Non-Invasive Real-Time Monitoring and Label-Free Sensing of Hydrogen Peroxide in Human Brain Cancer Cells Through Raman Spectroscopy

Darrell B. Tata* and Ilko K. Ilev

U.S. Food and Drug Administration, Center for Devices and Radiological Health, Office of Science and Engineering Laboratories, Silver Spring, MD 20993, USA

Abstract: We present evidence that real-time optical monitoring of human brain cancer cells through forward Raman spectroscopy can be used as a highly sensitive non-invasive label-free tool in acquiring real-time information on the cellular metabolic activity through the concentration levels of selective biomarkers. In this investigation, we quantified the concentration level of a natural by-product of cellular respiration, hydrogen peroxide (H_2O_2), through its characteristic Raman signature due to the transitions of O-O vibration energy level, resulting in a sharp Raman scatter frequency shift around 880cm^{-1}. The experimental findings revealed that 3.75×10^6 cells to have produced a substantial amount (3%) of H_2O_2.

Keywords: Raman spectroscopy, human glioblastoma, hydrogen peroxide, biomarkers, cellular metabolic activity.

INTRODUCTION

Real-time, non-invasive monitoring of the level of selective biochemical species (biomarkers) can aid detection of abnormalities in biological cells and tissues. Changes in the cellular physiological state are typically correlated with changes in the cellular metabolic activities within mitochondria. Reactive oxygen species (ROS) such as hydrogen peroxide (H_2O_2) are a natural by-product of cellular metabolic activity [1], and could potentially represent as an effective biochemical marker for the pathological state of cells and tissues. For example, large H_2O_2 levels have been reported from human cancer cells [2], and dysfunction in the mitochondria electron transport chain (resulting in large ROS generation) have been correlated to a range of serious human disease [3,4].

Furthermore, the real-time label-free detecting and monitoring of intracellular ROS generation is of keen interest also from the perspective of light-tissue interaction mechanisms and evaluation of safety and efficacy of recently developed optical therapeutic technologies and medical devices. A recently identified key mechanism of light-tissue interaction includes the functional role of intracellular light-induced ROS from mitochondria in cells through cytochrome c oxidase and the generation of superoxide anion O_2^{-} and its stable product H_2O_2 [5-7].

In this study, we present evidence that non-invasive real-time optical monitoring through Raman scattering can be used as an effective tool to acquire real-time information on the levels of selective native biomarkers. Raman spectroscopy is a technique which depends on inelastic scattering of photons from molecules. For the vast majority of monochromatic scattered photons, the incident and scattered photons have identical energy as with the incident photons (elastic scatting). However, there exists a very small fraction of scattered photons, with different energy than the incident photons. Therefore, in the case for an inelastic photon scattering with a molecule, the incident photon can either give up some of its energy or gain some energy from the molecule, thus resulting in a shift in the wavelength of the scattered photon, known as the Raman Shift. Each molecular species has a unique set of Raman signature. Consequently, we can identify biochemical species through its Raman spectrum.

The advantage of using Raman spectroscopy is that the Raman signature for biochemical species are label-free and independent of the probe beam wavelength. Thus, if the biological sample has a large absorption or fluorescence signal within a wavelength range, one has the flexibility to select the Raman probe wavelength far removed from this optical active wavelength region. In this first proof-of-concept study of real-time and label-free sensing of ROS in biological cells, we aimed to detect and quantify the constitutive levels of hydrogen peroxide from human glioblastoma cells *in-vitro* through Raman spectroscopy. We take advantage of the extra oxygen atom in H_2O_2, which results in a sharp characteristic Stoke shifted Raman line around

*Address corresponding to this author at the U.S. Food and Drug Administration, Center for Devices and Radiological Health, Office of Science and Engineering Laboratories, Silver Spring, MD 20993, USA;
E-mail: Darayash.Tata@fda.hhs.gov

ISSN: 1927-7210 / E-ISSN: 1927-7229/12 © 2012 Lifescience Global

880 cm^{-1} due to the oxygen – oxygen single vibration bond oscillations [8].

MATERIALS AND METHODS

Raman Scattering Detection System

The *in-vitro* sample was laser illuminated with intensity stabilized (0.3% stability) 532-nm-wavelength laser operating in a continuous-wave mode, delivering a 65-mW output optical power. The samples were illuminated in a standard 10×10×40 mm quartz cuvette within a Photon Technology International double-monochromator scanning spectrofluorometer system operating in a digital single-photon counting mode through the Hamamatsu (R928PMT) photomultiplier tube. The Raman signal was collected in the forward scatter direction and the double-monochromator and PMT were additionally protected from the excitation laser wavelength with a 532-nm notch filter (17-nm wavelength bandwidth and peak optical density in blocking region >6.0) which was placed in front of the scanning monochromator.

