

Study on DNA single-strand Double-strand Breaks Caused by Cell Radiation Damage

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Abstract

With the application of proton therapy, the damaging effect of proton beams on tumors has received widespread attention. The degree of radiation damage to the cell can be determined by the breaks of the single-stranded and double-stranded DNA in the cell. This paper builds cell structure based on MCDS simulation software, simulates the cell radiation damage such as DNA single and double strand breaks within the energy range of proton therapy, and studies the effects of different energy proton beams on cell radiation damage. Provide theoretical support for future proton radiation therapy.

Keywords

Cell radiation damage; Proton therapy; DNA single-strand damage; DNA double-strand damage.

1. Introduction

With the improvement of computer computing power, more and more scholars have begun to pay attention to radiation damage at the cellular level. In order to explore the degree of damage to human cells caused by different energies and different radioactive sources, the researchers studied the degree of radiation damage to cells from two aspects. The first is based on the Monte Carlo simulation method to construct different biological cells, [1] combined with the damage location to propose DNA single-strand breaks, double-strand breaks, protein damage and other radiation damage, and then determine whether the radiation damage leads to cell death, and proposes cell damage repair. [2] Under conditions where cells are damaged by radiation, failure to cause cell death will result in base pair repair and so on. Researchers continue to optimize cell models and propose more refined damage modes to simulate and judge cell radiation damage. The second is to use experimental methods to centrifuge the cells damaged by the radiation to separate the DNA double strands and observe the damage with a microscope to determine the final radiation intensity.

2. DNA Radiation Damage

There are two main types of radiation damage to DNA, one of which is single-stranded radiation damage and the other is double-stranded radiation damage. Single-strand radiation damage can be subdivided into simple single-strand radiation damage and complex single-strand radiation damage. Double-strand radiation damage can be divided into simple double-strand radiation damage and complex double-strand radiation damage. The method for judging single-stranded and double-stranded radiation damage is to limit the number of base pairs with a fixed length in the double-stranded DNA. If within this range, a single base pair break occurs in a single DNA strand, this situation is called Simple single-stranded radiation damage; If within this range, multiple base pair breaks occur in a single DNA strand, this situation is called complex single-stranded radiation damage.

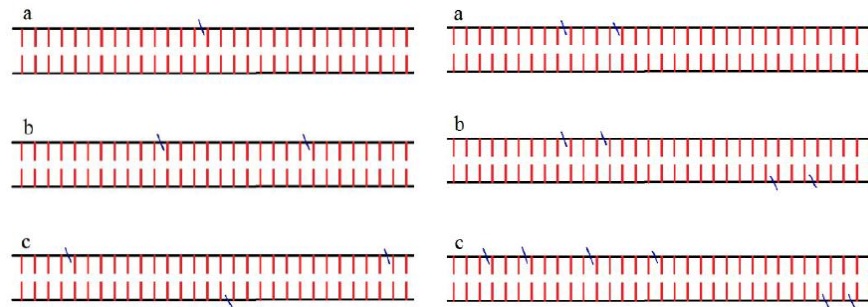


Figure 1. Single-stranded radiation damage

Similarly, within this range, if base pair breaks occur on both DNA strands, this situation is called simple double-stranded radiation damage; Breaks that produce multiple base pairs are called complex double-stranded radiation damage. In specific simulations and experiments, only single-stranded radiation damage and double-stranded radiation damage were studied, and no detailed division was made.

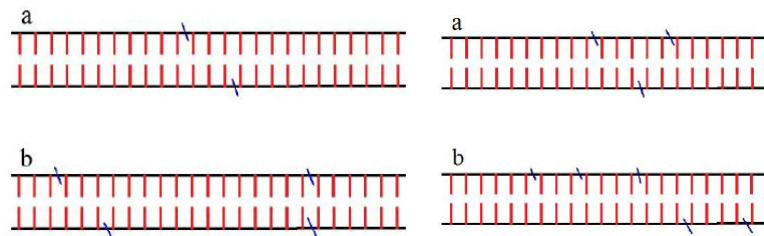


Figure 2. Double-stranded radiation damage

Next, the DNA radiation damage will be introduced from two aspects: experimental methods and DNA models.

