Metallized Carbon Nanotubes for Radiation Therapy Enhancement

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Research

Keywords: Carbon nanotubes, radiation enhancement, copper filled nanotubes, dose enhancement, nanoparticles, CNT, MWCNT.

Posted Date: October 23rd, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-81440/v1>

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Abstract

Background: Carbon nanotubes have been the focus of many in-vitro and in-vivo studies where they serve as cargo delivery vehicles. In this preliminary work, multi-walled carbon nanotube were impregnated with copper, to study the effect of metallized carbon nanotubes on enhancing radiation dose in external beam radiotherapy.

Methods: Using a 6x6x6 cm³ cube, filled with water or with water plus partially filled or unfilled carbon nanotubes, the dose enhancement effects of megavoltage photon beams were determined. Ion chamber readings were collected at 1 cm below the water cube. The beams were delivered from a TrueBeam Linac, at a 100 cm source to surface distance and 20x20cm² field size. In addition, film measurements were taken just below the water cube (i.e. at 0 cm distance) and compared to the ion chamber dose enhancement at 1 cm below, for 6 MV energy beam.

Results: For all studied photon energies, the ion chamber readings showed a dose increase when copper filled carbon nanotubes were added to the water medium. With copper filled CNTs concentration in water of 5 x 10⁻² mg/ml, the measured dose enhancement ratios (DER) were 1.025, 1.016, 1.017, 1.011 and 1.013 for 2.5MV, 6MV, 6MV FFF, 10MV and 10MV FFF energies, respectively. Furthermore, when copper filled MWCNTs are added to water, the film measurements (at 0 cm distance from the water cube) have also shown a dose enhancement ratio of 1.022 in comparison to 1.012 for ion chamber (1 cm away from the water cube).

Conclusion: The results show an increase of the radiation dose enhancement, with a DER up to 1.025 at 1 cm below the studied medium, when metallized carbon nanotubes are added to water versus when the beam is delivered to just water. This increase in DER decreases with the increase of x-ray beam energy. It also decreases with increasing the distance of the point of measurement from the medium where the metallized carbon nanotubes were added.

Background

Several studies have focused on the use of metallic nanoparticles for enhancing the radiation dose where the target lesions are located, while sparing the surrounding healthy tissue^{1,2,3,4}. Gold is the most-studied nanoparticle for dose enhancement, while very little has been published on the use of metallized carbon nanotubes as radiation dose enhancers in external beam radiotherapy. Carbon nanotubes (CNTs) are a carbon allotrope with unique physical and chemical properties. Due to their needle-like shape, they have superior cell permeability compared to other types of nanoparticles and a large aspect ratio, allowing a large surface area for functionalization and modification^{5,6,7}. They can also be shortened and functionalized to decrease toxicity and enable chemical specificity towards targeted sites. In addition, both the hollow interior and the outside of the CNTs can be utilized to carry treatment and targeting agents simultaneously. Because of these considerations, carbon nanotubes have been the focus of many in-vitro and in-vivo studies where they serve as cargo delivery vehicles. In this preliminary work, the

radiation enhancer of choice is copper, encapsulated in the multi-walled carbon nanotube (MWCNTs). This choice was made due to the cost effectiveness of copper salt such as Copper (II) Nitrate Trihydrate compared to gold analogs. Copper nanoparticles, however, have shown liver toxicity in rats due to oxidation⁹. Encapsulating the copper within carbon nanotubes walls can preserve it from oxidation¹⁰. In addition to the effect of metalized carbon nanotubes, the effect of pure MWCNTs was also studied

Methods

MWCNTs with > 98% purity were obtained from Sigma-Aldrich (Sigma-Aldrich, Saint Louis, MO). The MWCNTs possessed outer diameters of 6–13 nm and lengths of 2–20 μm .

The MWCNTs were opened and filled with copper in a two-step process⁸. 500 mg of the nanotubes were first refluxed in nitric acid (Fig. 1-a). When refluxing, the MWCNTs and the acid were combined in a flask on a hot plate with a spinning magnetic stirring rod. As the acid boils, the vapor rises and condenses in the refluxing tube, preventing it from boiling away. At the same time, the MWCNTs are being stirred in the hot acid. The heat, along with the mechanical force, breaks the carbon-carbon double bonds at the ends of the tube and simultaneously functionalizes the walls with an OH group (Fig. 1-b). After 24 hours of refluxing, the MWCNTs are washed with deionized water until neutral (PH = 7), then the tubes are dried in an oven at 60–100 °C for 24 hours. After the opened MWCNTs are dried, around 100 mg are sonicated in a copper nitrate solution, with molar concentration of 0.868 mol/L. Capillary forces draw the salt solution into the cavity of the MWCNTs (Fig. 1-c). The MWCNTs are then washed with DI water and left to dry in air.

