Electronic Supplementary Material (ESI) for Analyst.

Supplementary Information

Intracellular Detection of Singlet Oxygen Using Fluorescent Nanosensors

Peuli Nath [a], Sameer Sayel Hamadna [a], Leshern Karamchand [b], John Foster [c], Raoul Kopelman [b], Jacques G. Amar [a] and Aniruddha Ray*[a]

[a] *Department of Physics and Astronomy, University of Toledo, Toledo, Ohio, USA.*

[b] *Department of Chemistry, University of Michigan, Ann Arbor, MI, USA.*

[c] *Department of Nuclear Engineering, University of Michigan, Ann Arbor, MI, USA.*

Description	Size (nm)
20 mg PLGA_3µLSOSG_SDS_Sonication_15min_EtAC	135.3
50 mg PLGA_3µLSOSG_SDS_Sonication_1 hr_EtAC	118.1
30 mg PLGA_3µLSOSG_SDS_Sonication_15min_DCM	155.2
20 mg PLGA(75:25)_3µLSOSG_Tween20_Sonication_1 hr_EtAC	296.5

Table S1. Table showing size of PLGA_SOSG nanoparticle with different PLGA concentration, emulsifier and sonication time.

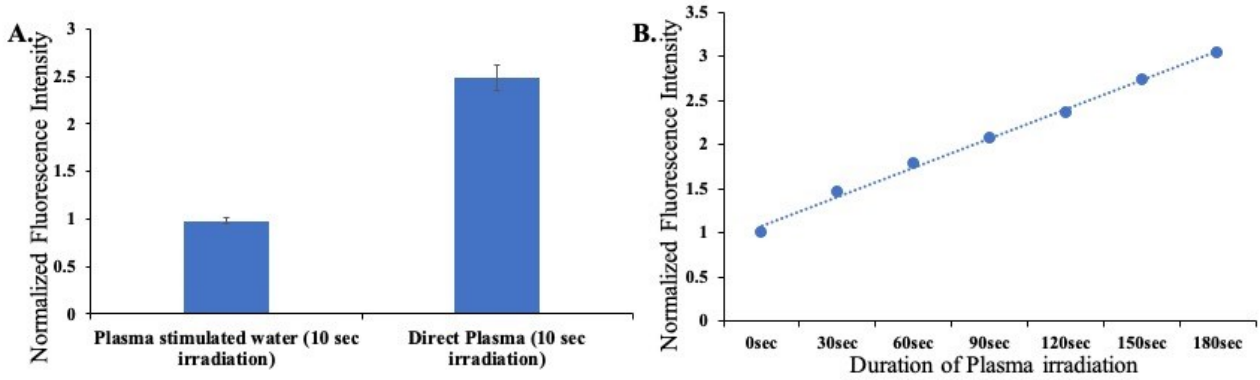

Figure S1. (A) Fluorescence intensity of SOSG molecule in presence of long lived reactive oxygen species after plasma irradiation at 0 and 10 sec. For this experiment, 200 µL of water was irradiated with plasma source for 10 sec to generate ROS in the system. Then, immediately 200 µL of 20 µM of SOSG dye solution was added and mixed properly. In another experiment, water containing same concentration of SOSG dye was irradiated for 10 sec. The samples were taken in a capillary and measured using FITC channel (ex. 495 nm and em. 530 nm; green) at same exposure and gain. The data is normalized to time "0", pre-exposure to plasma. (B) Fluorescence intensity of SOSG molecule (10 µM) at 525 nm in presence of Rose Bengal (5 µM) upon light exposure at 560 nm for different duration of time. For this experiment, the SOSG dye solution and Rose Bengal solution was mixed properly and taken in a capillary. The sample was irradiated using green light (560 nm) for singlet oxygen generation for 0, 30, 60, 90, 120, 150, 180 sec and fluorescence intensity of the SOSG dye was measured using FITC channel (ex. 480 nm; em.525 nm). The increasing fluorescence intensity indicate the reaction between SOSG and singlet oxygen produced by Rose Bengal. The data is normalized to time 0 sec.