2.1. Experimental Method

Yuri Kawashima, Department of Medicine, Oita University, Japan, used pulsed field gel electrophoresis (PFGE) and western blotting to observe the DSB effect of DNA. Pulsed field gel electrophoresis is a method of separating large molecules of DNA or chromosomes. In ordinary gel electrophoresis, large DNA molecules (>10kb) move at close speeds, and it is difficult to separate and form sufficiently distinguishable bands. In pulsed field gel electrophoresis, the electric field constantly changes in two directions (with a certain angle, rather than two opposite directions). DNA molecules are negatively charged and will move toward the positive electrode. Relatively small molecules can quickly change the direction of movement after the electric field is switched, while larger molecules are more difficult to turn in the gel. Therefore, small molecules move forward faster than large molecules. Pulsed field gel electrophoresis can be used to separate DNA molecules ranging in size from 10kb to 10Mb.

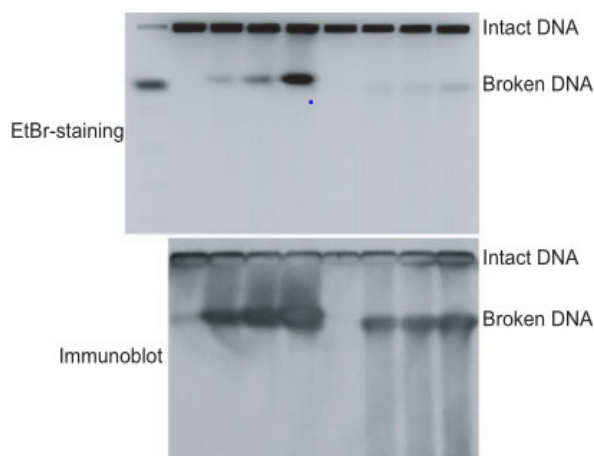


Figure 3. Pulse field gel electrophoresis

Mondal, the Indian Institute of Chemistry, uses a comet test combined with DNA fluorescently labeled probes to detect specific DNA damage. In the comet test, cells are embedded in agarose, lysed and untwisted, and electrophoresed in a buffer solution. Observed by fluorescence microscope, undamaged DNA is attached to the nuclear matrix and exists in the form of nucleoids, while the damaged DNA is derived from nucleoids. Diffuse out and move towards the anode.

Goutham of the University of Manipal in India relied on centrifugation, fluorescent dyes and microfiltration devices to place the cells on the filter membrane and dissolve them with a solution containing detergent, leaving only DNA fragments for observation.

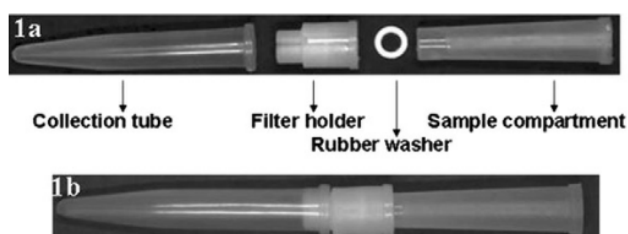


Figure 4. Centrifugal instrument

In addition to the above methods, there is also a ligation-mediated polymerase chain reaction method. This method uses a ligation reaction to attach double-stranded common adapters to both ends of the broken DNA fragments, and then use common primers to amplify all broken DNA fragments, and then observe the amplified DNA fragments.

2.2. DNA Model

The earliest used and most mature DNA model is the cylindrical volume model. This model equates the DNA double helix as a solid cylinder with a diameter of 2.3 nm, in which the small solid cylinder with an inner diameter of 1 nm represents a base pair; The remaining outer part represents the two DNA backbones (sugar phosphates). The cylinder is equivalent to the DNA spiral structure.