Cell Line

Human glioblastoma (brain cancer) cell line (CRL-2366) was purchased from the American Type Culture Collection (Manassas, VA) and grown and maintained in T-75 flasks under incubation conditions of 5% CO_2 at 37°C. The cells were taken from a 33 year old male with untreated malignant glioblastoma. The cells were maintained in ATCC formulated DMEM/F12 growth medium with 10% of fetal bovine serum and 50 Units/ml of penicillin and streptomycin antibiotics.

Cell Preparation

When the adherent glioblastoma cells reached 90 – 95% confluence within the T-75 flasks, the cells were trypsinized and brought into suspension. The cells were centrifuged and the (trypsin) supernate was discarded. The cells were re-suspended in fresh Dulbecco's Phosphate Buffer Solution (PBS) with calcium and magnesium, and centrifuged and washed three additional times. This ensured that the entire residual growth medium and the trypsin had been diluted out and removed. The final working cell

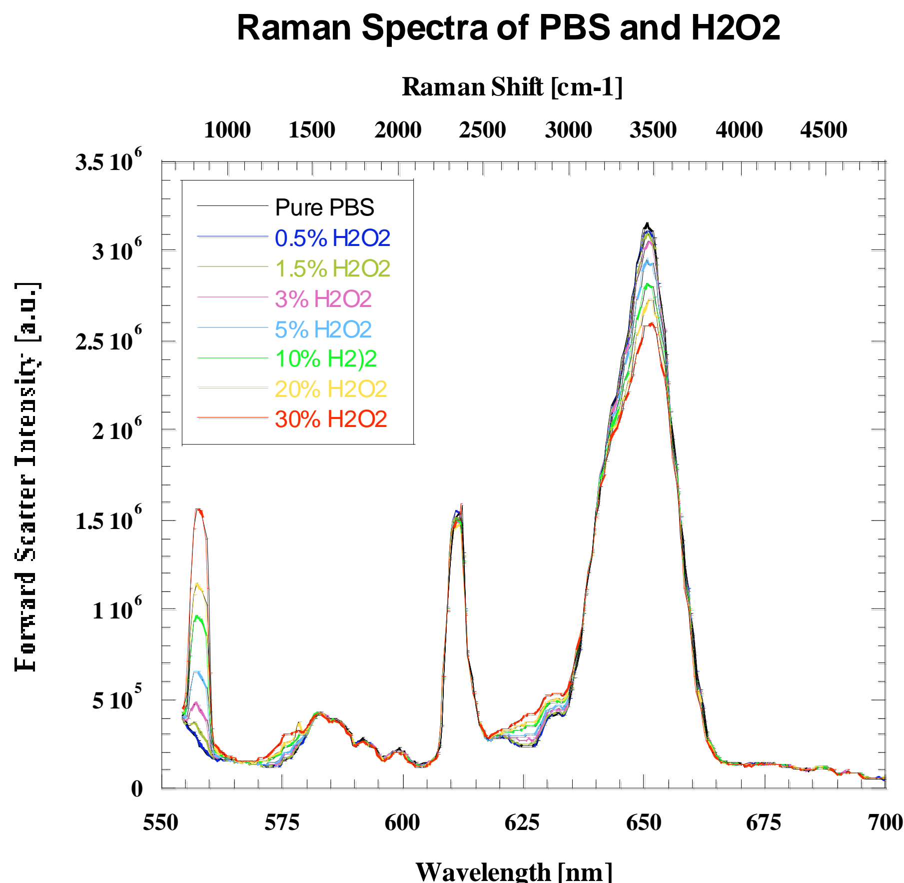

Figure 1: Raman spectra of pure phosphate buffer solution (PBS) and various concentrations of hydrogen peroxide (H_2O_2) in PBS, plotted as a wavelength in [nm] as well as a Raman shift in [cm^{-1}] relative to the excitation laser wavelength of 532nm.

suspension concentration was 1.25x10^6 cells/ml within a 3ml volume. The cell suspension was then directly transferred into a standard quartz cuvette for Raman spectroscopic analyses at room temperature.

RESULTS AND DISCUSSIONS

To experimentally evaluate the sensitivity of the suggested Raman spectroscopic-based approach for noninvasive label-free detection and monitoring of hydrogen peroxide, we measured Raman spectra calibration curves for hydrogen peroxide with various concentrations in PBS. Figure **1** illustrates typical Raman forward scatter spectrum of PBS alone and also spectra with several concentrations of hydrogen peroxide in PBS. We observed a number of specific Raman peaks associated with H_2O_2 in PBS. These peaks are centered at the following wavelengths (or Raman shift in wave numbers): 558 nm (880 cm^{-1}), 580 nm (1,555 cm^{-1}). While some of these H_2O_2 and H_2O related peaks completely overlap PBS alone, including 610 nm (2,400 cm^{-1}) and 652 nm (3460 cm^{-1}), the Raman peak centered at 558 nm (880 cm^{-1}) is a specific signature peak for H_2O_2 in PBS with no comparable Raman peak in the pure PBS solution. Figure **2** shows the concentration calibration curve for H_2O_2 at the selected Raman scatter maxima (at 558nm). We found that under the applied experimental exposure and collection conditions, the resolving power is approximately 1% H_2O_2.