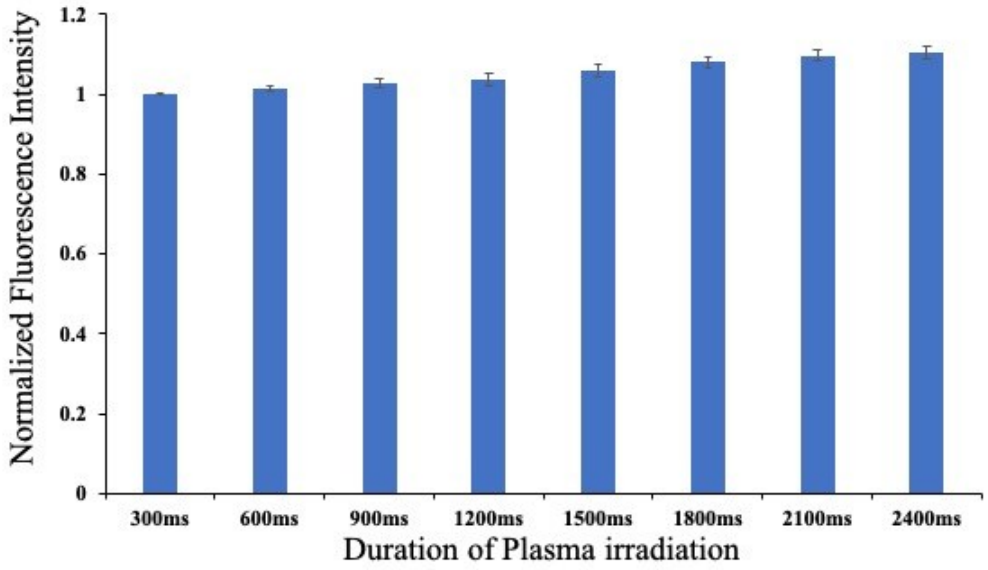

Figure S2. Bar graph showing fluorescence intensity of SOSG dye molecule resulting from the light excitation for the time duration used for microscopic imaging. Here, we irradiated the SOSG dye solution (10 µM) for 300 ms at a time, because the typical exposure time of the laser, for a single image, is <300 ms. The fluorescence signal is normalized with the first data point. Even after 8 image acquisition we do not observe a significant change in fluorescence. For our experimental studies we typically used single acquisition and the miniscule amount of singlet oxygen that may have been produced did not affect our measurements with plasma.

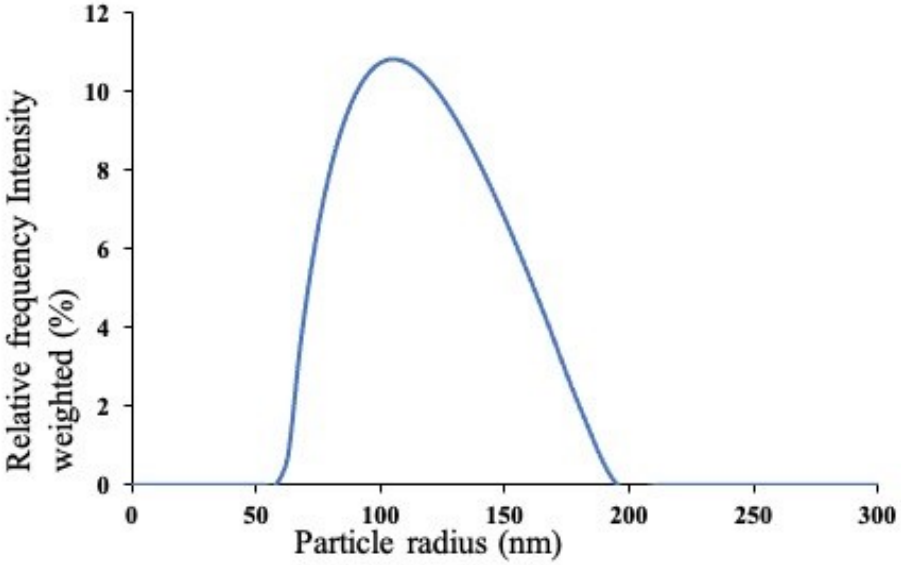

Figure S3. Size intensity of PLGA_SOSG nanosensor (~120 nm) synthesized using ethyl acetate (EtAc) with 1 hr sonication time as measured by dynamic light scattering technique.