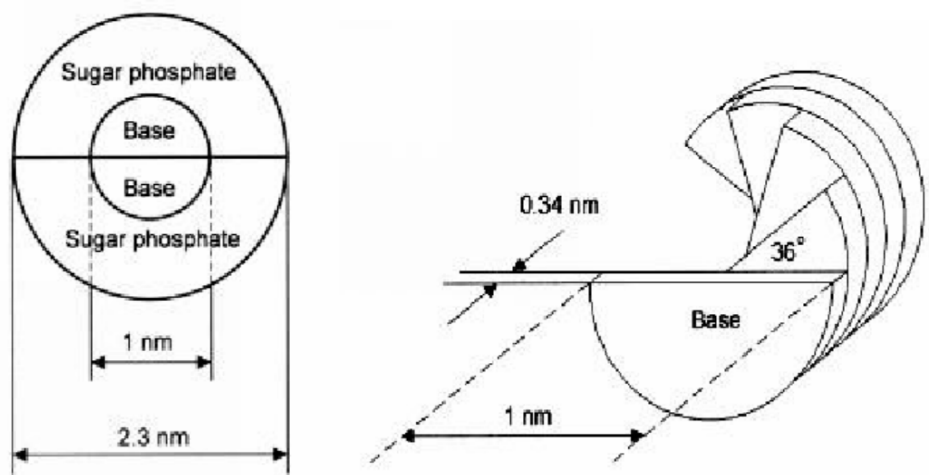


Figure 5. Cylindrical volume model

At the same time, the big round bead is subdivided into small slices of 0.34nm thickness, and each small slice is equivalent to the thickness of a base pair; Each adjacent small slice sequence rotates 36° along the central axis of the cylinder in turn.

For the placement of the cylindrical spiral structure. First set up a virtual sphere, and then set the particles in the center of the sphere. The size of this virtual sphere can ensure that all the particles are deposited in it. In the sphere, DNA fragments with random directions and random positions are set, and there is a cylindrical shell of free radicals with a diameter of 4nm outside the DNA. The purpose is to be able to divide the range of diffuse free radicals that may cause DNA damage.

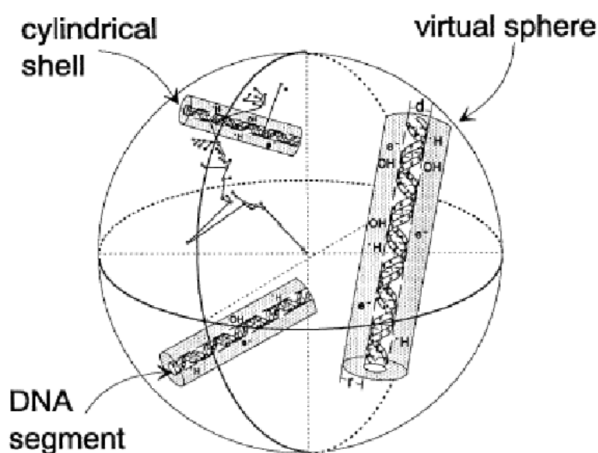


Figure 6. Cell model

The second is the nucleosome model proposed by Santos MD, which is formed by a cylinder with a radius of 1.2nm and a total length of 200bp. The 166 base pairs in the middle represent the core DNA, and the 17 at both ends represent the linker DNA; The material is water, with a density of 1.7g/cm^3 , and two turns are formed around another cylinder with a radius of 3.26nm and a height of 5.7nm, with a spacing of 2.7nm; the material is water with a density of 1.3g/cm^3 , and the cylinder is Central histone.

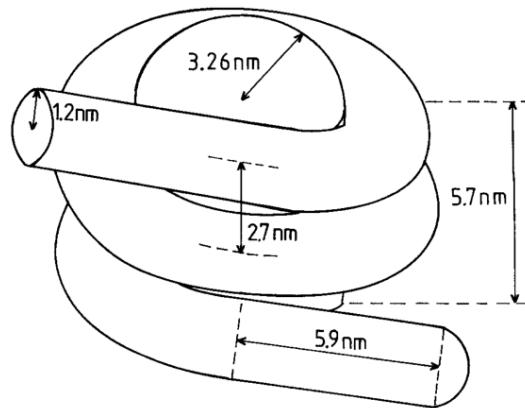


Figure 7. Nucleosome Model

The DNA model established by Nikjoo and Girard is encapsulated in the PHITS and KURBUC programs. The blue part is loop representing DNA, and the 30nm solenoid fiber is 7.5~175kbp in length; The red part is the factory, and the 50nm sphere contains the loop; the green part is the domain, and the size is generally determined by the number of factories. Two domains form a chromosome, so there are 46 chromosomes in an atomic nucleus, which means 92 domains.