Employing the reference calibration data presented above and the H_2O_2 signature Raman scatter peak found at 558nm, we tested the system's potential and sensitivity for intracellular H_2O_2 detection. Figure **3** exhibits the Raman spectrum of human glioblastoma cells at concentration of 1.25×10^6 cells/ml in PBS suspension. The change in the forward scatter intensity (ΔFSC) was evaluated at the characteristic Raman Stoke shift of 880 cm^{-1} for H_2O_2 (at 558nm) and the change in the forward scatter intensity was compared to the calibration curve of Figure **2**. The experimental findings revealed 3.75×10^6 cells to have produced a substantial amount (3%) of H_2O_2.

CONCLUSION

Based on highly sensitive label-free Raman scattering signature, we have demonstrated a proof-of-concept approach for detecting and monitoring cellular reactive oxygen species such as hydrogen peroxide in brain cancer cells. This technique can provide real-time measurements of ROS concentrations and my yield disease-specific biomarkers such as highly over expressed constitutive proteins in aggressive human cancers. The resolving power of this strategy is

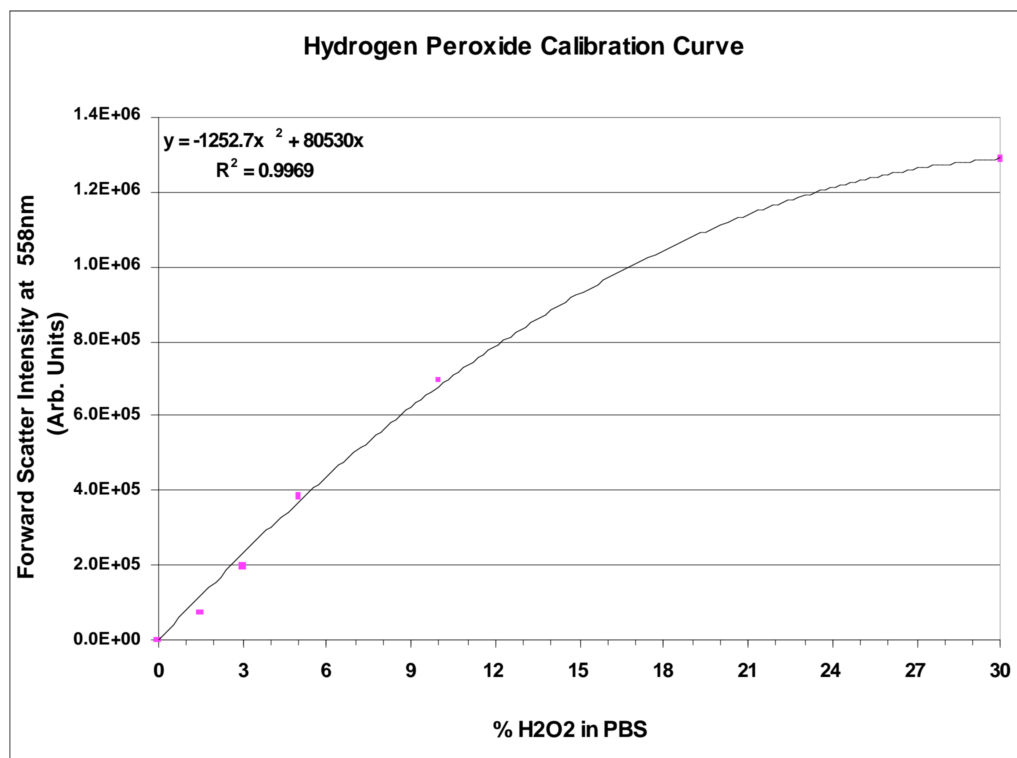

Figure 2: Calibration curve for H_2O_2 at the signature Raman shift at 558nm (880 cm^{-1}).

