Scanning force microscopy studies of X-ray-induced double-strand breaks in plasmid DNA

M. Brezeanu · F. Träger · F. Hubenthal

Received: 23 June 2008 / Accepted: 12 January 2009 /
Published online: 26 February 2009
© Springer Science + Business Media B.V. 2009

Abstract We present an analysis of X-ray-induced damage in Φ X174 plasmid DNA, applying doses between $D = 250$ and $1,500$ Gy. To analyse this damage in detail, the distribution of plasmid fragments after irradiation have been determined by scanning force microscopy. The results show that even for the lowest dose of $D = 250$ Gy, a significant amount of double-strand breaks are observed. For increasing dose, the percentage of small fragments increases and is accompanied by a shortening of the average fragment length from $\langle L \rangle = 1,400$ nm for a dose of $D = 250$ Gy to $\langle L \rangle = 1,080$ nm after irradiation with $D = 1,500$ Gy. The most crucial parameter, the average number of double-strand breaks per broken plasmid ($\langle \text{DSB}_b \rangle$) has been determined for the first time for the applied doses. The results show that the average number of DSBs per broken plasmid $\langle \text{DSB}_b \rangle$ increases almost linearly from a value of $\langle \text{DSB}_b \rangle = 1.3$ after irradiation with $D = 250$ Gy to $\langle \text{DSB}_b \rangle = 1.7$ after exposure to $D = 1,500$ Gy. The presented results show that the amount of DSBs induced by X-ray radiation in plasmid DNA can be calculated with high accuracy by means of scanning force microscopy, providing relevant information regarding the interaction of X-rays with DNA molecules.

Keywords SFM · Plasmid · DNA · X-rays · DSBs

1 Introduction

DNA can be considered the most important biological macromolecule, since it essentially contains all the genetic information necessary for life. Since DNA is also the principal

M. Brezeanu (✉) · F. Träger · F. Hubenthal
Institute of Physics and Center for Interdisciplinary Nanostructure Science and Technology—CINSaT,
University of Kassel, Heinrich-Plett-Str. 40, 34132 Kassel, Germany
e-mail: brezeanu@physik.uni-kassel.de

target for biological effects of ionising radiation, investigation of DNA damage has attracted much attention in recent years [1–9]. After irradiation, much serious damage, such as single-strand breaks, double-strand breaks or base damage can occur in a DNA molecule. The most serious damage is double-strand breaks (DSBs), i.e., the rupture of both DNA strands within a distance of only few base pairs. If this damage remains unrepaired or is misrepaired, it can cause cell death, carcinogenesis or mutation.

Ionising radiation is usually classified into sparsely and densely ionising radiation. Sparsely ionising radiation, e.g. X-rays, induces basically indirect damage in DNA structure mostly by free hydroxyl radicals, resulting from the ionisation of the surrounding water molecules [10–13]. These free radicals are able to induce mainly single strand-breaks (SSBs), i.e. only one of the DNA strands is broken. In contrast, densely ionising radiation, such as particle radiation, causes indirect as well as direct damage and, thus, the most serious damage, i.e. DSBs, occurs [6–9, 13, 14].

A standard method to observe SSBs and DSBs is to investigate conformational changes of plasmid DNA. While an intact plasmid is supercoiled, its conformation changes to relaxed circular, when at least one strand is broken, or to linear, when a DSB occurs [13]. To analyse these damages, electrophoresis methods [14–17] are usually applied. These common methods can provide information about the percentages of supercoiled (SC), relaxed circular (RC) and linear plasmid fragments (LF) or about the percentages of linear fragments within a certain length range. However, by electrophoresis methods, it is impossible to obtain simultaneously, and with high accuracy, the percentages of different plasmid conformations and the fragment distributions in a broad range, i.e. from several tens to thousands of base pairs. The reason is that a high concentration of each fragment length is necessary for electrophoresis measurements and cannot be obtained by sparsely ionizing X-ray irradiation with doses below $D = 1,500$ Gy. In addition, electrophoresis analysis is not a direct determination but relies on a model (and a calculation) in order to obtain the distributions [15–18]. These drawbacks can be overcome by scanning force microscopy (SFM) measurements. They permit direct visualisation and precise measurement of the length of individual DNA fragments with an accuracy of few nanometers. Thus, detailed investigation of fragment distributions yields information about the occurrence of multiple¹ DSBs in Φ X174 plasmid DNA.

