Application of Confocal Raman spectroscopy to measure the gold nanofilm dose enhancement

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1. Introduction

In radiotherapy, tumor is controlled by killing cancer cell while sparing healthy cells [1]. In parallel to conventional radiation therapy methods, researchers are considering other approaches to target only cancer cells. One such method includes the use of High Z-material nanoparticles [1]. Gold nanoparticles have been extensively studied because of its bio-compatibility [1].

Low energy electrons have subcellular ranges and interact adversely with chemical bonds triggering radiation damage [2]. Such low energy electrons can be generated in a bulk locally in cancerous cells by inserting metal nanoparticles nearby and bombarding with low energy x-rays [1-4]. Due to photoelectric interaction, photoelectrons and auger electrons are released in high Z-material (i.e. gold nanoparticles), which penetrate into surrounding unhealthy cells (Low Z-material) and causing cell killing [4].

A number of published Monte Carlo simulations for dose enhancement by metal nanoparticles are available; however, not many studies for their experimental determination [4]. Moreover, simulated dose enhancement values are much higher than experimental values [4]. Our work is an effort to find a suitable method for the experimental measurement of dose enhancement. While assuming that gold nanofilm is the planar arrangement of closely packed gold nanoparticles, we used the confocal μ-Raman spectroscope along with radiochromic film to measure the dose enhancement by a gold-nanofilm at cellular level.

2. Methods and Material

2.1. Un-laminated Gafchromic™ EBT-XD film

Measurements were performed with Gafchromic film model EBT-XD by International Specialty Products (ISP, Wayne, NJ) [5]. It is comprised of an active layer, 25 μm thick; comprising of the active component with marker dye.25 The active layer is packed in between two 125 μm matte polyester substrates. In this film type, the chromophores are about 1-2 μm in diameter and 2-4 μm in length, respectively. Films were stored below 25 °C, handled carefully to avoid unwanted exposure to radiation and sunlight and hands were gloved to avoid finger prints distorting measurements.

The film sheets were cut into 3 × 3 cm^2 pieces and each piece was marked with an arrow on the right corner to indicate orientation. To make the film un-laminated one, one of the two matte polyester layers was ripped off for direct contact of gold nanofilm and active layer of radiochromic film.

2.2. Gold nanofilms

Gold nanofilms of thicknesses 20, 30, 40, 50 and 100 nm on PET substrate (100 μm) were produced by applying thermal evaporation technique. In order to measure the film thickness, surface profiler alpha step was used.

2.3. Calibration curve

To obtain a calibration curve, 2 × 2 cm^2 PET substrates were attached directly to the active layers of 3 × 3 cm^2 pieces of EBT-XD films. The films were than irradiated for the 50 kVp beam by 320 kV biological x-ray irradiator (Precision X-Ray Inc, North Brantford, CT) with 2 mm Al filter. The X-RAD 320 biological irradiator was calibrated using an in-air technique as described in the AAPM TG61 report.

The films were placed at source to surface distance (SSD) of 50 cm on solid water phantom with a field size of 10 × 10 cm^2. For calibration films were exposed to 0.0, 0.3, 0.5, 0.7, 1.0, 1.5, 2.0, 2.5, and 3.0 Gy

2.4. Sample irradiation

Experimental setup is same as described in section 2.2 for film calibration. The samples were prepared by directly attaching the gold nanofilm layers of different thicknesses with the active layer of EBXD film. For each gold thickness, the films were irradiated to dose levels of 0.3, 0.5, and 0.7 Gy.

2.5. Raman spectroscopy

Raman spectroscopy is based on inelastic scattering of a monochromatic excitation source by molecules. It is a weak process as nearly 1 in 10^6 to 10^8 photons are scattered inelastically [6]. Raman spectrum is a plot of intensity of scattered photons versus energy difference and can be used to identify molecules, as each molecule provides a unique "molecular fingerprint" [6].

On the basis of previous knowledge of Raman spectrum of diacetylene polymer, Callens et al 2016 allocated bands in the Raman spectrum of EBT3 film [7]. In this work, we considered the variation in Raman spectra of C≡C stretching bands of diacetylene polymer at 2060 cm^{-1}, as a function of therapeutic X-ray dosage with spatial resolution of 10 μm.