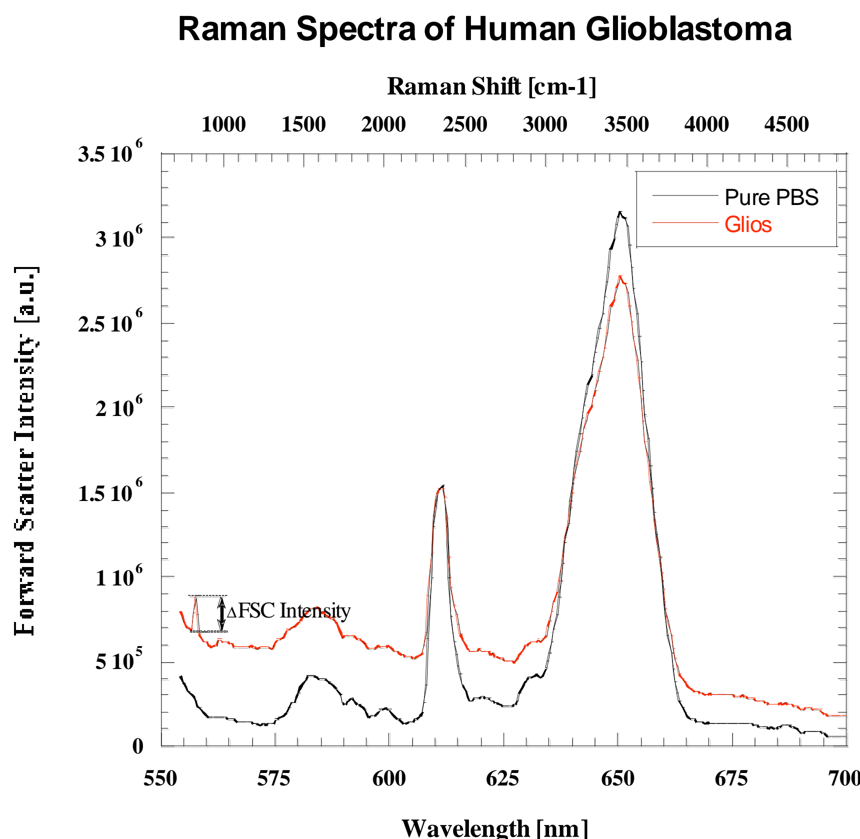

Figure 3: Raman spectrum of human glioblastoma cell suspension in PBS. Cell concentration = 1.25×10^6 cells/ml. The Raman spectrum of cell-free PBS is also shown.

dependent on two key parameters: (i) the intensity of the laser and (ii) the sensitivity of the detector. Great improvements in biochemical sensing through Raman spectroscopy can be made through ultra-short pulsed lasers which produce high intensity pulses within a short pulse duration.

DISCLAIMER

The mention of commercial products, their sources, or their use in connection with material reported here is not to be construed as either an actual or implied endorsement of such products by the U.S. Food and Drug Administration (FDA).

REFERENCES

[1] Brookes PS, Yoon Y, Robotham JL, Anders MW, Sheu S. Calcium, ATP, and ROS: a mitochondrial love-hate triangle. Am J Physiol Cell Physiol 2004; 287: C819-C833. http://dx.doi.org/10.1152/ajpcell.00139.2004

[2] Szatrowski TP, Nathan CF. Production of Large Amounts of Hydrogen Peroxide by Human Tumor Cells. Cancer Res 1991; 51: 794-98.

[3] Heales S, Bolanos JP, Stewart VC, Brookes PS, Land JM, Clark JB. Nitric oxide, mitochondria and neurological disease. BBA 1999; 1410: 215-28. http://dx.doi.org/10.1016/S0005-2728(98)00168-6

[4] Bolanos JP, Moro MA, Lizasoain I, Almeida A. Mitochondria and reactive oxygen and nitrogen species in neurological disorders and stroke: Therapeutic implications. Adv Drug Deliv Rev 2009; 61: 1299-15. http://dx.doi.org/10.1016/j.addr.2009.05.009

[5] Tata D, Waynant R. Laser therapy: A review of its mechanism of action and potential medical applications. Laser Photonics Rev 2011; 5: 1-12. http://dx.doi.org/10.1002/lpor.200900032

[6] Karu T. Mitochondrial signaling in mammalian cells activated by red and near-IR radiation. Photochem Photobio 2008; 84: 1091-99. http://dx.doi.org/10.1111/j.1751-1097.2008.00394.x

[7] Lavi R, Shainberg A, Shneyvays V, *et al.* Detailed Analysis of Reactive Oxygen Species Induced by Visible Light in Various Cell Types. Lasers Surg Med 2010; 42: 473-80. http://dx.doi.org/10.1002/lsm.20919

[8] Taylor RC, Cross PC. Raman Spectra of Hydrogen Peroxide in Condensed Phases. The Spectra of the Pure Liquid and Its Aqueous Solutions. J Chem Phys 1956; 24: 41-44. http://dx.doi.org/10.1063/1.1700850

Received on 20-10-2012 Accepted on 11-11-2012 Published on 01-12-2012

http://dx.doi.org/10.6000/1927-7229.2012.01.02.2