To examine the outcome of the partial filling process, scanning electron microscope (SEM) and transmission electron microscope (TEM) images were acquired along with energy dispersive x-ray spectroscopy (EDS) and x-ray photoelectron spectroscopy (XPS) data. SEM gives morphological information of the samples, EDS and XPS give chemical information and elemental composition, TEM verifies that the copper nanoparticles are located within the nanotubes and not attached to the outside walls.

For the dose enhancement study, we have added 10 mg of Cu-MWCNTs to 216 g of water, with < 1 mg of copper in the water cube. We studied the effect of adding unfilled and Cu-filled MWCNTs to an aqueous medium on the measured radiation dose. A Varian TrueBeam Linac (Varian, Palo Alto, CA), was the source of the megavoltage x-ray radiation. A $6 \times 6 \times 6 \text{ cm}^3$ cube from the Max-HD phantom (IMT, Troy, NY) was filled with water and placed at 100 cm from the x-ray target. Another cube with the same dimensions made of solid water material (a plastic-like material with electron density close to water's electron density) was placed below the water-filled cube, such that the beam passes through the water cube first, with radiation field size of $20 \times 20 \text{ cm}^2$ at 100 cm source to surface distance. This setup was chosen for its simplicity, while the field size was to assure consistency with future measurements that may require a large field size. The lower cube has several inserts allowing for micro ion chamber measurements.

An A16 ion chamber, with an active volume of 0.007 cc (Standard Imaging, Middleton, WI) was placed in the hole closest to the water cube, at 1 cm below the water cube; all other holes had solid water plugs inserted in them to minimize any effect of inhomogeneity (Fig. 2). After the initial readings were acquired, metallized or as-received MWCNTs were added to the water cube with concentration of 5×10^{-2} mg/cc, and readings were taken using the same setup within an hour from the original readings to avoid set up discrepancies and machine output variations. After the readings were complete, verification of the initial readings of the water filled cube with no metallized MWCNTs suspended in the medium were done by repeating the initial readings and comparing the two readings to each other.

These measurements were repeated using a Farmer-type ion chamber (active volume of 0.6 cc), placed at 1 cm below the water cube along the central axis of the radiation beam. This was to verify the effect of the off-center measurements, conducted by the A16 ion chamber, on the measured dose enhancement. The off-center positioning was mandated by the design of the phantom, where the closest insert to the top is shifted to the side of the phantom.

Measurements of 5 different x-ray energy spectra were conducted twice, for consistency check.

In addition, a gafchromic-type film (Ashland Advanced Materials, NJ) was sandwiched between the water cube and the lower cube, where the ion chamber is inserted. The phantom was irradiated with and measurements were taken with film and ion chamber, simultaneously. This was done for a 6MV energy beam, with the cube being filled with water only, water plus copper filled MWCNTs and water plus pure MWCNTs. The DERs were calculated for film and ion chamber, i.e. at 0 cm distance from the water cube and at 1 cm beneath it. This ratio is calculated for each setup, such that the DER for film is the ratios of film measurements and the DER for ion chamber is the ratio of ion chamber measurement.

JMP software (SAS Institute Inc., Cary, NC) is used for statistical analysis of the acquired data and the statistical significance is determined. We used one-way Anova with Tukey-Cramer post-hoc testing for all measurements and student's t-test for each pair of samples at a given energy.

Results

SEM images (Fig. 3, a) can be used to confirm that carbon nanotubes are collected on the substrate, but not whether a sample contains copper. However, SEM can be used in conjunction with EDS measurements. The EDS spectra (Fig. 3, b) showed the presence of copper at bright areas of the MWCNT SEM images. XPS spectra (Fig. 4) confirmed the presence of copper in the samples processed with the copper salt.

