Invited Paper

Indirect spectroscopic detection of singlet oxygen during photodynamic therapy

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ABSTRACT

Chemiluminescence (CL) and optical upconversion (OU) are used to measure photosensitizer singlet oxygen production indirectly. Both methods produce light that is blue-shifted relative to the normal photosensitizing wavelength. The emisson intensity is proportional to the concentration of singlet oxygen generated and is the result of either decomposition (CL) or energy transfer (OU) processes occuring inside a thin polymer film. Techniques for fabrication and characterization of CL and OU polymer films are discussed.

1. INTRODUCTION

Photodynamic therapy (PDT) of neoplastic tissue is based on the administration of phototherapeutic agents which are activated by light.[1] Upon irradiation, highly reactive oxygen intermediates, primarily singlet molecular oxygen, $^1O_2$, are generated. These toxic species contribute to tumor destruction by participating in the oxidation of essential cellular components.

We have pursued two general strategies in order to follow the efficiency of PDT *in situ.*: 1) monitoring fluctuations in tumor oxygen content (i.e. ground-state O_2) during irradiation and recovery[2] and 2) measuring the production of the cytotoxic oxygen intermediate, $^1O_2$. Information provided by each technique is complementary since singlet oxygen is produced at the expense of the ground-state species. We anticipate that *in situ* characterization of oxygen in both forms (i.e. O_2 and $^1O_2$) may improve our understanding of the overall process and perhaps lead to new means for determining treatment efficacy.

Previous efforts to record the appearance of $^1O_2$ in biological systems have involved either time[3] or phase-resolved[4,5] measurements of $^1O_2$ phosphorescence at at 1.27 μm. In this work we report the detection of singlet oxygen production indirectly. Two techniques are described, 1) Chemiluminiscence (CL) and 2) Optical Upconversion (OU). Both methods employ a highly specific reagent phase which responds either to $^1O_2$ oxidation (CL) or $^1O_2$ phosphorescence (OU) by producing visible light. This indirect emission is blue-shifted with respect to the therapeutic wavelength, and the intensity is proportional to the concentration of singlet oxygen. Fabrication and characterization of two different reagent phases (CL and OU) are examined using both conventional and, in the case of CL, fiber optic sensor instrumentation.

2. MATERIALS AND METHODS

The chemiluminescent material bis(2,2'-biphenylene) (BPE) was synthesized from fluorenone (Aldrich), according to Turro *et al.*[6], and immobilized in a 200-μm thick polystyrene film. The optical density of the film at the BPE absorption maximum (λ = 463 nm) was typically maintained between 0.5 and 1. In order to scan the chemiluminescence spectrum, the film was placed in a 45-degree-angle cuvette in the sample compartment of a spectrofluorometer (SLM/Aminco, model 48000-MHF, Urbana, ILL). The cuvette was filled with the photosensitizer methylene blue (MB, Sigma). Excitation at the absorption maximum of MB (664 nm) was provided by the instrument's 450-Watt Xe-arc lamp coupled to a single-grating, f/4.2 monochromator with a 4 nm/mm bandpass. The CL emission was scanned using a second (motorized) monochromator which had a photomultiplier tube (Hammamatsu R928) mounted at the exit port.

A CL fiber optic sensor was fabricated by epoxying a portion of the BPE polymer to the flat-cut face of a single quartz optical fiber (Figure 1). The sensing terminus of the fiber was placed in a cuvette containing 2.2 x 10^{-6} M MB/water.

Singlet oxygen was generated at the sensing tip by directing a 664 nm, 175 mW laser beam from an Argon-pumped dye laser (DCM dye, Coherent Innova 20/599) just beneath the BPE film (beam diameter approximately 2 mm). The CL signal was isolated with appropriate filters (a 520 nm broad bandpass filter and a 590 nm short-pass filter) and recorded using a photomultiplier tube (Hammamatsu R928), picoammeter (Keithly model 485, Taunton, MA), and strip chart recorder.

Optical upconversion reagent phases were fabricated from ESPI C97C (Electronic Space Products International, Agoura, CA) phospor particles cast in a 200-μm thick polystryrene film. The film was characterized in a 45-degree-angle cuvette using the spectrofluorometer described above. The photosensitizers MB and chloroaluminum sulfonated phthalcyanine (CASPc, Ciba Geigy) were evaluated. Both Xe-arc lamp and laser excitation were employed during OU studies (λ = 664 nm for MB and 672 nm for CASPc), however a fiber sensor was not constructed.