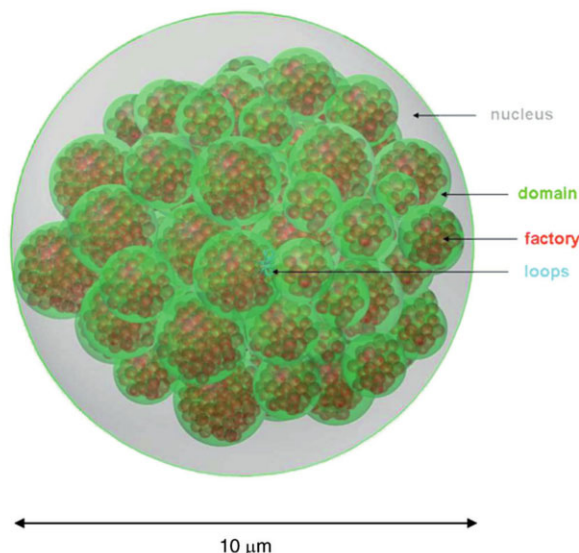


Figure 8. DNA Model

3. Introduction of MCDS Software

MCDS is a simulation software based on Monte Carlo experiment method, which can simulate DNA radiation damage by setting cell and radiation parameters.

Parameter setting of MCDS software

There are mainly 4 parameters in the MCDS program, among which the CELL parameter mainly determines the cell characteristics and parameters. The main setting method is CELL: DNA={R8} NDIA={R8} CDIA={R8} WEM={R8}; Wherein DNA is the content of DNA in the nucleus; NDIA is the diameter of the nucleus; CDIA is the diameter of the cell; WEM is the equivalent water distance;

SIMCON is an analog control parameter, the main setting method is SIMCON: seed={I} nocs={I}; Where seed is the seed of the random number generator; nocs is the number of MC simulations; RADX is the radiation parameter, the main setting method is RADX: FN={C240} PAR={C5} KE={R8} MeV/A={R8} AD={R8}; Among them, FN is to set the mixed radiation field; PAR is to

set the particle type; KE is to set the kinetic energy of the particle; MEV/A is to set the kinetic energy of each nucleus; AD is the absorbed dose.

EVO2 is the ambient oxygen concentration, the main setting method is EVO2: $Po2=\{R8\}$ $mmHg=\{R8\}$ $m0=\{R8\}$ $k=\{R8\}$ $q=\{R8\}$ $r=\{R8\}$. Where $Po2$ is the percentage of oxygen concentration; $mmHg$ is an alternative method of oxygen concentration; $m0$ is the maximum OER induced by SSB; k is the concentration when half of the maximum OER occurs; q is the first parameter related to the temporary correction of radiation quality; r is the first parameter related to the temporary correction of radiation quality;

4. DNA Damage Simulation Results

This article mainly uses two methods to study cell radiation damage. One is based on Geant4 to simulate particle tracks and energy deposition in water with different energies, and propose a DNA base distribution calculation method, which combines the energy deposition points of protons to simulate cell radiation damage; The other is to use MCDS programming software to simulate the radiation damage of proton DNA.

4.1. Geant4's Method of Simulating Radiation Damage

In 1994, led by CERN, hundreds of scientists from all over the world jointly developed the Monte Carlo simulation calculation data package Geant4 (Geometry And Tracking) based on C++ programming. [3] Geant4 can simulate the reaction process of different particles and substances, and collect the particle's motion track and energy loss, and then calculate the dose and position distribution. Geant4 has a high degree of freedom, from radioactive sources, physical processes to detectors, which can be set according to your own requirements to meet the simulation requirements under various conditions. In addition, Geant4, as an open source program, can add external interfaces to process the data obtained by simulation, such as the data processing toolkit ROOT, [4] which is also developed for CERN, and is a software for simulation of gas detectors. Garfield++, [5] Soildworks^[6] based on 3D modeling, etc.

