infrared Photodynamic Activity.....	99
Introduction.....	99
Experimental.....	102
Results and Discussion.....	111
Acknowledgments.....	124
References.....	125
5. Summary and Future Directions.....	129
Summary.....	129
Future Directions.....	132
Appendices.....	135

List of Figures

- Figure 2.1: The reaction mechanism of DPIBF with singlet oxygen. Fluorescent DPIBF can be transformed to non-fluorescent endoperoxides (DPIBFO₂) by reacting with singlet oxygen.....16
- Figure 2.2: SEM image of synthesized DPIBF embedded Ormosil PEBBLE nanoprobe showing an 80-140 nm diameter range. SEM image was obtained with XL 30 FEG SEM device with 40 seconds gold sputter coating on the sample surface.....30
- Figure 2.3: Determination of the amount of DPIBF embedded in the Ormosil PEBBLES: a) fluorescence emission spectra of DPIBF with various known concentrations and unknown DPIBF Ormosil PEBBLE nanoprobe, b) fluorescence intensity vs. DPIBF concentration calibration curve for estimation of dye loading capacity; ●: known, □: unknown31
- Figure 2.4: Examination of dye leaching from PEBBLE nanoprobe: Comparison of DPIBF's fluorescence emission between a nanoprobe solution in DI water (blue line in left chart) and the filtrate solution in DI water (pink line in left chart), showing no dye leaching out of the nanoparticles and numerical comparison of fluorescence intensity at maximum peak.....32
- Figure 2.5: Comparison of auto-oxidation between DPIBF free dye and DPIBF Ormosil PEBBLE nanoprobe in various compositions of water-EtOH mixtures. The reduction of DPIBF's and DPIBF Ormosil PEBBLE nanoprobe's fluorescence intensity at emission maxima (around 455 nm) were measured for 5 minutes with increasing water content. DPIBF free dye showed larger auto-oxidation effect as water content increased. PEBBLE nanoprobe exhibited significantly lower such effect than free dye in all solvent compositions.....34
- Figure 2.6: Relative comparison of singlet oxygen sensitivity among ADPA free dye, DMA Ormosil PEBBLE nanoprobe, and DPIBF Ormosil PEBBLE nanoprobe under identical condition. Singlet oxygen was generated by 5 μM MB free dye in DI water by 660 nm irradiation for 5 minutes and quenched by these three singlet oxygen probes individually. The decay of fluorescence intensity for each probe was compared after the normalization of I_t (fluorescence intensity at time t) / I_0 (fluorescence intensity at time 0).....36
- Figure 2.7: The exponential decay of DPIBF-SDS micelles' and DPIBF Ormosil PEBBLE nanoprobe's fluorescence intensity by reaction with singlet oxygen generated by photosensitizer MB in various concentrations in DI water. The results were normalized by converting the fluorescence intensity to concentration

of each probe and the auto-oxidation and light scattering effects were subtracted. [a) DPIBF-SDS micelles, and b) DPIBF Ormosil PEBBLE nanoprobe, ■: 9.091 μM MB, □: 4.545 μM MB, ▲: 0.909 μM MB, Δ: 0.455 μM MB, and ●: 0.091 μM MB].....37

Figure 2.8: Comparison of k values determined for DPIBF-SDS micelles and for DPIBF Ormosil PEBBLE nanoprobe in various photosensitizer MB's concentrations in 5 minutes photosensitization. It showed a consistent trend that DPIBF Ormosil PEBBLE nanoprobe have roughly 50 % reduced sensitivity towards singlet oxygen compared to DPIBF-SDS micelles in overall situations.....39

Figure 2.9: Fluorescence emission change of DPIBF Ormosil PEBBLE nanoprobe measured from *in vitro* PDT for C6 glioma cells using photosensitizer MB PAA DNPs for 5 minutes, 4 different conditions using 2 DNP concentrations and 2 cell densities were examined and control experiment for each DNP concentration was performed as well without the presence of cells. a) 1 mg/mL MB PAA DNP with high cell density, b) 0.1 mg/mL MB PAA DNP with high cell density, c) 1 mg/mL MB PAA DNP with low cell density, d) 0.1 mg/mL MB PAA DNP with low cell density, e) 1 mg/mL MB PAA DNP without cells (control), and f) 0.1 mg/mL MB PAA DNP without cells (control).....45