Nucleic acids and especially DNA have been extensively investigated with SFM [5–9, 19–23], since its invention by Binning and collaborators in 1986 [24]. So far, SFM studies of DNA focused in particular on visualising the DNA structure [19, 25, 26], manipulating DNA on surfaces [27, 28] or using it as a conductive material for future molecular electronics [29–31]. In contrast, few SFM studies of X-ray-induced fragmentation of DNA molecules have been published [7–9]. In particular, for doses of less than $D = 1,500$ Gy, detailed SFM investigations of fragment distributions and a precise analysis of the induced DSBs are still lacking.

The aim of this contribution is the direct observation of DSBs in plasmid DNA, induced by X-ray irradiation with doses below $D = 1,500$ Gy. Here, we have analysed and quantified the fragment distributions of Φ X174 plasmid DNA by means of SFM. The distributions

¹The expression *multiple* means, that more than one DSB have occurred in the investigated Φ X174 plasmid DNA with 5,386 bp. However, the number of induced DSBs per broken plasmid depends on the length and, thus, on the number of base pairs of a plasmid. Therefore, the expression multiple refers only to Φ X174 plasmid DNA. For a detailed comparison with published data, the average number of DSBs per mega base pair will be given in Section 3.

have been measured for plasmids irradiated with X-rays for doses ranging from $D = 250$ and 1,500 Gy. We show that after irradiation, even with the lowest dose of $D = 250$ Gy, a significant amount of short DNA fragments occurs.

2 Materials and methods

The Φ X174 plasmid DNA (5,386 bp, length $\approx 1.83 \mu\text{m}$, Promega GmbH, Germany) has been used as purchased and was afterwards diluted to a concentration of $C = 25 \text{ ng}/\mu\text{l}$ in 20 mM HEPES radioprotective buffer solution. Five Eppendorf tubes were prepared for irradiation, each with 20 μl solution. One tube remained non-irradiated and was used as a control solution, while the other four tubes were irradiated with X-rays at doses of $D = 250$, 500, 1,000 and 1,500 Gy, respectively. The irradiation has been performed under anoxic conditions using an X-ray generator operated at 250 kV, with a dose rate of 7 Gy/min. During the irradiation, the DNA samples were stored in ice.

For the agarose gel electrophoresis measurements, we have used a similar method as those described by Milligan et al. [18] and Taucher-Scholz and Kraft [32]. Using a G-box system from Syngene Europe, the image of the gel was recorded with a resolution of 512×512 pixels and 8 bits/pixel, in 1/3 s. The Gene Tools software package (Syngene Europe) was used to analyse the amount of plasmid DNA in each of its three forms.

For the SFM measurements, parts of the non-irradiated control solution as well as of the irradiated plasmid solutions have been further diluted in 10 mM MgCl_2 and 10 mM HEPES, to a DNA-concentration of $C = 0.5 \text{ ng}/\mu\text{l}$ and deposited on freshly cleaved mica, which served as substrate. The MgCl_2 has been added in order to produce Mg^{2+} cations, which bind the negatively charged backbone of the DNA molecule to the negatively charged surface of the mica after its cleavage [19, 22, 26]. The concentration of MgCl_2 has been chosen high enough to bind a large amount of fragments on the mica substrates. For each sample, 3 μl of the diluted solution has been deposited on a mica substrate and rested in air for several minutes. Afterwards, the substrates have been rinsed with double distilled water and gently dried in a nitrogen stream. Five samples have been prepared for the control as well as for each irradiated solution. Ten images per sample have been recorded by SFM. The images were recorded in tapping mode in air at a relative humidity of 35–40% and at room temperature, using a CP-Research microscope from Veeco Instruments. The scanning area was $3 \times 3 \mu\text{m}$ and the resolution 512×512 pixels. Tips with an average frequency of 325 kHz, from MikroMasch, have been used. The length of the fragments was analysed for each image with ImageJ software from NIH, USA [33].