Using water as an alternative medium for cells, Geant4-DNA currently only has the cross section of the substance and water reaction, so the distribution of macromolecules is not considered. Focus on the spatial distribution of base pairs, and then compare it with the proton track to study DNA damage effects. Set the base pair radius r_1 , the damage radius r_2 of the radiation effect, and draw a circle with r_1 and r_2 as the radius, and construct the damage location in the overlapping part of multiple circles. As shown in Figure 9.

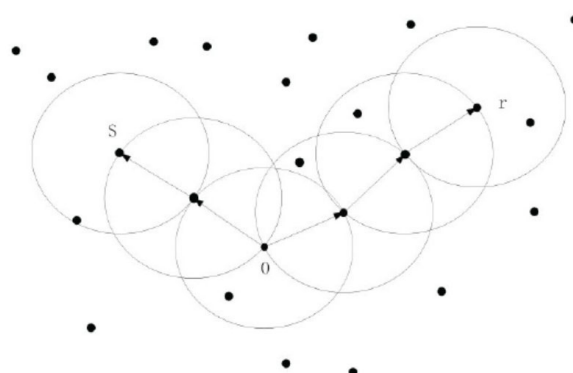


Figure 9. Radiation damage geometric construction model

After constructing the DNA damage model, Geant4 was used to simulate the particle tracks of protons in water, as shown in Figure 10.

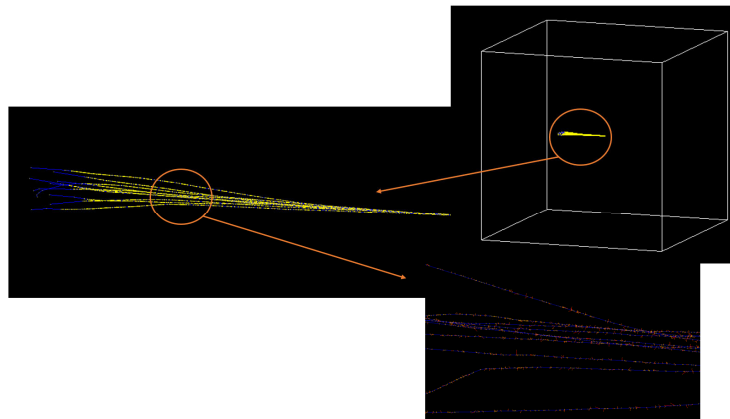


Figure 10. Geant4 simulates the track of protons in water

Combining the energy deposition and position of the protons, the points exceeding the damage threshold are judged, and the overlapping areas in the geometric model are compared to judge the degree of DNA radiation damage.

4.2. MCDS Cell Radiation Damage Simulation Results

Because the proton beam energy used in proton therapy is 70-235MeV, it simulates the radiation damage of cells under this range of energy conditions. These include DNA single-stranded radiation damage, DNA double-stranded radiation damage, and clustered damage. Set the simulation parameters as: CELL: DNA=1 ndia=5 cdia=10; the simulation results are shown in Table 1.

Table 1. MCDS cell radiation damage simulation results

ENERGY (Mev)	DSB	SSB	OTHER	ALL CLUSTERS
70	8.418430E+00	1.883760E+02	4.235120E+02	6.203060E+02
90	8.402930E+00	1.885120E+02	4.244160E+02	6.213310E+02
110	8.355880E+00	1.885990E+02	4.249250E+02	6.218800E+02
130	8.341030E+00	1.886780E+02	4.252740E+02	6.222930E+02
150	8.314980E+00	1.887290E+02	4.255350E+02	6.225790E+02
170	8.309900E+00	1.887730E+02	4.258370E+02	6.229200E+02
190	8.308900E+00	1.887880E+02	4.260060E+02	6.231020E+02
210	8.317150E+00	1.887630E+02	4.261650E+02	6.232450E+02
230	8.297350E+00	1.888150E+02	4.261840E+02	6.232960E+02
250	8.305170E+00	1.888030E+02	4.262470E+02	6.233550E+02

From the results, it can be seen that with the increase of energy, DNA double-strand radiation damage is continuously decreasing, and DNA single-strand radiation damage is continuously increasing, but the magnitude of increase and decrease is less. In order to solve the problem of small change amplitude, simulation was performed at the cell level at the same time to reduce the energy of protons. The simulation results are shown in Figures 11.