Figure 2.10: k value determination for estimation of steady-state singlet oxygen concentrations for *in vitro* PDT experiment performed. The change of fluorescence intensity at emission maxima of DPIBF Ormosil PEBBLE nanoprobe were measured for 5 minutes and the obtained fluorescence intensity values were normalized as I_t (fluorescence intensity at time t) / I_0 (fluorescence intensity at time 0) for comparison. Right: PDT was performed with 1 mg/mL MB PAA DNPs in 3 sets of samples: high cell density, low cell density, and no cells (control). Left: PDT was performed with 0.1 mg/mL MB PAA DNPs in same 3 sets of samples. Besides DNP concentration and cell density, all other experimental conditions were identical.....46

Figure 2.11: Linear relationship between normalized concentration of photosensitizer MB PAA DNP and normalized consumption of singlet oxygen by cells determined from *in vitro* PDT experiments described above.....48

Figure 2.12: Bright field – fluorescence overlaid images to show cell damage indicated by PI staining, observed during *in vitro* PDT using MB PAA DNP for 5 minutes. The fluorescence of PI staining was measured in Olympus IMT II fluorescence microscope with standard green filter cube set. The cell density dependence of cell damage was indicated by delay of appearance of PI stained cells when there were more cells in the system under same concentration of DNPs.....51

Figure 2.13: Tumor regrowth profiles after *in vitro* PDT using MB PAA DNP for 5 minutes for 2 different cell densities and comparison with growth of non-treated cells (controls). The C6 glioma cells were continued to be cultured for 3 days after PDT treatment and the number of surviving cells were counted by hemacytometer with trypan blue exclusion. All the results were averaged out of 4 measurements for consistency. Significant reduction of cell numbers were observed from PDT treated cells compared to un-treated controls.....53

- Figure 2.14: A summary of cell regrowth profiles over 3 days after in vitro PDT. The number of cell for each case was normalized by adjusting the initial cell count (day 0) to 0 and final cell count (at day 3) of each control (un-treated cells) to 100. Both controls showed similar pattern of growth after normalization. The treated cells with high density showed 31 % regrowth and the ones with low density showed 4 % regrowth compared to controls, indicating significant suppression of cell growth by PDT treatment.....54
- Figure 3.1: Schematic for ROS detection mechanism of DCFDA free dye in biological systems. Lipophilic DCFDA can readily penetrate cell membrane and be trapped by being transformed to hydrophilic DCFH. DCFH can be oxidized by ROS producing strong fluorescent product DCF.....64
- Figure 3.2: SEM image of DCFDA loaded Ormosil nanopores. SEM imaging was performed with DCFDA post-loaded PEBBLE nanopores in XL 30 FEG SEM device after 50 seconds gold sputter coating on the sample surface.....74
- Figure 3.3: Schematic description of the post-loading method based on the modified solvent displacement method, using pre-formed Ormosil nanoparticles. Hydrophobic Ormosil blank nanoparticles and DCFDA dye were suspended together in a volatile organic solvent, chloroform. The suspension was mixed into aqueous media immiscible with chloroform, forming an oil-in-water type emulsion. The DCFDA loaded Ormosil PEBBLES were resulted by evaporating chloroform under stirring and ultrasonication.....75
- Figure 3.4: Time resolved acquisition of fluorescence increase of DCFDA Ormosil PEBBLE nanopores induced from oxidization by H_2O_2 with various concentrations in PBS buffer (pH 7) (a). The fluorescence of DCFDA Ormosil PEBBLE nanopores were monitored at 523 nm emission wavelength with 505 nm excitation for 5 minutes after addition of H_2O_2 . The PEBBLE nanopores showed nearly linear increase of fluorescence intensity for 5 minutes in concentration dependent manner (proportionally). In 8.7 nM H_2O_2 , the PEBBLE nanopores showed only negligible difference in response from control measurement with addition of water instead H_2O_2 solution, indicating detection limit of low 10s of nM (b). All results were normalized as I_t (fluorescence intensity at time t) / I_0 (initial fluorescence intensity) to adjust starting intensity to be same.....76
- Figure 3.5: Comparison of fluorescence emission increase induced by H_2O_2 addition between DCFDA Ormosil PEBBLE nanopores in PBS buffer (pH 7.4) and the filtrate solution from the nanopores to examine dye leaching effect. While PEBBLE solution showed rapid increase of fluorescence, the filtrate did not exhibit any notable response by addition of same amount of H_2O_2 , proving the reaction between DCFDA dye and H_2O_2 only occurred inside of Ormosil matrix.....77
- Figure 3.6: Examination of interference by HRP: The fluorescence intensity change was monitored to test the interference from HRP when HRP, H_2O_2 , or H_2O_2 +HRP was added to DCFDA Ormosil PEBBLE nanopores suspension in PBS buffer (pH 7.4). HRP alone did not cause any change in DCFDA Ormosil PEBBLE