3 Experimental data and discussion

As explained before, an SC plasmid transforms into the RC form, when it suffers at least one SSB, or into LF, if a DSB occurs. Figure 1 shows the percentages of these three plasmid conformations as a function of dose, obtained by means of gel electrophoresis. From these measurements we have calculated that the control solution contained 73% supercoiled and 27% relaxed circular plasmids, but no linear fragments. A rapid increase of the percentage of the RC form to 75% is observed for a dose of $D = 250$ Gy, followed by a saturation at 87% at higher doses. This increase is accompanied by a similar drastic decrease of the SC form from initially 73% to 0% at $D = 1,000$ Gy. In contrast, the percentage of LFs increases

Fig. 1 Percentages of RC, LF and SC forms, after irradiation with X-rays, as a function of dose

only moderate from 2% at the dose of $D = 250$ Gy to 14% for the highest applied dose. However, in particular, in the downstream direction the LF bands appeared very unclear in the gel electrophoresis images. The unclear regions, which could not be quantified, result very likely from small linear fragments of different lengths, produced by multiple DSBs in a single plasmid.

Before investigating the fragment distributions and the induced DSBs in the irradiated plasmid DNA, we verified that the SFM studies are consistent with the electrophoresis measurements. For this purpose, 20 SFM images of the non-irradiated control solution were analysed. A typical SFM image of the control solution is depicted in Fig. 2. In all obtained images, no linear fragments have been observed, in accord with the electrophoresis measurements. Therefore, we can exclude systematic errors because of DSBs that could have been induced due to the SFM imaging.

To investigate the induced DSBs, we have performed a detailed SFM analysis of the linear fragments of the irradiated samples. As mentioned before, $3.0 \mu\text{l}$ of each plasmid solution, corresponding to 1.5 ng of plasmid DNA, has been deposited on mica substrates and subsequently imaged. Figure 3 depicts four SFM images of plasmid DNA after irradiation with doses of $D = 250$, 500, 1,000 and 1,500 Gy, respectively. Although the number of observed plasmids per image is different,² the general trend, i.e., increasing fragmentation for increasing dose, can be observed.

In order to determine the fragment distribution of the irradiated plasmids, the length of each plasmid fragment has been measured with ImageJ software. To obtain sufficient statistical accuracy, 652 fragments were analysed (48 fragments for the sample irradiated with $D = 250$ Gy, 225 fragments for $D = 500$ Gy, 185 fragments for $D = 1,000$ Gy and 194 fragments for $D = 1,500$ Gy).

Histograms, showing with the probability of producing (measuring) a fragment with a certain length at different irradiation doses, are presented in Fig. 4. Although fragments

²The number of observed plasmids per image is different, because the DNA is statistically distributed over the entire sample and SFM images have been recorded randomly.

Fig. 2 SFM image of plasmid DNA of the control solution

have been measured with a resolution of 10 nm, for clarity, each column in the histogram represents fragments in a length interval of 100 nm. For example, the columns centered at $L = 1,700$ nm contain all measured fragments with lengths between $L = 1,650$ and $1,750$ nm. With this notation, the columns at $L = 1,800$ nm correspond to the full contour length $L = 1,831$ nm of the $\Phi X174$ plasmid.

The histograms show that the amplitude at $L = 1,800$ nm, i.e., the percentage of plasmids with only one DSB, drops off steadily for increasing dose. Further analysis reveals that the percentage of fragments with lengths between 100 and 600 nm increases from 7.8% after irradiation with $D = 250$ Gy to 20.0% after irradiation with $D = 1,500$ Gy. However, even for the lowest dose of $D = 250$ Gy, a significant number of short fragments are observed, demonstrating that multiple DSBs have occurred in some plasmids. This result confirms our former expectation that short plasmid fragments have been produced and that these fragments are responsible for the smeared gel electrophoresis images. From the data presented in Fig. 4, the average fragment length $\langle L \rangle$ has been calculated using the following equation:

$$\langle L \rangle = \frac{\sum_{i=1}^n L_i}{n}, \quad (1)$$

where L_i is the length of an individual fragment, and n is the number of counted fragments. The obtained results are depicted in Fig. 5 as a function of dose.

