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(Multi-dimensional clustering of alpha particle-induced DNA double-strand breaks)

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Abstract. Radiation causes damage in DNA structure via energy deposition while passing through a cell nucleus. Low-LET radiation causes relatively homogeneous DNA lesions, but high-LET ions induce multiple double-strand breaks (DSBs) in proximity due to highly localized energy depositions along the track. Hardly repairable clustered DSBs possibly lead to cell mutation or death. On the other hand, such clustered DSBs may not be differentiated from a simple DSB due to limitations in conventional measurement techniques. In this paper, we defined a spherical nucleus volume in which the spatial distribution of DSBs induced by alpha particles was estimated considering particle energy and absorbed dose to the nucleus. The distributions of DNA damage were based on the track structure of alpha particles simulated using the Geant4-DNA. The distribution of DSB density and the clustered DSBs falling within a certain distance were assessed. Consequently, a correlation was observed between the calculated number of DSB clusters and the measured number of alpha particle-induced gamma-H2AX foci in laboratory.

KEYWORDS: Alpha particle, clustered DNA damage, Geant4-DNA

1 INTRODUCTION

Radiation of high-linear energy transfer (LET) deposits energy in a highly localized manner around the primary track. Energy depositions in genome material in cells may induce double-strand breaks (DSBs). Energy depositions concentrated at proximate sites induce greater complexity of DNA damage. These clustered DNA lesions are repaired less efficiently than isolated simple damages [1], and thus results in increased biological effectiveness in cellular death and mutation [2, 3].

A common method to evaluate the radiation-induced damage in laboratory is to measure the number of gamma-H2AX foci which locate at DSB sites during the cellular response of repair [4]. Clustered DSBs would be detected as a single DSB (focus) due to the limited image resolution in microscopy. The yield of foci can be further underestimated in two-dimensional (2D) image taking due to overlapped foci.

In this study, the distribution of DSB density as a function of the separation distance between DSBs in the nucleus, and the number of DSB clusters formed within a certain distance (clustering radius) were calculated. In addition, we tested the correlation between the number of clusters evaluated by the 2D-clustering of DSB sites and the measured number of gamma-H2AX foci induced by alpha particles.

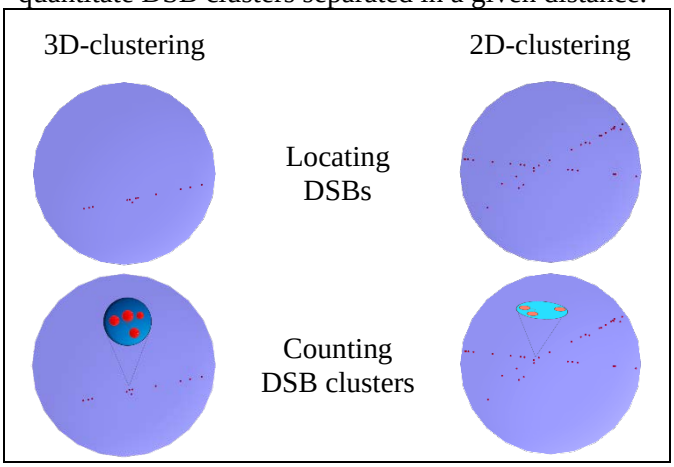
2 METHODS

Geant4-DNA [5] was used to simulate the tracks of alpha particle with different initial energies in cell nucleus. A total of 2×10^3 up to 3×10^4 samples were simulated for each energy ranging from 2MeV up to 300MeV. Alpha particles were directed toward a spherical cell nucleus of 10μm in diameter vertically from random positions on a circular disk tangent to the surface of the nucleus.

