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DNA dosimeter measurements of beam profile using a novel simultaneous processing technique

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Abstract

A DNA dosimeter (DNAd) was previously developed that uses double-strand breaks (DSB) to measure dose. This dosimeter has been tested to measure dose in scenarios where transient-charged particle equilibrium (TCPE) has been established. The probability of double strand break (PDSB₀), which is the ratio of broken double-stranded DNA (dsDNA) to the initial unbroken dsDNA in the dosimeter, was used to quantify DSBs and related to dose. The goal of this work is to produce a new technique to process and analyze the DNAd and quantify DNA-DSBs. This technique included simultaneously processing multiple DNAds and also establishing a new form to the probability of double strand break (PDSB_n), which was then used to test the DNAd in a non-TCPE condition by taking beam penumbra measurements. The technique utilized a 384-well plate, and the measurements were made at the edge of a 10 × 10 cm field and compared to film measurements. During these penumbra measurements, while observing the positional differences in the higher gradient region at 4.1 and 4.55 cm from the center of the radiation field, the distance to agreement of PDSB₀ to film were 0.38 cm and 0.26cm while the distance to agreement of PDSB_n to film were 0.11 cm and 0.06 cm, respectively. Finally, the developed new separation technique reduced the time needed for the analysis of 25 samples from 200 minutes to 30 minutes.

Keywords

DNA dosimetry; DNA double-strand break; percent depth dose; beam profile

1. Introduction

Radiation dosimeters are used to measure and verify dose when commissioning and calibrating equipment for radiotherapy patient treatment. The precision of these measurements is important because it will significantly affect interpretations of machine outputs and delivered dose¹. Improvements in this area will create more favorable and safer radiotherapy techniques². While the therapeutic effect of radiation comes from the production of biological damage, conventional dosimeters have not employed this to measure dose. Among the types of known biological damage, DNA double strand breaks

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(DSBs) are the most cytotoxic ones³. This can occur in both cancer and healthy cells during radiation treatment⁴, and since the role of DNA DSBs remains a common and important effect throughout radiotherapy, it is desirable to pursue it for dosimetry measurements. The long-term hypothesis is that using a dosimetry technique that is more closely related to biological damage than conventional dosimeters will produce more meaningful measurements.

Various types of DNA damage have been explored with techniques that would be adaptable to dosimetry^{5-6,7,8,9,10,11,12,13,14,15,16}. However, only one focused on quantifying DSBs. This technique utilized relaxation of supercoiled circular DNA¹⁷. Its procedure, however, required gel electrophoresis separation, which is a time-consuming process. This then led to a new DNAd technique based on a faster magnetic separation method¹⁸. The hope is that this faster separation technique makes it more practical to eventually implement a DNAd clinically.

There has been progress with the DNAd's development and optimization¹⁹. For a point of reference, each DNAd costs approximately \$6 to produce, which is cheaper than purchasing an optically stimulated luminescent detector, but it is not reusable. The DNAd has also been used as a benchmark for the Geant4 DNA toolkit²⁰. However, there are still many practical issues that must be improved before the DNAd could potentially be used clinically. Currently, one limitation of the dosimeter is a lack of rapid analysis capability. The current analysis process requires many manual steps involving transference of dosimeter. This is ultimately time consuming and likely contributes to a larger uncertainty. Due to the nature of the measurements in this study, it offers an opportunity to test a new method we developed that requires only a single 384-well plate. The DNAd remains in the plate for irradiation and analysis, which drastically reduces the overall dosimeter handling time. The response with this new method was also benchmarked against our current preparation method. Furthermore, while previous quantified measurements used the DNAd as point measurements to obtain dose responses within TCPE, measurements in the following sections are made with DNAds set laterally and at the penumbra. By comparing and benchmarking the DNAd response in this scenario, we can provide a larger picture of the DNAd's capabilities and reveal potential areas to further optimize the dosimeter.

2. Methods

2.1 DNA dosimeter fabrication

Fabrication of the dosimeter and details has been previously reported¹⁸. Briefly, a polymerization chain reaction (PCR) was first used to combine oligonucleotide-attached fluorescein amidite (oligo-FAM) and biotin-labeled oligonucleotides with a pRS-316 vector as the template. The result is 4 kbp double stranded DNA with FAM on one end and biotin on the other on the other end. Gel electrophoresis was then used to verify strand length. After confirmation, these strands are then attached to streptavidin-coated magnetic MyOne T1 Dynabeads (Thermo Fisher Scientific, Waltham, MA) using the recommended binding buffer from the Dynabeads kilobase BINDER Kit (Thermo Fisher Scientific, Waltham, MA). After completing the attachment, unattached strands are finally removed from the samples.