Raman spectra of each film piece were measured by a Ramboss-star microscope Raman system (Dongwoo Optron Co. Ltd, Korea) with the Raman shift range from 50 cm^{-1} to 4200 cm^{-1}. The Raman spectroscope was calibrated with a silicon wafer at 520 cm^{-1} prior to measurements. Raman spectra of each film were recorded at minimum of 24 hrs post-irradiation. Data acquisition and processing were done using Andor SOLIS 4.16.30002.0 and Ramboss mapping 1.02, respectively.

2.6. Data acquisition and analysis

Raman spectra at different points on Gafchromic films were acquired by placing the films on the Marzhauser motorized XY mapping stage with electrically powered Z-axis focus (smallest step size of stage is 0.05 µm). Raman mapping technique developed earlier (Mirza et al 2016) was used to map a region of interest (ROI) of 10 × 10 μm^2 on each film with a sampling resolution of 1 µm [8]. Through this, Raman spectrums of 121 pixel points per ROI were obtained. C≡C peak of each film were preprocessed, which included band selection, smoothing and baseline removal. The sample generated dose dependent fluorescence, (i.e. background) was removed by creating a smooth curve as baseline, using data points from two ends of selected band comprising of C≡C peak, and then subtracting it from the original Raman spectrum of this selected band. Thereafter the subtracted peak was fitted with Lorentzian peak function. Finally, the band heights (i.e., Raman intensity values at the peak of Lorentzian distribution) for all dose levels were obtained to draw calibration curves. Microsoft Excel and Origin Pro 9 were used for this analysis.

2.7. Dose profile

Horizontal axis dose profile of 40 nm gold nanofilm exposed to 0.5 Gy was measured in a ROI of 900 µm along x-axis and 10 µm along y-axis on EBT-XD film with attached gold nanofilm. Each point in the profile was of size 10 × 10 μm^2. The ROI was selected such that some portion was PET only while reaming was consisted of gold nanofilm coated PET substrate.

2.8. Dose enhancement factor

Dose enhancement factor (DEF) is defined as

$$\frac{Dose\ with\ gold\ film}{Dose\ without\ gold\ film}$$

3. Results and discussion

3.1. Calibration curve

Mean band height values against various dose levels were used to draw a calibration curve for a type EBT-XD film as shown in Fig. 1. All the peaks were preprocessed by removing baselines and Lorentzian function fittings. The curve was then fitted with an exponential function (R^2 = 0.994).

3.2. Dose profile

Dose profile along horizontal axis for 40 nm thick gold nanofilm exposed to 0.5 Gy is shown in Fig. 2. The data was then fitted with sigmoidal function (red line in Fig. 2).

3.3. Measurement of Dose enhancement factors

Dose enhancement factors for various Gold thicknesses are summarized in Table I. For each thickness, the films were exposed to 0.3, 0.5, and 0.7 Gy. Dose enhancement factors for each dose levels were averaged to get the DEF of specific thickness. For 50 kVp beam, DEF increases with gold thickness as shown in Fig. 3. The plot was best fitted with an exponential function (R^2 = 0.980).

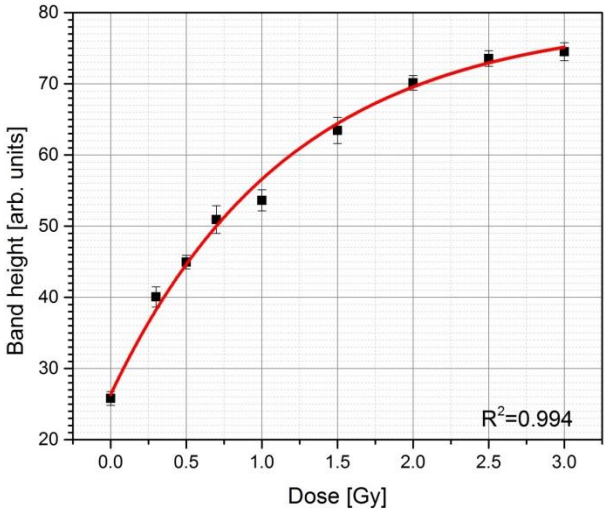

Figure 1: Calibration curve for Un-laminated EBT-XD film when irradiated with 50 kVp x-ray beam.