nanoprobes' fluorescence while H_2O_2 alone and H_2O_2 +HRP showed noticeable changes, indicating that HRP interference was effectively excluded by encapsulation in PEBBLES.....79

Figure 3.7: Examination of interference by superoxide anion radical (O_2^-) towards DCFDA free dye and DCFDA Ormosil PEBBLE nanoprobes: O_2^- was produced by mixing potassium superoxide (KO_2) with aqueous media (PBS buffer) containing DCFDA free dye and DCFDA Ormosil PEBBLE nanoprobes based on ionization of KO_2 . While DCFDA free dye showed certain increase of fluorescence intensity by addition of O_2^- , DCFDA Ormosil PEBBLE nanoprobes only showed constant fluorescence intensity under same condition, indicating that PEBBLE nanoprobes can have resistance to the interference by O_2^-80

Figure 3.8: Examination of peroxyntirite ($ONOO^-$) interference with use of DCFDA free dye and of DCFDA loaded Ormosil nanoprobes. The fluorescence change of DCFDA free dye in PBS buffer and DCFDA Ormosil PEBBLE nanoprobes in PBS buffer were monitored after addition of $ONOO^-$ for 5 minutes. DCFDA Ormosil PEBBLE showed slight increase by reaction with $ONOO^-$ but DCFDA free dye showed rapid and significantly higher increase than PEBBLE nanoprobes, indicating PEBBLE can effectively exclude the interference by $ONOO^-$. Because of the strong basic pH of the $ONOO^-$ stock solution which can interfere the response of DCFDA as well, control measurements were performed for DCFDA free dye and PEBBLE nanoprobes, using just a pure basic solution and these measurements showed that the rapid increase observed from DCFDA free dye was truly by $ONOO^-$ and the slight increase observed from DCFDA Ormosil PEBBLE nanoprobes was induced by basic pH.....82

Figure 3.9: Examination of nitric oxide (NO) interference with use of DCFDA free dye and of DCFDA Ormosil PEBBLE nanoprobes. NO stock solution with pH 6 was prepared by dissolving NO gas in DI water and the stock solution was mixed with DCFDA free dye in PBS buffer (pH 7.4) and DCFDA Ormosil PEBBLE nanoprobes in PBS buffer (pH 7.4) under gas-tight sealing. While DCFDA free dye displayed certain increase, the PEBBLE nanoprobes showed constant fluorescence intensity by addition of NO solution, indicating effective exclusion of interference by NO.....84

Figure 3.10: *In vitro* H_2O_2 detection in fMLP stimulated live macrophages. DCFDA Ormosil PEBBLE nanoprobes were incubated with macrophage cells for an overnight and washed away. The oxidative burst from macrophages was triggered by addition of stimulating agent, fMLP, and the fluorescence intensity of DCFDA PEBBLE nanoprobes were monitored for 6 hours. a) TAT-conjugated DCFDA loaded Ormosil nanoprobes with stimulation, b) TAT-conjugated nanoprobes without stimulation for control (no H_2O_2 generation), c) nanoprobes without TAT-conjugation with stimulation for examine the difference between active cytosolic delivery by TAT and passive uptake by phagocytosis, and d) summarizing plot of fluorescence change of DCFDA for all three cases.....88