The resulting dosimeter can be seen in Figure 1, and the samples can be stored until they are used.

2.2 DNA dosimeter analysis techniques

Some common quantities are significant and will be seen utilized in both the old and new methods of analysis. The upcoming short reiteration of the older analysis in 2.2a serves to reintroduce some of these values as well as the reasoning to evaluate them. For example, in 2.2b, a different correction factor is determined for use in the new method with the same purpose as the attenuation factor used in 2.2a.

2.2a The old method—For analysis after irradiation, the contents of each dosimeter were separated by individually placing them against a DynaMag magnet. The magnet collected the beads so that the supernatant can be transferred into a well in a 384-microwell plate. The beads were then re-suspended and placed into wells of the plate adjacent to the respective supernatants. A Biotek Synergy 2 Multi-mode Microplate Reader was then used to measure the 520 nm fluorescence signal from the wells containing dosimeters and beads. A fixed gain setting was used for the experiments presented here.

The signal from the wells containing supernatant with broken DNA-FAM (I_S), the wells containing beads with unbroken DNA-FAM (I_B), and wells containing only PBS (I_P) are used in Eq. 1 to calculate the probability of double strand break ($PDSB_o$) used in this method¹⁸. The subscript “o” denotes the older handling method of the dosimeter. An attenuation factor (A) is included to account for opacity effects due to the beads, shown in Eq. 2. The variable I_{PFAM} is the fluorescence intensity of wells containing only oligo-FAM suspended in PBS, the variable I_{BBFAM} is the fluorescence intensity of wells containing blank beads, which are Dynabeads with nothing attached, and oligo-DNA both in suspension. Lastly, the variable I_{BB} is the fluorescence of wells with only blank beads. The presence of the beads affects the attenuation of the 520 nm fluorescence signal within the wells during measurements. As shown in Eq. 1, this was accounted for by an attenuation factor.

$$PDSB_o = \frac{I_S - I_P}{I_S - I_P + A \cdot (I_B - I_{BB})} \quad (1)$$

$$A = \frac{I_{PFAM} - I_P}{I_{BBFAM} - I_{BB}} \quad (2)$$

2.2b Finding correction factors to use with the new method—A simpler separation method while keeping complete dosimeters in wells is utilized here to help avoid the need for manually separating the broken-unbroken DNA. This method is most effective if the dosimeter is irradiated and remains in the well plate. In Figure 2A, which shows the original technique, samples are separated into the two respective components; the supernatant containing broken DNA-FAM and the precipitate of beads with unbroken DNA-FAM. This required time to both transfer the components and check for any loss of

materials. Figure 2B shows the newer and simpler method separating the components while keeping them in the same well. For this new method, a magnet is placed against the bottom of the well to separate in place. Before the separation in Fig 3B, the broken DNA strands are affected by bead opacity, whereas afterwards, they are suspended above the beads and therefore not affected. The opacity of the beads then only affects the fluorescence reading of the beads and objects connected to the beads.

The inherent opacity of the beads can be used with this new separation technique to determine the fluorescence of the broken-unbroken DNA components. This phenomenon is used to measure DSBs and dose. To observe the influence of the beads when suspended and when magnetically separated, blank beads with no suspended DNA-FAM were compared with blank beads with suspended DNA-FAM. Along with PBS, 50 μ l of each were placed in 5 wells within a 384-microwell black-walled plate. Each well is 1 mm thick, and the center of each well is 4.5 mm apart. Fluorescence output at 520 nm was read after the contents were suspended in each respective well. Afterwards, a magnet was placed underneath the well for 4 minutes to collect the magnetic beads to the bottom of the plate before taking another fluorescence reading. These two readings were then analyzed to determine the contribution factors due to suspension and collection by magnet. The following is the mathematical formalism used to calculate the influence of the beads when suspended and not separated and when magnetically separated and collected at the bottom of the wells.