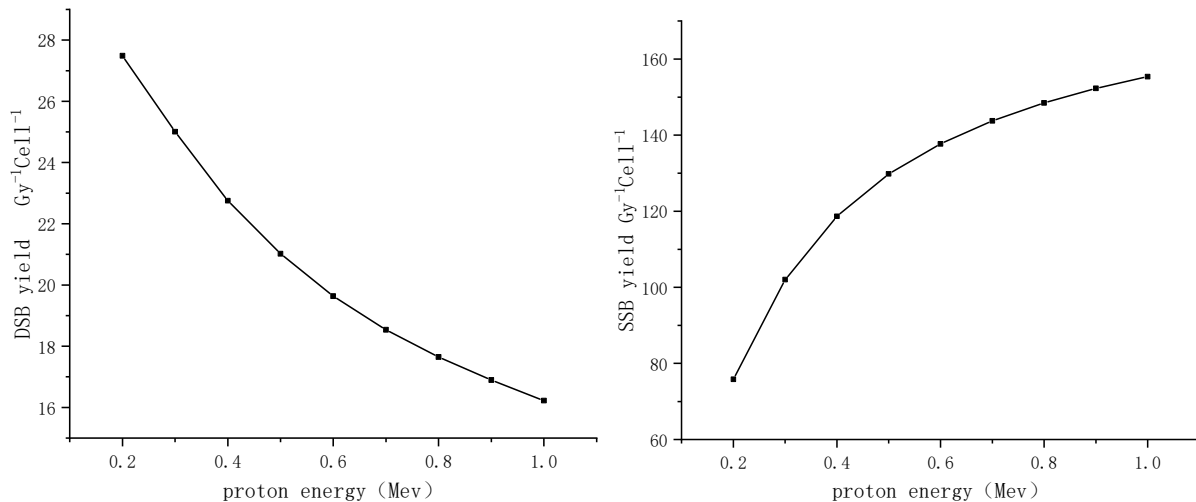


Figure 11. DNA single-stranded and double-stranded radiation damage results

It can be seen from the results that as the energy of protons increases, the energy deposited by protons across the cell scale will decrease, which leads to a decrease in DSB yield and an increase in SSB yield, which is in line with the existing experimental results.

5. Summary and Outlook

This article introduces two methods for judging cell DNA radiation damage through investigation. Geant4 is used to simulate the trajectory and energy deposition of protons in water. A base pair space model combined with Geant4 simulation results is proposed to obtain the final cellular DNA radiation damage. And use MCDS to simulate DNA single-strand radiation damage and double-strand radiation damage to provide a theoretical basis for future proton therapy.

References

- [1] Lin Hui, Xu Yuanying, Wu Dongsheng, Jing Jia, Xu Liangfeng. Application of Monte Carlo simulation in cell radiation damage[J]. Journal of Radiation Research and Radiation Proces, 2009, 27(04): 218-223.
- [2] Yuan Yaping, Park Tiefu, Xu Yaokui, Li Guoquan. Research progress in DNA damage, repair and mutation [J]. Journal of Jilin Agricultural University, 1995(03): 91-95.
- [3] Allison J, Amako K, Apostolakis J, et al. Geant4 developments and applications[J]. IEEE Transactions on Nuclear Science, 2006, 53:270-278.
- [4] Kasanda E et al. GEANT4 simulation of a range verification method using delayed γ spectroscopy of a ^{92}Mo marker[J]. Physics in medicine and biology, 2020, 65: 245047-245047.
- [5] Dorothea Pfeiffer, Lennert De Keukeleere, Carlos Azevedo, et al. Interfacing Geant4, Garfield++ and Degrad for the simulation of gaseous detectors[J]. Nuclear Inst. and Methods in Physics Research, 2019, 04:1-10.
- [6] Li Jingkui, Jing Yingyi. Parametric design based on SolidWorks[J]. Journal of Tianjin University of Technology, 2009, 25:26-28.