Table 1: Dose enhancement factors at gold nanofilm thicknesses of 20, 30, 40, 50 and 100 nm.

Au thickness [nm]	Dose (PET) [Gy]	Dose (Au) [Gy]	DEF	Avg. DEF (RSD/RPD [%])
20	0.30	0.57	1.90	1.95 ±2.35
	0.50	0.98	1.96	
	0.70	1.39	1.99	
30	0.30	0.74	2.48	2.23 ±15.42
	0.50	0.92	1.84	
	0.70	1.66	2.38	
40	0.30	0.76	2.53	2.39 ±4.84
	0.50	1.17	2.34	
	0.70	1.62	2.32	
50	0.30	0.80	2.67	2.47 ±11.98
	0.50	1.31	2.61	
	0.70	1.49	2.13	
100	0.30	1.00	3.35	3.12 ±13.73
	0.50	1.45	2.89	

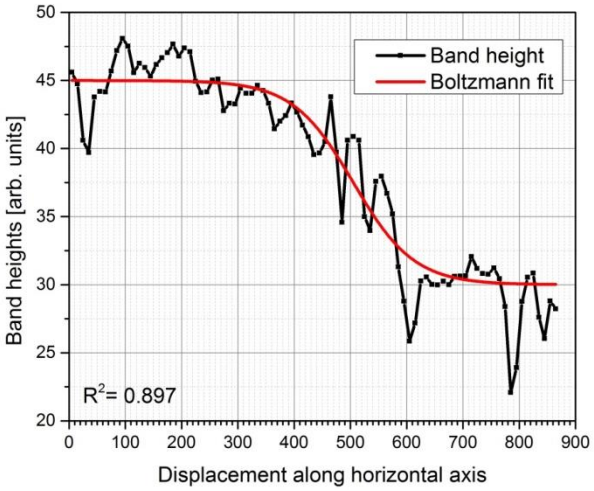

Figure 2: Dose profile along horizontal axis of EBT-XD film attached with 40 nm gold nanofilm during 0.5 Gy exposure. ROI is consisted of both PET only and PET substrate coated with gold nanofilm. The data points were then fitted with sigmoidal Boltzmann function with R^2 = 0.897.

To estimate the dose deposited by the gold nanofilm on EBT-XD film, same calibration curve is used i.e. curve for 50 kVp (see Fig. 1). From Fig. 1, it can be seen that response tends to saturate after 2.5 Gy and estimated dose value beyond it might be more ambiguous. Therefore, we did not include the estimated dose value of 0.7 Gy, for 100 nm gold film. Large variation in 30 nm film might be due to the non-uniformity created in the ROI of film exposed to 0.5 Gy, during the manual removal of matte polyester layer. In case of 50 nm and 100 nm film, estimated doses of 0.7 Gy and 0.5 Gy respectively were wrongly predicted. More experiments will be carried out in future for more accurate dose enhancement factor at microscopic level.

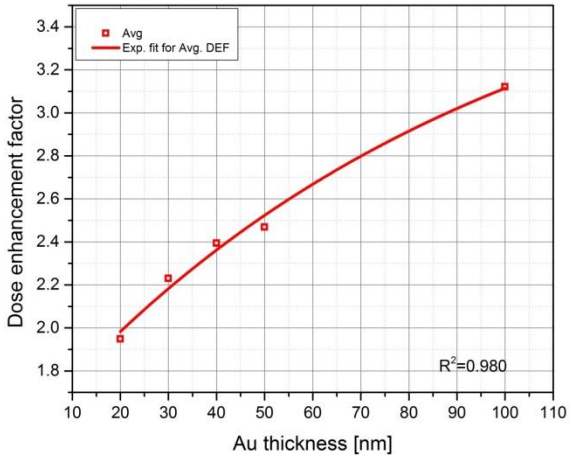

Figure 3: Gold nanofilm thickness vs dose enhancement factor.

4. Conclusions

The results of this work suggest that Raman mapping technique developed earlier can be used to determine the dose enhancement factor at cellular level (10 μm). Large standard deviations in DEF were observed for 30, 50 and 100 nm thick gold nanofilms. More experiments will be carried out in near future to find the root cause of these large errors.

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