The DNA structure in the nucleus was defined according to Francis, et al.'s model [6]. Instead of an explicit description of DNA molecules, Francis et al. employed a uniform probability of sensitive volume in nucleus. The density-based spatial clustering of applications with noise (DBSCAN) algorithm [7] was employed to identify the clusters, that consist of at least two SSBs in opposite strands within a 10 base-pair (3.3 nm) radius. An additional DBSCAN clustering algorithm was developed and applied to both 3D DSB sites and their 2D projections in order to find the number of DSB clusters within a given distance [Fig. 1]. The 3D-clustering was employed to count the DSB clusters, within sensitive volumes,

formed by a single track of alpha particle at a varying LET. The 2D-clustering was employed to count the number of DSB clusters (2D) by 6 MeV alpha particles at varying nucleus doses.

Figure 1: Clustering algorithms employed to quantitate DSB clusters separated in a given distance.



3 RESULTS AND DISCUSSION

DSB clusters on a single alpha track

Number of DSBs per track increases with the LET of alpha particle. The DSB density in this study equals the number of neighboring DSBs separated by a given distance from each other. The DSB density was counted with a varying distance of DSB separation for every alpha track of varying LET as presented in Fig. 2. Increased density of DSBs may result in a higher probability of mis-repair [8]. The probability of multiple DSB production by a single track of alpha particle carrying high energy is extremely low. As the particle energy decreases, the LET increases, and the yield of DSBs and thus the probability of DSBs being at proximity increases [Fig. 2]. This would lead to an increased number of clustered DSBs within a small sensitive volume in cell nucleus. Clustered DSBs within spherical volumes of different radii were merged into a single DSB cluster. Fig. 3 depicts the number of DSB clusters within sensitive volumes of different radii as a function of the LET of alpha particles. The number of DSB clusters increases with the LET of alpha particles and saturates at high LET. The number of DSB clusters saturates at a smaller value of the LET as the clustering radius increases (Fig. 3). However, the number of DSBs within individual clusters increases because of the high DSB density.

Figure 2: Distribution of DSB density as a function of separation distance in nucleus hit by a single alpha particle of varying LET.

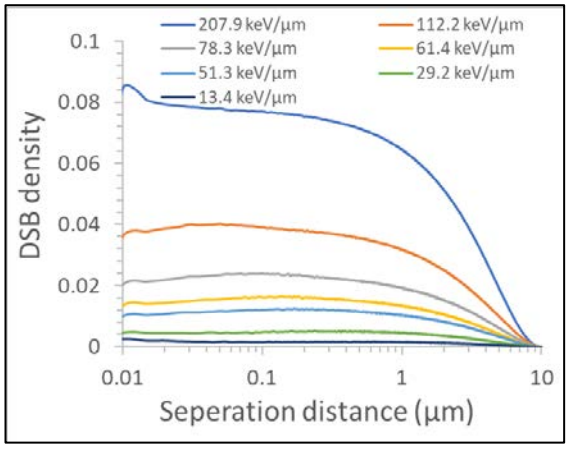
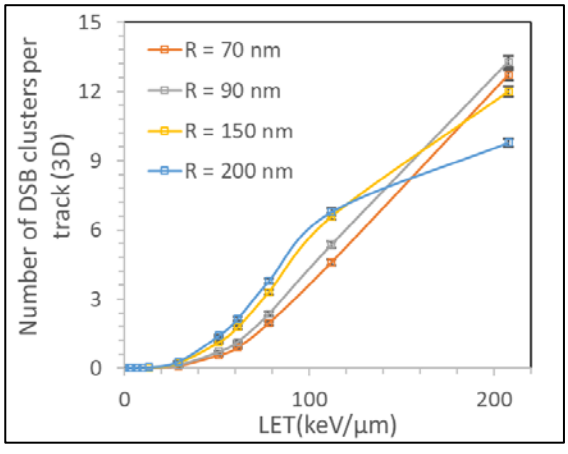


Figure 3: Number of DSB clusters as a function of the LET of alpha particles for different clustering radii. Error bar indicates 1.96 standard deviations.