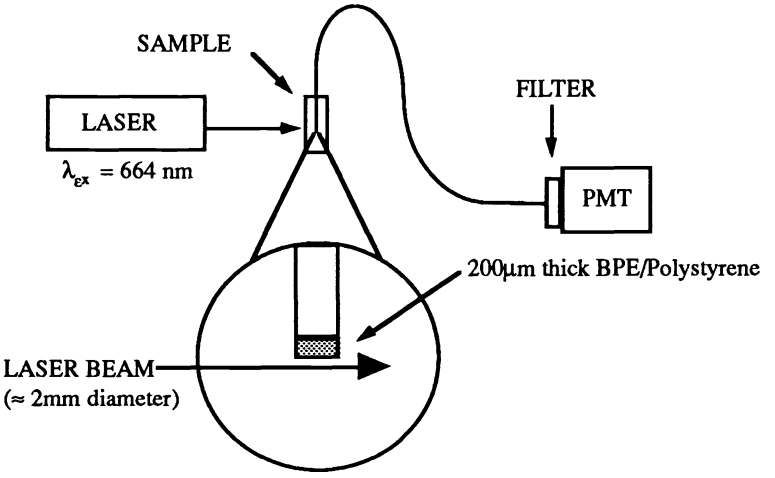

Figure 1. Fiber sensor configuration.

3. RESULTS AND DISCUSSION

3.1 Chemiluminescence

The follwing scheme depicts the steps involved in BPE chemiluminescence:

1. photosensitizer + hν (630-675 nm) → $^1O_2$
2. $^1O_2$ + BPE → (BPE)O_2
3. (BPE)O_2 → 2(Fluorenone) + hν (525 nm)

Red light (630-675 nm) is launched in to the system, and green light (525 nm) is measured. The blue-shifted emission spectrum for BPE/MB is recorded in Figure 2. There is a clear intensity dependence on photosensitizer concentration (2x MB = 2.2 x 10^{-6} M), and the emission maximum is centered at the CL maximum. The small signal observed for the water blank can be attributed to second-order excitation of incompletely dimerized fluorenone in the film. This contribution is reduced when the arc lamp/monochromator source is substituted for a laser.

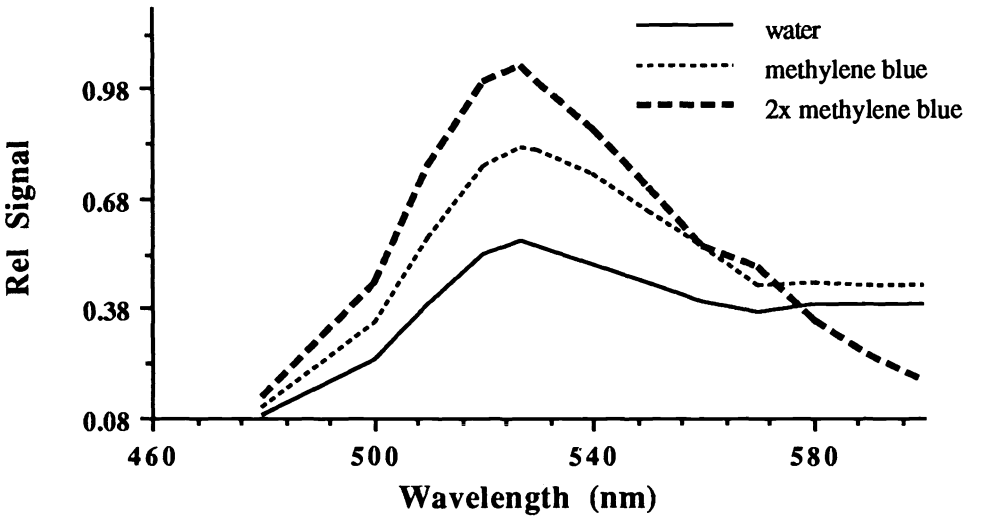

Figure 2. Chemiluminescence spectrum of BPE/polystryene film.