The variable α_1 is a fluorescence collection efficiency factor associated with the reading before magnetic separation, and the variable α_2 is a collection efficiency factor associated with the bead-connected fluorescence after magnetic separation. These factors serve a similar role as the A attenuation factor and are considered as the correction factors differentiating between before and after separation. As seen in Table 1, the variable I_1 in Eq. 3 is the fluorescence intensity of a well with only beads suspended in solution. It is defined as the product of the intensity from the suspended beads (I_{BB}) and α_1 . The variable I_2 in Eq. 4 is the fluorescence intensity of the same well that obtained I_1 with only beads but magnetically collected to the bottom of the well. It is defined as the product of the intensity from the collected beads (I_{BB}) and the variable α_2 . The variable I_3 in Eq. 5 is the fluorescence intensity of a well with beads suspended with unattached oligo-FAM. Since both oligo-FAM and beads are suspended, the variable I_3 is defined as α_1 multiplied by the sum of both intensities from oligo-FAM (I_F) and I_{BB} . Lastly, the variable I_4 in Eq. 6 is the fluorescence intensity of the same well that obtained I_3 but with the beads magnetically collected to the bottom. Since only the beads are collected to the bottom of the well and only oligo-FAM remains suspended, the variable I_4 is defined as the sum of I_F and the collected beads ($\alpha_2 I_{BB}$). The known variables in Eqs. 3–6 are the fluorescence values (I_1 through I_4). These equations can be manipulated to express the correction factors (see Eqs. 7 through 10). The variables I_{BB} and α_2 are used in future analyses.

$$I_F = I_4 - I_2 \quad (7)$$

$$\alpha_1 = \frac{(I_3 - I_1)}{I_F} = \frac{(I_3 - I_1)}{(I_4 - I_2)} \quad (8)$$

$$I_{BB} = \frac{I_1}{\alpha_1} = \frac{I_1 I_F}{(I_3 - I_1)} = I_1 \frac{(I_4 - I_2)}{(I_3 - I_1)} \quad (9)$$

$$\alpha_2 = \frac{I_2}{I_{BB}} = I_2 \frac{\alpha_1}{I_1} = \frac{I_2 (I_3 - I_1)}{I_1 I_F} = \frac{I_2 (I_3 - I_1)}{I_1 (I_4 - I_2)} \quad (10)$$

2.3 Profile measurements using the old method and the new method

To make profile measurements at a beam penumbra, DNAds were placed in a way to make lateral measurements. Keeping the dosimeters in individual centrifuge tubes and maintaining their alignments would be unnecessarily cumbersome in addition to giving an inadequate resolution, as the closest distance the dosimeters could be spaced would be 1.3 cm. A 384-well plate enabled a 4.5 mm resolution. This allowed multiple dosimeters to be placed adjacent to each other and be irradiated simultaneously. A Varian Clinac 23EX linear accelerator was used to irradiate 50 Gy with a 6 MV beam, at an SSD of 100 cm, a depth of 1.5 cm, a 10×10 cm field, and with a half beam block (HBB) to remove divergence at the dosimeter plane (see Fig. 3). All experiments used water-equivalent Gammex solid water due to its ease of setup. The monitor units delivered for this irradiation were based on that needed to deliver 50 Gy to the detector location in water. Moving beyond making point measurements one at a time as done in previous works, the 384-microwell plate was used to hold multiple dosimeters side by side and then placed within the phantom to irradiate them simultaneously. Figure 3A shows the location of the dosimeters, and Fig. 3B and 3C show how the microwell plate is placed within the plastic phantom at the penumbra. In this experiment, the newer method of handling the dosimeter was benchmarked with the older method, and film was compared against the two. After the dosimeters were placed in the 25 wells, irradiated, and separated, samples were then extracted and prepared to be read in the same way as previously done using Eq. 1 to calculate $PDSB_o$.

The newer separation method used the following formalism to determine $PDSB_n$. Here, the subscript “n” denotes the newer handling method of the dosimeter. The variable α_2 is the collection efficiency factor, found in the fluorescence factor experiment from Eq. 10, when beads were collected to the bottom of the plate. The variable I_r is the measured fluorescence signal of the irradiated and separated dosimeters, the variable I_A is the measured fluorescence signal from still attached strands, and I_S and I_{BB} are defined similarly as before. The variable I_r is defined as the sum of the three variables I_S , $\alpha_2 I_A$, and $\alpha_2 I_{BB}$ (as shown in Eq. 11). After magnetically separating the beads with attached DNA-FAM to the bottom of the wells, the broken DNA-FAM remains suspended in the supernatant, and α_2 accounts for the fluorescence signals coming from the bottom of the wells. In Eq. 12, I_o is the fluorescence signal from everything not irradiated and magnetically collected, and α_2 is factored in for all terms because no irradiation has taken place. With this separation method,

$PDSB_n$ can now consider only the fluorescence from the broken and unbroken DNA-FAM.