Although the amount of copper within a sample can't be measured directly, given that the values given by the EDX and XPS software may need interpretation, we can estimate the mass via calculations. In the filling process, we start with 500 mg of MWCNTs and 1 mL of $\text{Cu}(\text{NO}_3)_2$ solution with a concentration of 0.868 mol/L, this corresponds to 187.56 g/mol of $\text{Cu}(\text{NO}_3)_2$. 33.88% of the salt's weight is Cu, hence there

would be 63.55 g/mol of Cu, which is equivalent to 55 mg of Cu in the sample. The mass of Cu in a Cu-CNT sample would not exceed 10% of the total mass.

We used TEM to determine the location of the copper with respect to the MWCNTs. The TEM images such as in Fig. 5 shows that the MWCNTs are partially filled with copper.

The dose increase for each studied beam energy is plotted in the graph below (Fig. 6).

To demonstrate the specific effect of adding copper filled nanotubes, MWCNTs with partial copper filling were compared to plain water with respect to dose enhancement.

There is a statistically significant ($P < 0.05$, Student's t-test) difference between the water doses with and without metallized MWCNTs suspended in the medium. They are statistically significant to better than 95% confidence, for all five energies.

Furthermore, the effect of distance on the measured radiation dose enhancement was studied, using an EBT2 – type radiochromic film and an ion chamber. The results are shown in Fig. 7, such that the film measurements are averages of 3 pixel values of exposed areas in the film to 300MUs and that the ion chamber measurement is a reading at 300 MUs, with the film in the way. The ion chamber reading was verified after removing the film.

In addition to the effect of metalized carbon nanotubes, the effect of pure MWCNTs was also studied. Figure 7, below, shows an increase in both cases at two different distances from the water cube.

The off-axis measurement was confirmed to not impact the results with measurements taken along the central axis.

Discussion

The copper filling of the MWCNTs is confirmed via a combination of various modalities, where EDS and XPS data showed the existence of copper within samples, and the TEM images provided proof of the filling of the MWCNTs with copper.

Following the confirmation of the copper filling of the carbon nanotubes, the effect of

these Cu-filled MWCNTs on enhancing the radiation dose in a water cube is studied, in megavolt x-ray beams where the dominant type of radiation interaction in that energy range (between 2.5 and 10 MeV) is Compton scattering, an interaction between the incident photon and a free electron¹¹. This type of interaction is independent of the atomic number “Z” of the absorbing material, as opposed to the photoelectric effect, which has a mass-dependent cross-section but is a less likely interaction in the studied energy range. Figures 6 and 7 show the dose increases when the MWCNTs are filled with copper, which has a higher atomic number than carbon and water equivalent, suggests that the photoelectric interaction is responsible for this dose enhancement. Both photoelectric effect and Compton scattering

decrease with increasing photon energy, the decrease in the dose enhancement shown in Fig. 6 is consistent with both types of interactions.

Furthermore, the dose increase just below the water cube was measured by placing a radiochromic film between the cube and the solid water slab (Figs. 7 and 8). We see a dose increase with values that are different than the ion chamber measurements. When unfilled MWCNTs are added to water, there's no photoelectric effect introduced, given that the density of the MWCNTs is less than the effective density of water, therefore the main increase would be due to Compton scattering. This increase is equal at two different distances from the water cube, at 0 cm and at 1 cm away. When the pure MWCNTs are replaced with copper filled ones, for the same photon energy (6 MV in this case), we notice a large increase in the proximal measurements (the film that is placed just under the cube) and a small increase in the distal point of measurement (the ion chamber, 1 cm below the water cube). This suggests that at the vicinity of the interaction, photoelectrons due to the high Z of the copper contribute most to the dose increase with this effect decreasing with distance. Further measurements will be done to support this hypothesis.

The dose increase when pure carbon nanotubes were added to water (Figs. 7 and 8) can be explained by the probability of Compton scattering. Compton scattering probability is a function of the classical radius of the electron¹², as in the Klein-Nishina formula such that:

Compton cross section per unit solid angle ($d\sigma/d\Omega$) $\propto r^2$

with "r" being the electron's classical radius, which is inversely proportional to the rest mass of the electron. The effective mass of the electron in carbon nanotubes was studied by Roy and Maksym¹³ in units of the electron's rest mass, a function of the radius of the MWCNT. This leads to an expected increase in the probability of Compton scattering.

Adding copper filled carbon nanotubes to water would increase the measured dose, due to higher probability of photoelectric effect from the low energy photons in the spectrum. As we increase the energy, this probability decreases and so does the probability of Compton effect, therefore the increase in the measured dose (due to dose enhancement), decreases with increasing the energy of the beam. In the studied material, pair production is negligible. Also, secondary electrons in the beam react with nanoparticles in a different way than they would in bulk material (Krasheninnikov and Nordlund 2004), owing to the large surface area of the nanotubes the increased probability of interaction.