The fiber sensor was fabricated and measurements were conducted in 2.2 x 10^{-6} M MB. The oxygen content of this 2-ml sample was varied by bubbling with either O_2 or N_2 for at least 15 minutes. Air-saturated samples were estimated to be approximately 20% at 22 C (2.7 x 10^{-4} M).[7] Sensor response (Figure 3) is clearly oxygen-dependent, however the dynamic range is poor. We also observed memory effects due to adosorbed/entrapped sensitizer in the polystyrene matrix. Response time for a "fresh" sensor was approximately 40 seconds.and we routinely followed our signals for as long as 2 minutes without observing depletion of BPE. However, due to the irreversibility of this reaction and the fundamental limitation that $^1O_2$ must diffuse to and react with BPE, we investigated an alternate detection scheme based on optical upconversion.(OU).

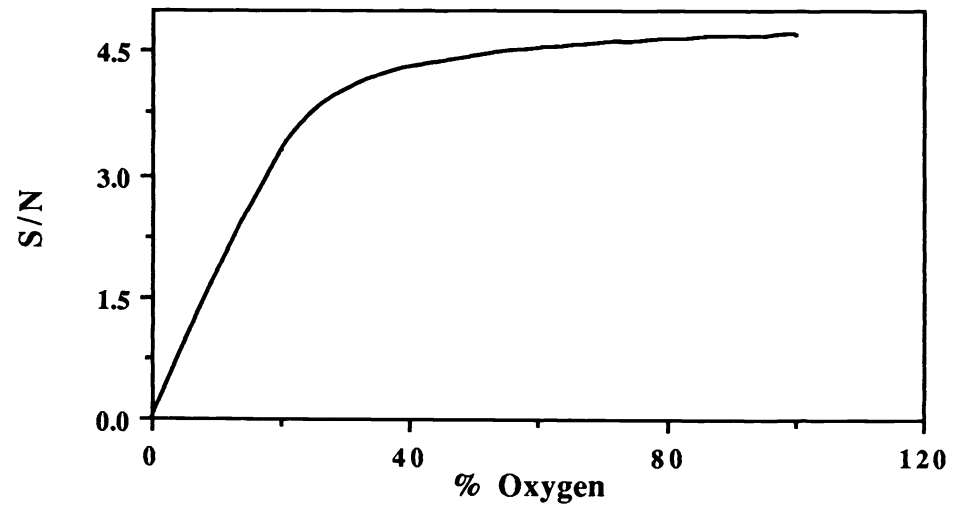

Figure 3. Fiber sensor oxygen response.

3.2 Optical Upconversion

Using infrared phosphors, the near-infrared (NIR) $^1O_2$ emission can be optically-upconverted, thus permitting the use of conventional detectors. The principals of upconversion have been reviewed.[8] The general scheme is described below:

1. photosensitizer + $h\nu_1$ (630-675 nm) $\rightarrow$ $^1O_2$
2. $^1O_2 \rightarrow O_2 + h\nu_2$ (1.27 μm)
3. $h\nu_2$ (1.27 μm) + phosphor* $\rightarrow$ phosphor + $h\nu_3$ (visible)

The spectral response of the ESPI C97C/polystyrene film is illustrated in Figure 4. In order to create sufficient phosphor*, the film was charged by exposure to 350 nm light for 3-5 s, then placed in 4 x 10^{-6} M MB. The prominent feature is a broad emission band centered at about 520 nm.

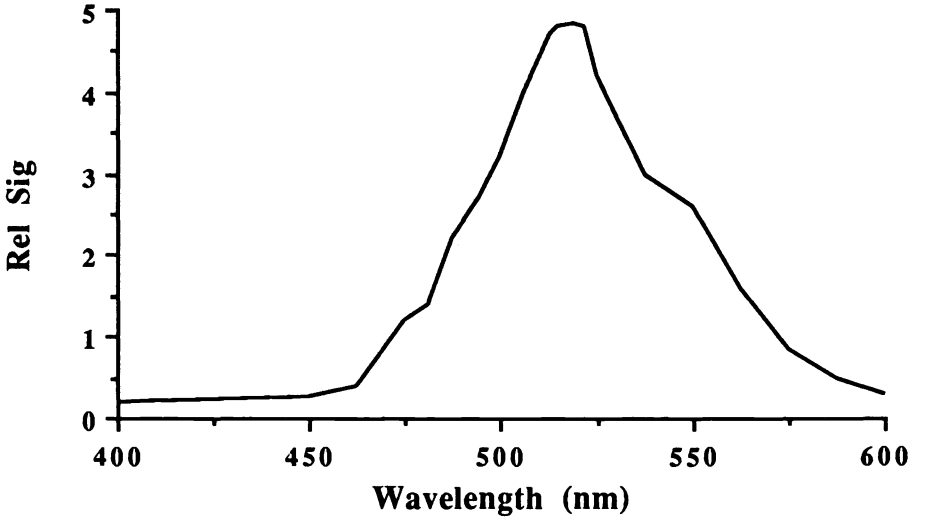

Figure 4. Optical upconversion spectrum.