Specifically, $PDSB_n = \frac{I_S}{I_S + I_A}$.

To find $PDSB_n$ from Eqs.11 and 12, we subtract $\alpha_2 I_{BB}$ from both sides of each expression and then take the ratio of the corresponding left and right sides as shown in Eq.13. We add and subtract $\alpha_2 \cdot I_S$ in Eq.14 to organize terms as seen in Eq. 15. After rearranging to Eqs. 15 and 16, we arrive at the equation for $PDSB_n$ as seen in Eq.17. This formulation considers the magnetically collected parts of the dosimeter and its contribution to the fluorescence signals from the wells. It is important to note that there needs to be 0 Gy sample measurements and the characterization of factors α_2 and I_{BB} before $PDSB_n$ can be calculated. Below 50 Gy, the dosimeter response is linear with dose, and both PDSBs can be normalized by the max PDSB. The profile measurements were compared with EBT3 film, as the 2D volume averaging could be matched with the DNAd.

$$I_r = I_S + \alpha_2 \cdot I_A + \alpha_2 \cdot I_{BB} \quad (11)$$

$$I_O = \alpha_2(I_S + I_A) + \alpha_2 \cdot I_{BB} \quad (12)$$

$$\frac{(I_r - \alpha_2 I_{BB})}{(I_O - \alpha_2 I_{BB})} = \frac{I_S + \alpha_2 I_A}{\alpha_2(I_S + I_A)} \quad (13)$$

$$= \frac{I_S - \alpha_2 I_S + \alpha_2 I_S + \alpha_2 I_A}{\alpha_2(I_S + I_A)} \quad (14)$$

$$= \frac{I_S(1 - \alpha_2) + \alpha_2(I_S + I_A)}{\alpha_2(I_S + I_A)} \quad (15)$$

$$\frac{(I_r - \alpha_2 I_{BB})}{(I_O - \alpha_2 I_{BB})} = (PDSB_n) \left(\frac{(1 - \alpha_2)}{\alpha_2} \right) + 1 \quad (16)$$

$$PDSB_n = \left(\frac{\alpha_2}{1 - \alpha_2} \right) \left(\frac{(I_r - \alpha_2 I_{BB})}{(I_O - \alpha_2 I_{BB})} - 1 \right) \quad (17)$$

3. Results

From the experiment with the correction factors for fluorescence measurements, Table 2 shows the fluorescence values and uncertainties read from wells containing only PBS, blank beads without oligo-FAM, blank Dynabeads with oligo-FAM, before magnetic separation and after magnetic separation. Table 3 shows the values calculated from the formalism for this experiment.

Figures 4 and 5 show the plot of the $PDSB_o$ and $PDSB_n$ with dose measured with film. The y-axis shows normalized units, and the x-axis shows the distance from the center of the field. While penumbra characterization with the DNAd, as can be seen in Figs. 4 and 5, seems to match closely with film, the resolution for these measurements are limited by the spacing of the plates used. The 384-microwell plate contains wells with centers that are approximately 0.45 cm apart. Table 4 shows the original and normalized $PDSB_o$ and $PDSB_n$ calculated using Eqs. 2 and 17, respectively, and dose measured with and interpolated from film.

4. Discussion

The old separation technique, where the beads are separated and suspended in different wells from the supernatant with broken DNA-FAM, required multiple steps. These steps increase the chances of losing material between transfers. From transferring between tubes and waiting for magnetic separation, approximately 8 minutes would be used per dosimeter to complete preparing for analysis. The new separation technique significantly improves this point by removing the necessity to transference, only requiring 4 minutes to prepare for analysis regardless of the number DNAd used, and placing the dosimeters in a more structured container. These important points enable future experiments that will require a large quantity of dosimeter, a more evenly spaced sample holder, while maintaining the goal of developing the DNAd for a faster paced clinical setting. As seen in the profile measurements, using the original separation method for the 25 samples would require approximately a total of 200 minutes. This amount of time including with the multiple transfers would contribute to a higher coefficient of variance (COV). Using the plate and the new separation method only required 1 minute per sample to place into the well, and, with the short time to magnetically separate the magnetic beads, less than 30 minutes was needed to prepare for fluorescence analysis. This 85% reduction in processing time is a significant improvement for this dosimeter.