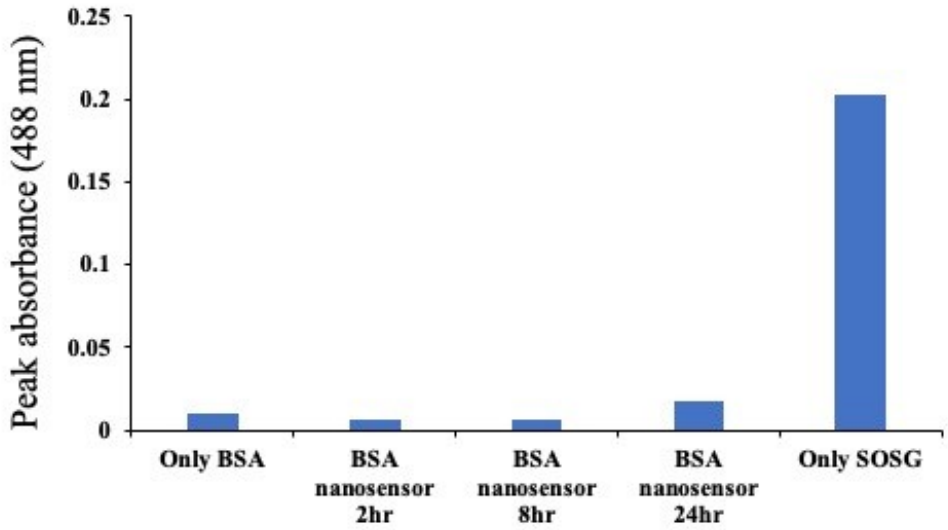

Figure S4. Bar graph showing absorbance intensity at 488 nm for BSA (~35mg/mL), supernatant collected from nanosensor stirred in BSA solution for 2hr, 8hr, 24 hrs respectively and SOSG (10 µM). For dye leaching study, 5 mg/mL PLGA_SOSG nanosensor was stirred in BSA solution (~35mg/mL). 1 mL SOSG nanoparticle in BSA solution was collected after 2 hrs, 8 hrs and 24 hrs stirring and centrifuged. The supernatant was collected and its absorbance at 488nm was monitored using an UV-Vis spectrophotometer (Shimadzu UV-2450).

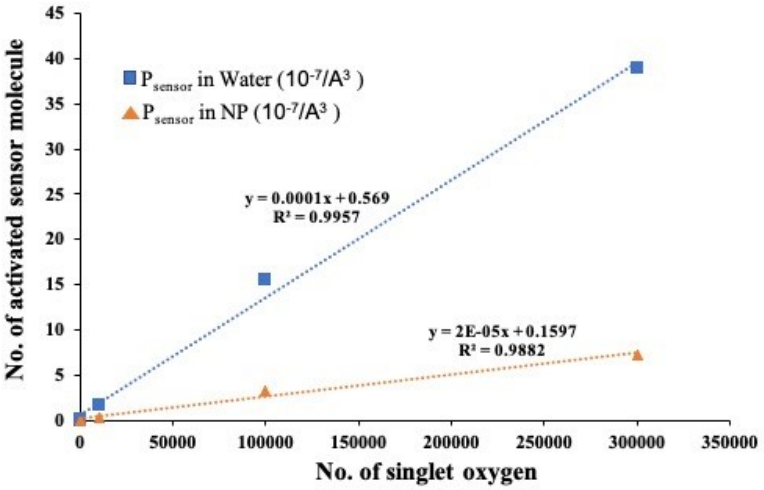

Figure S5. Plot showing the linearly fitted curve for SOSG activation in aqueous solution and inside the nanosensors. The slope of the curve for SOSG activation in water is ~5 time higher than SOSG in nanosensors. SOSG concentration (P_{sensor}= $10^{-7}/A^3$).

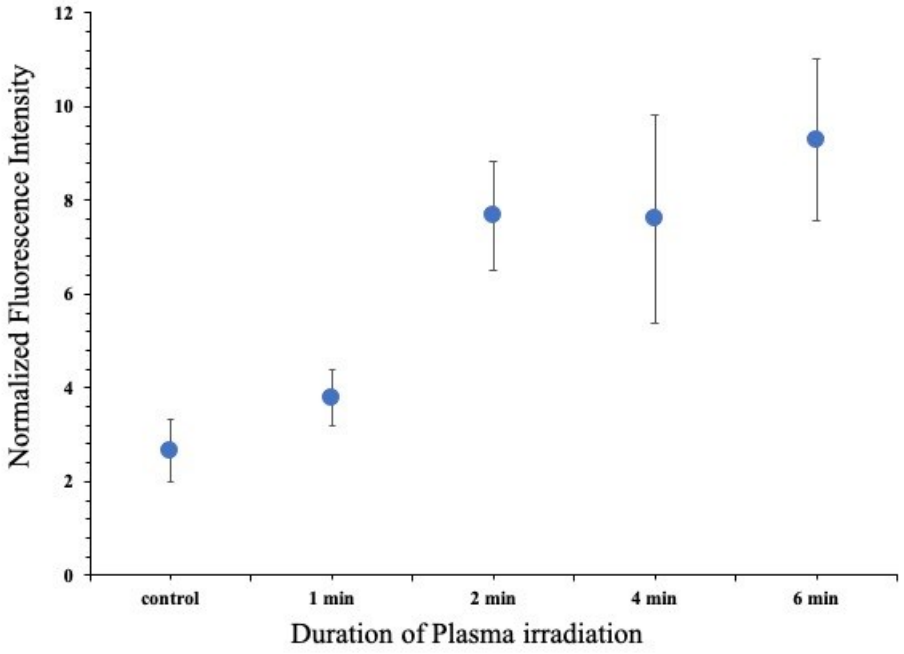

Figure S6. Plot showing oxidative stress build up in cells upon plasma irradiation at different time frame. The experiment was performed using fluorescence DCF (Dicholorofluorescein) probe which showed increased fluorescence intensity proportional to the ROS production in cells.