The peak OU intensity is determined, in part, by the concentration of photosensitizer. This relationship is demonstrated in Figure 5. At high MB concentrations (greater than O.D.= 0.6), the upconversion signal is reduced, probably due to an inner filter effect (i.e. reabsorption of $h\nu_3$ by the sensitizer).

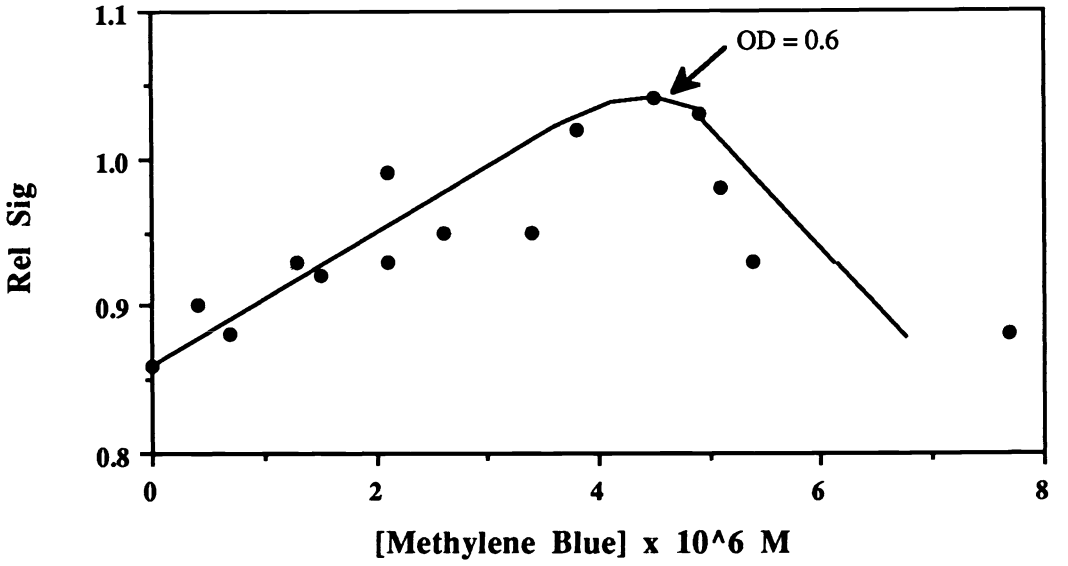

Figure 5. MB concentration.dependence.

When CASPc was used to generate singlet oxygen (Figure 6), dynamic range improved due to reduced photosensitizer absorption in the region of $h\nu_3$.

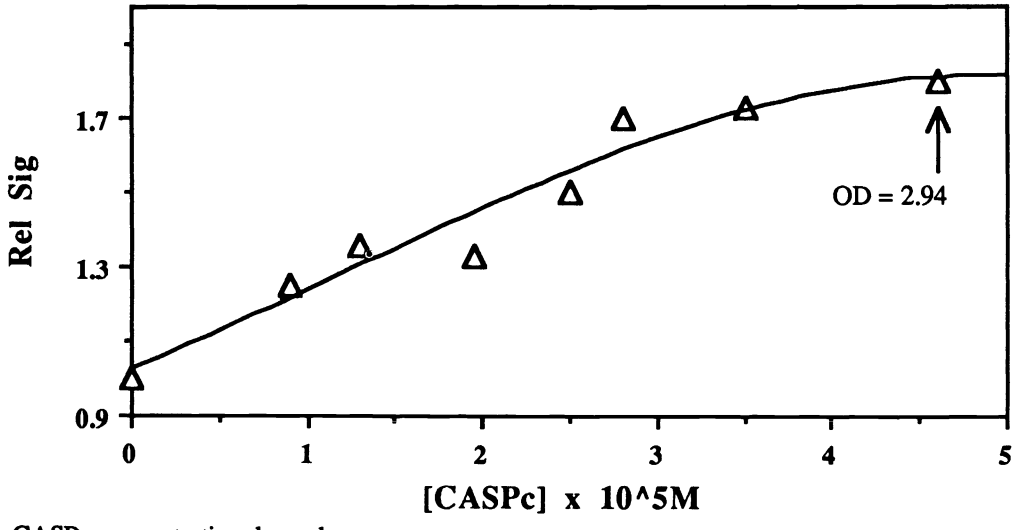

Figure 6. CASPc concentration dependence.