When comparing penumbra profiles between $PDSB_o$ and $PDSB_n$ against film, the COV for the measurements within the field for $PDSB_o$ were 17.6% and 22.4% for $PDSB_n$ were 3.0% and 4.0%. Comparing the two PDSBs to the film at each point in Figs. 4 and 5, the new method appears to be closer to the film measurements than the old method. While observing the positional differences in the higher gradient region, the distance to agreement of $PDSB_o$ to film were 0.38 cm and 0.26 cm, and the distance to agreement of $PDSB_n$ to film were 0.11 cm and 0.06 cm. This suggests that the new separation method used for this experiment was more reliable than the old method. The large uncertainties from $PDSB_o$ are likely due to issues of retrieving the samples from the wells for separation. Solution adheres to the well walls and is difficult to completely remove, which adds a high variation to the resulting dosimeter signals. While removing the dosimeter from the well plates after irradiation from the old method was a necessity for the profile experiment, this was not required for previous experiments because irradiation did not take place in wells. Additionally, in contrast to taking point measurements one at a time in previous experiments, measuring a beam profile requires multiple DNAd to be placed in a way to make lateral measurements and maintain a better resolution.

5. Conclusion

An alternative and improved method of processing the DNAd was explored. Instead of using DNAd in individual tubes as point measurements and then processed one by one as was done previously, a 384-microwell plate enabled the ability to irradiate multiple DNAd at the same time and then separated and analyzed simultaneously. This method was used to measure beam profile, specifically the penumbra, by positioning the laterally placed DNAd in a multi-well plate along the beam edge. The new separation technique significantly decreased the processing time of the dosimeters for analysis from 200 minutes to less than 30 minutes. By accounting for separation within the wells instead of in two different wells per dosimeter, this resulted in a much faster and more accurate measurement of the penumbra. The new and quicker separation technique is a favorable indication for the variable application of the DNA dosimeter.

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Figure 1.

A diagram of biotinylated and fluorescently tagged double stranded DNA attached to magnetic streptavidin-coated beads. After radiation, induced DSBs cause broken DNA-FAM to separate from the rest.

Figure 2.

A) The previous method physically separates the supernatant and beads into two separate wells for reading. B) The new method magnetically separates the two while keeping the whole sample in the original well.

Figure 3.

A. DNA placed in a 384-microwell plate for irradiation. B. Experimental Setup with Solid water as the phantom. C. A side view of the use of split-beam for penumbra profile measurements.

Figure 4. Penumbra profile comparison between PDSBo and film. DNAd shows a distance to agreement of 0.38 cm and 0.26 cm in the higher gradient region

Figure 5. Penumbra profile comparison between PDSB_n and film. DNAd shows a distance to agreement of 0.11 cm and 0.06 mm in the high gradient region with film.

Table 1.

Expressions of fluorescence readings while considering two different states.

Fluorescence signals	Before separation	After separation
Beads without FAM	$\begin{matrix} I_1 \\ = a_1 \\ \cdot I_{BB} \end{matrix} \quad (3)$	$\begin{matrix} I_2 \\ = a_2 \\ \cdot I_{BB} \end{matrix} \quad (4)$
Beads with unattached FAM	$\begin{matrix} I_3 \\ = a_1 \\ (I_F \\ + I_{BB} \\) \end{matrix} \quad (5)$	$\begin{matrix} I_4 \\ = a_2 \\ \cdot I_{BB} \\ + I_F \end{matrix} \quad (6)$

Table 2.

Values from the fluorescence factor experiment

	Before separation	After separation
PBS	246.6± 2.15	239± 1.79
w/o oligo-FAM	624.8± 2.93	294.2± 3.31
w/ oligo-FAM	4176± 123	47870± 1510

Table 3.

Values and uncertainties calculated from Eqs. 2 – 5

I_F	47600 ± 1510
a_1	0.0746 ± 0.0035
I_{BB}	8370 ± 395
a_2	0.0351 ± 0.0017

Table 4.

Raw and normalized values from film and dosimeters.

		Distance from center of field (cm)				
		4.1	4.55	5	5.45	5.9
Raw values	PDSB _o	0.062±0.018	0.062±0.017	0.050 ±0.012	0.010±0.0030	0.013±0.0047
	PDSB _n	0.036 ± 0.0011	0.031± 0.0014	0.019± 0.00032	0.005 ± 0.0029	0.002 ± 0.0011
Normalized values	PDSB _o	100±23.0	99.42 ±28.0	80.27 ±19.0	15.49 ±4.9	21.07±7.7
	PDSB _n	100±3.3	87.47±4.0	52.88 ± 0.88	13.73±8.0	5.301± 3.0
	Film	100± 0.31	96.15± 0.36	59.13 ±2.4	10.80 ±2.4	2.520 ±0.56