Based on the histograms of the fragment distributions, a decrease of average fragment length from $\langle L \rangle = 1,400$ to $1,080$ nm is observed (Fig. 5), i.e., the number of DSBs per plasmid increases for increasing dose. This is consistent with data already presented in the literature [8, 34], where, in general, a decrease of the average length of plasmid fragments for increasing dose has been observed. In particular, in the dose range between $D = 500$ and $1,500$ Gy, our results for the average fragment length coincide to within 15% with the values given of Psonka et al. [8].

Fig. 3 SFM images of plasmid DNA after irradiation with X-rays with doses of $D = 250$ Gy (**a**), $D = 500$ Gy (**b**), $D = 1,000$ Gy (**c**) and $D = 1,500$ Gy (**d**)

For further detailed analysis, the average numbers of DSBs per plasmid ($\langle \text{DSB}_t \rangle$) and per broken³ plasmid ($\langle \text{DSB}_b \rangle$) have been calculated using the method by Pang et al. [35]:

$$\langle \text{DSB}_t \rangle = n / \left[\left(\sum_{i=1}^n L_i / \hat{L} \right) + N_u \right], \quad (2)$$

$$\langle \text{DSB}_b \rangle = n / \left(\sum_{i=1}^n L_i / \hat{L} \right), \quad (3)$$

³In our nomenclature, a broken plasmid is a plasmid that has suffered at least one DSB. Plasmids which have suffered only single strand breaks and plasmids without any strand break are called unbroken.

Fig. 4 Histograms of the fragment distributions of plasmids after irradiation with X-rays at doses of $D = 250$, 500, 1,000 and 1,500 Gy. No fragments have been observed for the control solution ($D = 0$ Gy), and, thus, no fragment distribution can be displayed. For details, see text

where N_u is the total number of unbroken plasmids (SC and RC forms), counted from the AFM images, and $\hat{L}$ is the length of an unbroken plasmid. The results are plotted as a function of dose in Fig. 6.

Figure 6a depicts the number of DSBs per plasmid ($\langle \text{DSB}_t \rangle$) as a function of dose. It shows that no DSBs have been registered for the control solution (non-irradiated solution, $D = 0$ Gy). After irradiation, the number of DSBs per plasmid increases quickly to $\langle \text{DSB}_t \rangle = 0.19$ for the dose of $D = 500$ Gy. For higher doses, a further, though less steep, increase to a value of $\langle \text{DSB}_t \rangle = 0.28$ for the highest dose of $D = 1,500$ Gy is observed. The values are rather small but are in the expected range for the applied doses. For example, Murakami et al. [34] found values of $\langle \text{DSB}_t \rangle = 0.14$, 0.21 and 0.71 for doses of $D = 1,900$, 5,600 and 8,300 Gy, using gel electrophoresis methods. These values are in good agreement with our results obtained with gel electrophoresis, where the percentage of $\langle \text{DSB}_t \rangle$ amounts to $\langle \text{DSB}_t \rangle = 0.1$ for a dose of $D = 1,500$ Gy (Fig. 1).

Fig. 5 Determined average fragment length plotted as a function of dose. The line is to guide the eye

Fig. 6 Number of DSBs per plasmid (**a**) and per broken plasmid (**b**) after irradiation with doses between $D = 250$ and $1,500$ Gy. The *lines* are to guide the eye. Note the difference in the Y-scale. For the control solution ($D = 0$ Gy), no broken plasmid has been observed and consequently, no data can be given in (**b**). The *errors bars* for $\langle \text{DSB}_b \rangle$ are smaller than the data points (*solid squares*)

In order to compare our experimental results with published gel electrophoresis data, we have calculated the average number of DSBs ($\hat{A}$) per mega base pair (Mbp) and dose, as follows:

$$\hat{A} = \frac{\langle \text{DSB}_t \rangle}{N_b D}, \quad (4)$$

where D is the irradiation dose and N_b represents the number of mega base pairs of the ΦX174 plasmid used in this study.