Figure 3.11: Estimation of H_2O_2 production from macrophages. Final product of DCFDA's oxidization, DCF are commercially available compound and the

	concentration of H ₂ O ₂ was able to be determined by comparing the obtained results from in vitro detection to the standard curve constructed using DCF. a) Fluorescence emission spectra of DCF in various concentrations and b) calibration curve obtained from a).....	90
Figure 3.12:	The experimental result showing the relationship between the DCFDA Ormosil PEBBLE nanoprobe concentrations and the H ₂ O ₂ response at a fixed H ₂ O ₂ concentration. The rate of fluorescence of DCFDA Ormosil PEBBLE nanoprobe increased proportionally to the concentration of the PEBBLE nanoprobe.....	91
Figure 3.13:	Bright field-fluorescence overlay image of DCFDA loaded Ormosil nanoprobe in macrophages after overnight incubation, obtained from Alexa 568 labeling. a) TAT-conjugated nanoprobe and b) nanoprobe without TAT conjugation. The unbound free PEBBLE nanoprobe were washed away by repeated rinsing with fresh cell media. The images were obtained at the same position in which the H ₂ O ₂ detections were performed.....	92
Figure 3.14:	Quantification of DCFDA Ormosil PEBBLE nanoprobe in monitored area using constructed standard curves. 2 calibration curves were constructed using TAT-conjugated PEBBLE nanoprobe (left) and PEBBLE nanoprobe without TAT conjugation (right). The concentration of PEBBLE nanoprobe used in in-vitro detections was fit to the calibration curve for estimation (pink and yellow color dots on the trend line).....	93
Figure 4.1:	A high resolution photoacoustic scanning system configuration.....	107
Figure 4.2:	Schematic of the photoacoustic imaging setup based on a clinical ultrasound scanner and a doubled YAG pulsed laser pumping an OPO. The beam is expanded (BX) before illuminating the phantom (PH). An ultrasound array probe (UA) detects the laser generated acoustic waves. The ultrasound scanner (US) acquires signals in synchronization with the laser pulses. The scanner employs a beam forming algorithm to form the photoacoustic image.....	109
Figure 4.3:	Schematic for tissue mimicking phantom for 2-D and 3-D photoacoustic imaging.....	111
Figure 4.4:	SEM image of ICG Ormosil PEBBLES (a) and particle size distribution obtained from dynamic light scattering particle sizer (b). Both results consistently showed that ICG Ormosil PEBBLES are nearly monodisperse, 100 nm in diameter.....	112
Figure 4.5:	Dye loading inside a PEBBLE particle was tested by comparing optical absorption of PEBBLE particles with optical absorption of free ICG dye at different concentrations (left). Linear curve fitting for the peak absorbance (at $\lambda=790$ nm) was evaluated, so as to estimate the dye concentration in the PEBBLE particle (right).....	114
Figure 4.6:	Comparison of UV-VIS absorption spectra between ICG Ormosil PEBBLES and filtrate at the absorption maxima near 800 nm. Filtrate solution showed only a trace of absorbance at 800 nm, verifying negligible dye leaching.....	115