In addition, CNTs would potentially have a different effect, compared to other nanoparticles (e.g. Au-NPs) due to their unique honeycomb like shape, allowing electrons to escape with no self absorbance¹⁶.

Cu-MWCNTs concentration in the water cube is 5×10^{-2} g/cc, this is much lower than the recommended concentration of nanoparticles to achieve dose coverage at the cellular level¹⁴.

However, these recommendations were given to nanoparticles of sizes ranging between 2 nm and 100 nm and carbon nanotubes have diameters in this range but lengths in μm range. The metal particles

are also distributed along the axis of the nanotubes. This could alter the recommended concentrations; further studies will be conducted to find the optimum number.

Conclusion

Carbon nanotubes were chosen as vehicles due to their superior properties, including high aspect ratio, ability to utilize the hollow inside and the outside walls simultaneously, and documented ability of protecting encapsulated materials within them from oxidation¹⁰, thus reducing the toxicity of the copper. Future studies are planned to support this hypothesis. Copper was chosen, as filler, for novelty given that the published data on its role as a radiation dose enhancer is very limited.

Successful filling of MWCNTs with copper has been demonstrated. The exact amount of copper within the carbon nanotubes can not be measured directly at this time but an estimate was calculated. In the future, other tools will be explored for more accurate measurement of the filling material's volume or mass. Ion chamber measurements have shown that adding Cu-filled MWCNTs to an aqueous medium increases the measured dose, relative to water readings by up to 2.5%. This is a proof of concept that metallized carbon nanotubes can be used for radiation dose enhancement in external beam therapy with the effect of the dose enhancement being most significant at lower beam energies in the range tested.

The findings from this study show dose enhancement, but the underlying reasons are not well-understood yet. More investigation in the effect of the unique properties that carbon nanotubes possess is in our future work. In addition, there is limited understanding of the biological effect of carbon nanotubes and their risk-benefit factor. Many investigators are actively researching this field.

Future directions for this work include measuring the dose enhancement for variable concentrations of Cu-MWCNTs in water and comparison between simulation values of the dose enhancement vs. measured data. In addition, it's intended to study the effect when the copper filling is replaced with a higher Z material. This study addresses only the radiation dose enhancement, which is a physical property, and does not approach the radiosensitization effect. Given the importance of biological models, in the future we will study the radiosensitization of metal-filled carbon nanotubes and it's radiobiological and clinical effects.

Declarations

Ethics approval and consent to participate: Not applicable

Consent for publication: I give "Cancer Nanotechnology" permission to publish this manuscript.

Availability of data and materials: All data is available and the material is reproducible.

Competing interests: Not applicable

Funding: Not applicable

Authors' contributions: Each author has contributed to this research.

Acknowledgements: We appreciate the support given by the radiation oncology team at Albany Medical Center. A sincere gratitude is extended to Miguel Rodriguez at SUNY Polytechnic

Institute for his tremendous help with acquiring TEM images.

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Acknowledgment

Many thanks to Dr. Afghan, Dr. Satti and the radiation oncology team at Albany Medical Center, for offering their tools and facility for this research work and for their continuous guidance and support. We'd also like to extend our gratitude to Umar Baharom at Integrated Medical Tech

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Figures

(a)

(b)

(c)

Figure 1

(a) The reflux of the MWCNTs in acid at 120°C. (b) CNTs ends open and CNTs walls are functionalized as a result of applying heat in an acidic environment. (c) Due to capillary effect, the copper salt solution (blue) enters the hollow CNTs.

Figure 2

(a) A16 ion chamber inserted below the water cube. (b) The water cube after adding the MWCNTs to it. (c) A schematic of the lower cube

Figure 3

(a) An SEM image and (b) an EDS spectrum (of a Cu-filled MWCNTs sample)

Figure 4

An XPS spectrum of a Cu-filled MWCNT sample

Figure 5

TEM images demonstrating a partially filled MWCNT

Figure 6

The radiation dose enhancement effect with variation of photon beam energy.

Figure 7

Dose enhancement ratios of filled vs. unfilled MWCNTs, at two distances: 0 cm (film measurement) and 1 cm (lon chamber)