From the $\langle \text{DSB}_t \rangle$ values presented Fig. 6a, we get an average number of $\hat{A} = (0.054 \pm 0.012)$ DSBs/(MbpGy). This result is in the expected range and consistent with the findings of Jones et al. [36]. They found a value of $\hat{A} = 0.052$ DSBs/(MbpGy). However, $\hat{A}$ depends strongly on the experimental methods, and in most cases, direct comparison is not possible.

It was shown in a recent review article by Terato [37], that $\hat{A}$ varies from around $\hat{A} = 0.006$ DSBs/(MbpGy) [3, 38, 39] to $\hat{A} = 0.14$ DSBs/(MbpGy) [40] for X-ray irradiation.

The more interesting parameter is $\langle \text{DSB}_b \rangle$, because its value reflects the average amount of DSBs per broken plasmid and, thus, the occurrence of multiple DSBs. As depicted in Fig. 6b, the average number of DSBs per broken plasmid increases from $\langle \text{DSB}_b \rangle = 1.3$ for the dose of $D = 250$ Gy to $\langle \text{DSB}_b \rangle = 1.7$ for a highest dose of $D = 1,500$ Gy. The results demonstrate that, even for the lowest X-ray dose, a significant number of plasmids have suffered more than one DSB. Although it is obvious that for doses close to $D = 250$ Gy, the value of $\langle \text{DSB}_b \rangle$ must be higher than 1, it is hard to estimate how the value of $\langle \text{DSB}_b \rangle$ evolves for doses significantly below $D = 250$ Gy. The data presented in Fig. 6 show that the linear dependence of $\langle \text{DSB}_b \rangle$ as a function of dose holds only for doses between $D = 500$ and 1,500 Gy.

A detailed analysis of the data presented in Figs. 1 and 6 reveals that more DSBs are measured by means of SFM, as expected from the gel electrophoresis measurements. For example, for a dose of $D = 250$ Gy only 2% of linear fragments have been observed by gel electrophoresis. Taking the value of $\langle \text{DSB}_b \rangle = 1.3$ for this dose into account, one would determine a value of $\langle \text{DSB}_t \rangle = 1.3 \times 2\% = 0.026$. This is significantly less than $\langle \text{DSB}_t \rangle = 0.07$ obtained from the SFM analysis. The reason for this difference is that, with gel electrophoresis measurements not all DSBs are counted. It has been mentioned before that the LF bands in the gel electrophoresis images were smeared in the downstream direction and that the smeared region could not be quantified. Hence, small plasmid fragments are not measured and consequently the calculated percentage of LF given in Fig. 1 is too small. In contrast, with SFM all linear fragments, independent of their length, are detected. Thus, the amount of DSBs per plasmid obtained by means of SFM is significant higher. This result demonstrates clearly that, for a precise analysis of the number of induced DSBs, SFM has to be applied.

In order to determine whether the multiple DSBs have been induced by multiple hydroxyl radicals attacks or if perhaps a single radical causes more than one strand break, the dose response curve for SSBs has been obtained from the data presented in Fig. 1 and is displayed in Fig. 7. Subsequently, the dose causing an average of one SSB in a single plasmid (D37)

Fig. 7 Dose response curve of loss of supercoiled form

has been determined from the dose response curve. Taking into account that the initial solution already contained 27% of plasmids with SSBs, we extract a D37 value of 240 Gy, which is smaller than the smallest dose used in this study. Hence, doses between $D = 250$ to 1,500 Gy cause significantly more than one SSB in a plasmid. Consequently, multiple DSBs are induced not by a single hydroxyl radical but rather by multiple attacks of radicals to one plasmid. To the best of our knowledge, the average number of X-ray induced DSBs per broken plasmid $\langle \text{DSB}_b \rangle$ has not been determined before. Thus, comparison of the number of DSBs per broken plasmid presented here with data previously presented in the literature is not possible at present.