Figure 4.7: Relative optical absorption of free ICG dye (diamonds) and ICG PEBBLE (triangles), measured over 5 days. Stabilization due to PEBBLE encapsulation is evident.....	117
Figure 4.8: Fluorescence intensity of singlet oxygen detecting probe, DPIBF with free ICG dye (a), DPIBF with ICG Ormosil PEBBLES (b), and pure DPIBF (c). As mentioned in chapter 2, DPIBF can lose its fluorescence by reacting with singlet oxygen. In each case the intensity was measured before (solid line) and after (dashed line) Ti-Sapphire laser illumination tuned at a wavelength of 800 nm, for 30 seconds. Reduced fluorescence intensity indicates singlet oxygen generation and ICG Ormosil PEBBLES and ICG free dye both showed notable fluorescence reduction while control did not shown any decrease under same illumination...118	118
Figure 4.9: Photoacoustic images of LNCaP cells incubated with antibody-conjugated PEBBLES (top), and LNCaP cells incubated with un-conjugated PEBBLES (bottom). In both cases cells were incubated for 1 hour and than washed twice. Signal gray scale is in dB. 22 dB stronger PA signal intensity was obtained from ab-conjugated PEBBLES than un-conjugated PEBBLES, indicating efficient tumor targeting.....	120
Figure 4.10: Photoacoustic image of a gel phantom with three, 1.5 mm diameter, cylinders of different PEBBLE concentrations: $1 \cdot 10^{10}$ (PEBBLES/cm ³) (left), $1 \cdot 10^{11}$ (PEBBLES/cm ³) (middle), and $1 \cdot 10^{12}$ (PEBBLES/cm ³) (right). The dynamic range of the gray scale is 40 dB.....	121
Figure 4.11: 3-D photoacoustic imaging of ICG embedded Ormosil PEBBLE phantoms designed as figure 4.3. 4 pieces of gel containing ICG Ormosil PEBBLES with random shape were planted inside tissue mimicking background gel cylinder and imaged. The illumination and acoustic signal reading were both achieved through more than 1 cm thickness of background gels.....	122-123
Figure A1.1: UV-VIS absorption spectra of PF PAA DNPs, showing difference in dye loading capacity.....	139
Figure A1.2: Singlet oxygen production efficiency by various PF PAA DNPs at different PEBBLE concentrations (1 mg/mL and 0.5 mg/mL DNPs in water). The singlet oxygen production from various DNPs was significantly reduced when they were evaluated by DPIBF Ormosil PEBBLE nanoprobe compared to when they were evaluated by DPIBF free dye.....	141
Figure A2-1: Intracellular localization of Ormosil PEBBLES based on DiI fluorescence. a) confocal imaging of DiI Ormosil PEBBLES to simulate DPIBF Ormosil PEBBLE nanoprobe, and b) a Z-series scanning, indicating highest distribution of PEBBLES in the central region of intracellular space.....	145
Figure A2.2: Fluorescence time change of DPIBF Ormosil PEBBLE nanoprobe, due to singlet oxygen produced from macrophage: a) response from PMA stimulated macrophage, b) response from non-stimulated macrophage (control), and c) comparison of fluorescence intensity changes between a) and b). The fluorescence intensity was normalized as I_t/I_0	147

Figure A2.3: A calibration curve showing the relation between the DPIBF Ormosil PEBBLE nanoprobe concentration and their fluorescence intensity for estimation of singlet oxygen production from stimulated macrophages.....148

List of Tables

Table 2.1: Numerical comparison of 1 st order fluorescence decay rate (k) by reaction with singlet oxygen between DPIBF-SDS micelles and DPIBF Ormosil PEBBLE nanoprobcs in various situations examined	38
Table 2.2: Numerical summary of β and k_c values for DPIBF Ormosil nanoprobcs. a) determination of β and k_c values for DPIBF Ormosil nanoprobcs by comparison with known values of DPIBF-SDS micelles [21,30], and b) comparison of nanoprobe's sensitivity towards singlet oxygen with those of other common singlet oxygen probes [21].....	40
Table 2.3: Steady-state singlet oxygen concentrations produced by photosensitizer MB with various concentrations in aqueous media estimated by equation (12), $[^1\text{O}_2]_{s-s} = k/k_{c,\text{nanoprobcs}}$ using measured fluorescence decay rate (k) and established sensitivity of DPIBF Ormosil nanoprobcs (k_c).....	42
Table 2.4: Quantification of steady-state singlet oxygen and consumption by cells. Steady-state singlet oxygen concentration for all 6 experiments (1 mg/mL DNP – high cell density, 1 mg/mL DNP – low cell density, 1 mg/mL DNP – control, 0.1 mg/mL DNP – high cell density, 0.1 mg/mL DNP – low cell density, and 0.1 mg/mL DNP – control) were estimated using equation (12) from k values experimentally determined. From the difference between each control measurement and actual measurement, the steady-state singlet oxygen concentrations used for attacking cells were calculated for each case. Then, quantities of singlet oxygen used for attacking a single cell were calculated by dividing the determined concentration values by number of cells.....	47
Table A1.1: Estimated dye loading (mg PF/mg PEBBLE) of PF in PAA DNPs based on the UV-VIS absorption intensity shown in figure A1.1.....	140

List of Appendices

1. PEBBLE to PEBBLE Detection of Singlet Oxygen in PDT.....	136
Experimental.....	137
Results and Discussion.....	138
References.....	142
2. Singlet Oxygen Detection in Live Macrophage <i>in vitro</i>.....	143
Experimental.....	143
Results and Discussion.....	144
References.....	149

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 nanoprobos 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 nanoprobos 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*.