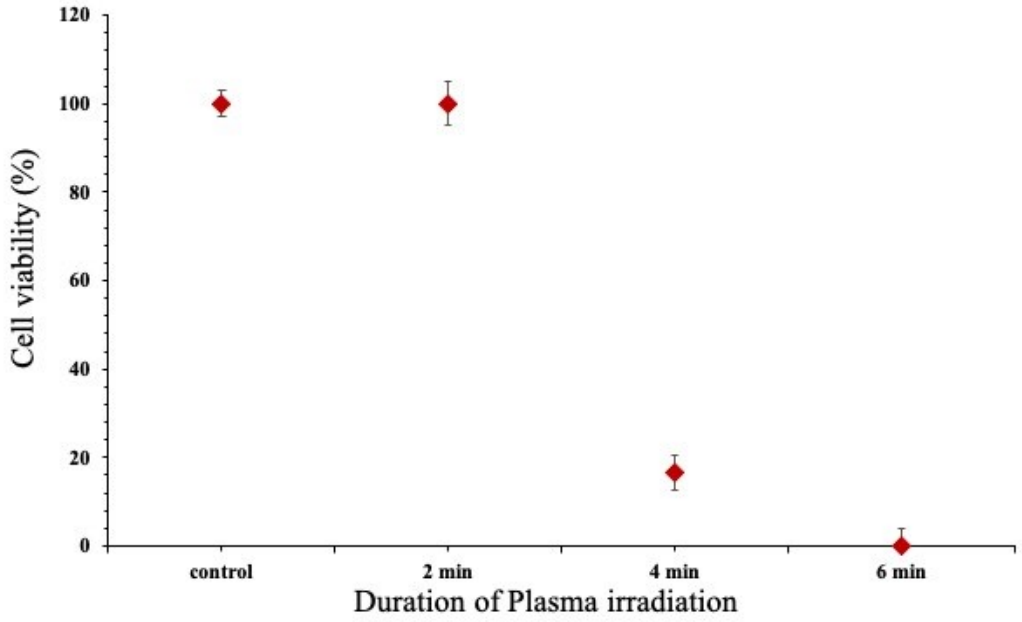

Figure S7. Plot showing cell viability percentage of 9L rat gliosarcoma cell line at different time of plasma irradiation. Cell viability study was performed using CCK-8 assay.

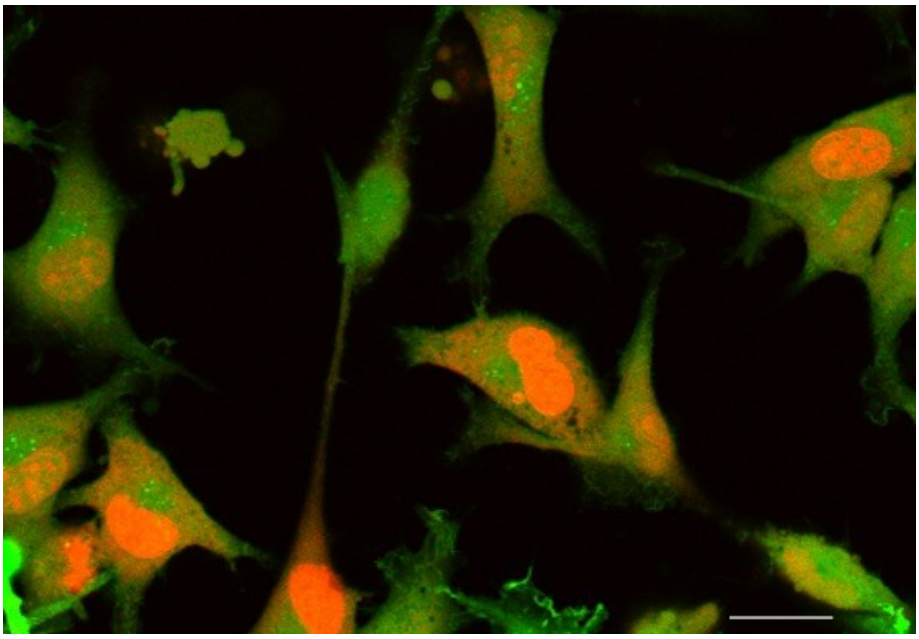

Figure S8. Confocal image of 9L cell after 6 min of plasma irradiation showing necrosis of cell as indicated by the propidium iodide (PI) staining of the nucleus of the necrotic cells (Red). Scale bar: 10 μm