Clustered DSB formation at varying doses by alpha particles

The number of gamma-H2AX foci induced by X-ray and neutrons was predicted in an earlier study [9] that simulated the 2D-clustering of DSBs using the PARTRAC code. Fig. 4 shows the simulated DSB density as a function of separation distance between DSBs induced by 6 MeV alpha particles delivering varying nucleus doses. The number of DSBs and thus the DSB density increases with nucleus dose regardless of separation distance between DSBs. The number of DSB clusters is much smaller than the total number of DSBs in the nucleus. The random direction of alpha track entering a cell nucleus was simulated by rotating the cell nucleus around its center by a random solid angle under mono-directional alpha beam tracks. The 2D-clusters of DSB were counted from the 2D images of DSB sites projected in parallel to the beam direction.

We also calculated the number of clusters formed by the 2D-projection of DSB sites induced by 6 MeV alpha particles at varying nucleus doses [Fig. 5]. The number of DSB 2D-clusters increases with the nucleus dose at low doses and saturates at high doses. The calculated number of DSB 2D-clusters was compared with the measured number of gamma-H2AX foci in an earlier study [10]. Best matching to the measured number of foci was obtained in the number of DSB 2D-clusters counted with the clustering radius of 1.5 μm. The ratio of the number of DSB 2D-clusters to total DSBs decreases with the nucleus dose. Reaching 5 % at 1 Gy, the the number of DSB 2D-clusters saturates.

Figure 4: Distribution of DSB density by 6 MeV alpha particles delivering different nucleus doses as a function of the separation distance.

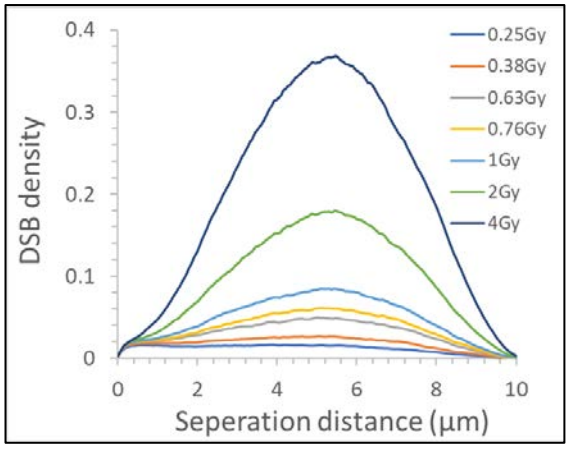
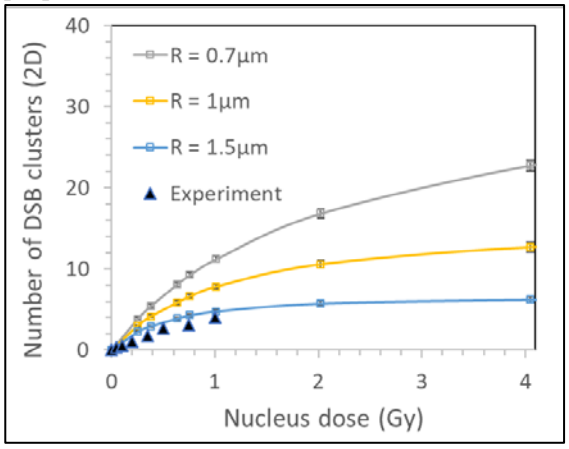


Figure 5: Calculated number of DSB 2D-clusters induced by 6 MeV alpha particles at different nucleus doses in comparison to experimental data [10]. Error bar indicates 1.96 standard deviations.



4 CONCLUSIONS

The DSB density and the number of clustered DSBs resulting from a single track of alpha particle increases with the LET of alpha particle. A good correlation was observed between the calculated number of DSB 2D-clusters, with the clustering radius of 1.5 μm, and the measured number of alpha particle-induced gamm-H2AX foci in laboratory. The number of DSB 2D-clusters saturates at doses higher than 1 Gy.

5 REFERENCES

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