The OU process depletes phosphor* during the course of $h\nu_3$ emission. This population can be restored simply by "recharging" the phosphor with high-energy (blue/UV) light. Since the phosphor cannot practically be recharged during upconversion, we investigated the working lifetime of the film. The film was immersed in a 4 μM sample of MB and continuously irradiated (at 664 nm) by the arc lamp. The OU signal, recorded at 525 nm, indicates that the ESPI C97C phosphor/polystrene film has a half-life of approximately 5 minutes. These results, displayed in Figure 7, indicate that short measurement times are acceptable, however the phosphor should be recharged when used for relatively long periods.

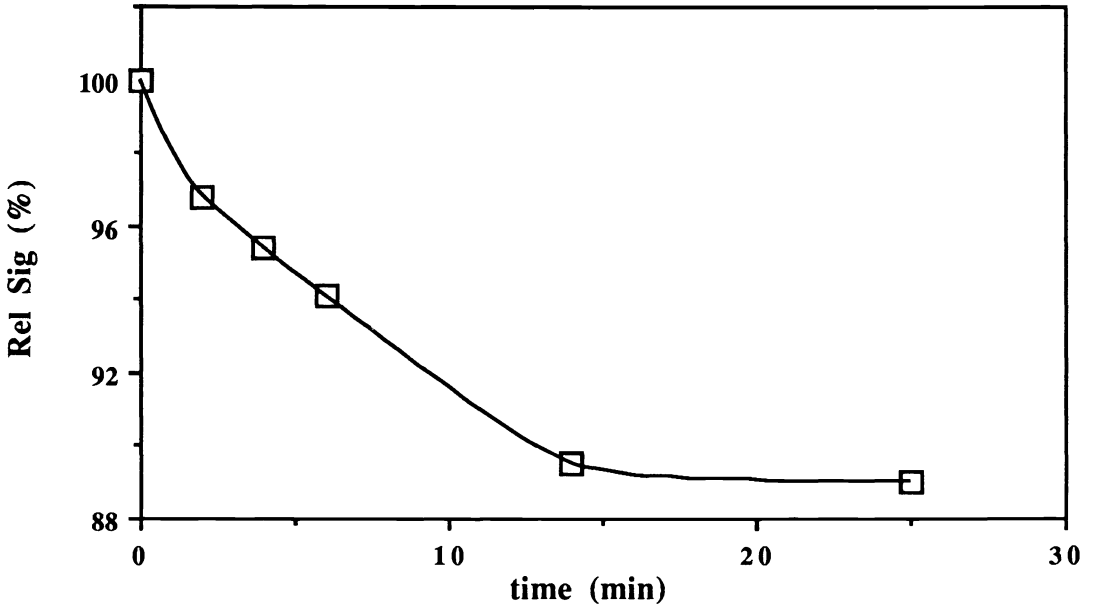

Figure 7. Film discharge rate.

In order to further demonstrate OU performance, we examined the influence of varying the concentration of a singlet oxygen precursor (O_2) and a singlet oxygen quencher (Furfuryl alcohol). These results are illustrated in Figures 8 and 9, respectively. The upconversion signal increases until the sensitizer solution is roughly 30% oxygen. Above these levels, oxygen sensitivity is greatly reduced. When furfuryl alcohol (FURF) is added to a film immersed in 25 μM CASPc, FURF competes for the short-lived $^1O_2$ and the OU signal is diminished. These oxygen- and FURF-dependent responses further underscore the sensitivity of the phosphor film to the $^1O_2$-generating capability of the photosensitizer.

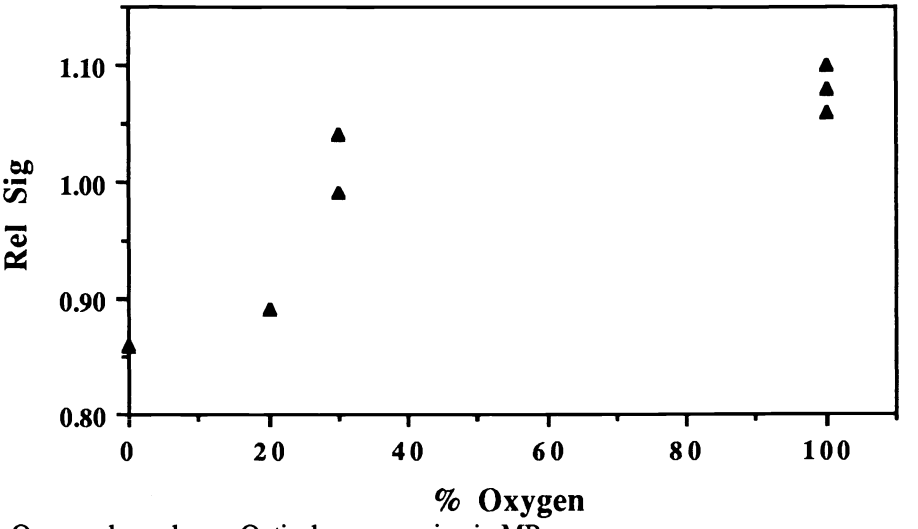

Figure 8. Oxygen dependence: Optical upconversion in MB.

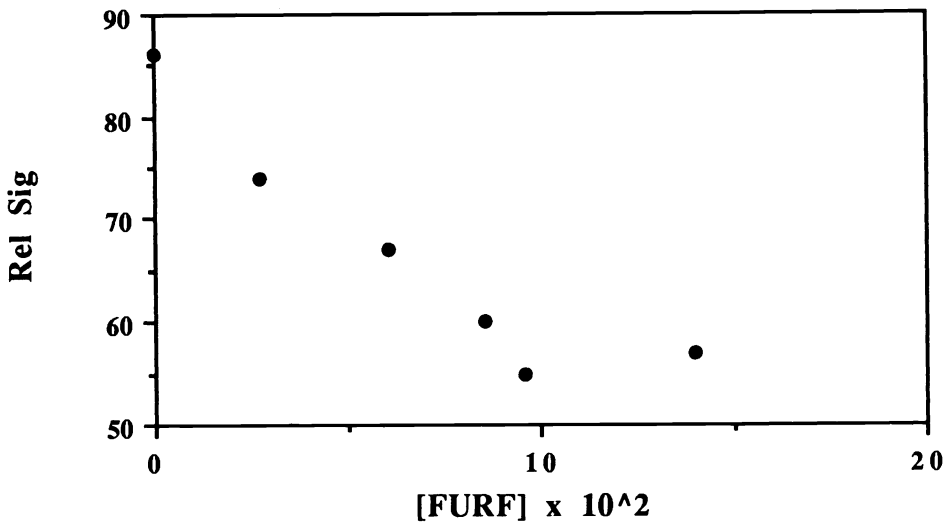

Figure 9. Furfuryl alcohol dependence: Optical upconversion in CASPc.

In fact, these measurements can be used to evaluate singlet oxygen-generating quantum efficiency, $\Phi(^1O_2)$, for different photosensitizers, provided a standard is used in the determination. The upconversion film was placed in MB and CASPc solutions of equivalent optical densities (at 672 nm). A 75 mW beam was used to stimulate $^1O_2$ production and the MB-generated signal was twice that produced by CASPc (after correction for a small water background due to phosphor* absorption of incident light). Since $\Phi(^1O_2)$ for CASPc has been independently determined to be 0.085-0.15.[9] , $\Phi(^1O_2)$MB is calculated to range range from 0.17 - 0.30.

4. CONCLUSIONS

In summary, we have explored the possibility that CL and OU can be used to monitor singlet oxygen production during PDT. Optical upconversion seems to be particularly promising since, in contrast to CL, diffusion of a short-lived species ($^1O_2$) through a polystyrene matrix is not required. In addition, OU films are rechargeable and can be used repeatedly. Both techniques are relatively simple and benefit from tremendous blue-shifting of the therapeutic light to a low-background region of the spectrum. Of course, these methods suffer from the practical limitation that, *in vivo*, much of the available $^1O_2$ participates in quenching/oxidation reactions and may not readily contribute to CL or OU signals. However, indirect sensors may be of practical value when a complete oxygen picture is required (i.e. both O_2 and $^1O_2$ are monitored) and, perhaps during the *in vitro* assessment of $^1O_2$ quantum efficiency

5. ACKNOWLEDGEMENTS

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6. REFERENCES

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