4 Conclusions

In this contribution, we have presented investigations of X-ray-induced fragmentation of ΦX174 plasmid DNA in 20 mM HEPES radioprotective environment, using doses between $D = 250$ and 1,500 Gy. After irradiation, SFM was applied for a detailed analysis of the plasmid fragment distributions. We have shown that X-ray radiation at doses between $D = 250$ and 1,500 Gy induces a significant amount of DSBs, enough to quantify the fragments by means of SFM. From the detailed analysis of the fragment distributions, the average fragment length has been determined as a function of dose. We observed a steady decrease from $\langle L \rangle = 1,400$ to 1,080 nm. Subsequently, the average numbers of DSBs per plasmid ($\langle \text{DSB}_t \rangle$) and per broken plasmid ($\langle \text{DSB}_b \rangle$) have been determined based on the SFM data. While the average number of DSBs per plasmid shows a strong initial increase up to a dose of $D = 500$ Gy and a further moderate increase as a function of dose, the average number of DSBs per broken plasmid increases almost linearly but moderately from $\langle \text{DSB}_b \rangle = 1.3$ at $D = 250$ Gy to $\langle \text{DSB}_b \rangle = 1.7$ at $D = 1,500$ Gy. In addition, the average number of DSBs per mega base pair and dose ($\hat{A}$) has been determined in order to compare our results with published data. We found a value of $\hat{A} = (0.054 \pm 0.012)$ DSBs/(MbpGy). In summary, the results demonstrate the biological impact of X-ray radiation on plasmid DNA molecules, at doses between $D = 250$ and 1,500 Gy, by quantifying by means of SFM the amount of multiple DSBs induced in single ΦX174 plasmid.

Acknowledgements The authors acknowledge financial support from GSI—Darmstadt under the project KS/HUB. We are thankful to Katarzyna Psonka, from the Department of Biophysics from GSI—Darmstadt, for technical support.

References

1. Brons, S., Taucher-Scholtz, G., Scholz, M., Kraft, G.: A track structure model for simulation of strand breaks in plasmid DNA after heavy ion irradiation. *Radiat. Environ. Biophys.* **42**, 63–72 (2003)
2. von Sonntag, C.: *Free-Radical-Induced DNA Damage and Its Repair: A Chemical Perspective*. Springer, New York (2006)
3. Durante, M.: Focus on heavy ions in biophysics and medical physics. *N. J. Phys.* **10**, 075002 (2008). doi:[10.1088/1367-2630/10/7/075002](https://doi.org/10.1088/1367-2630/10/7/075002)
4. Ke, C., Jiang, Y., Mieczkowski, P.A., Muramoto, G.G., Marszalek, P.E.: Nanoscale detection of ionizing radiation damage to DNA by atomic force microscopy. *Small* **4**(2), 288–294 (2008). doi:[10.1002/sml.200700527](https://doi.org/10.1002/sml.200700527)
5. Pang, D., Rodgers, E.J., Berman, B.L., Chasovskikh, S., Dritschilo, A.: Spatial distribution of radiation-induced double-strand breaks in plasmid DNA as resolved by atomic force microscopy. *Radiat. Res.* **164**, 755–765 (2005). doi:[10.1667/RR3425.1](https://doi.org/10.1667/RR3425.1)

6. Brezeanu, M., Taucher-Scholz, T., Psonka, K., Träger, F., Hubenthal, F.: SFM studies of carbon ion induced damages in plasmid DNA. *J. Mol. Rec.* **20**(6), 502–507 (2007). doi:[10.1002/jmr.852](https://doi.org/10.1002/jmr.852)
7. Boichot, S., Fromm, M., Cuniffe, S., O'Neill, P., Labrune, J.C., Chambaudet, A.: Investigation of radiation damage in DNA by using atomic force microscopy. *Radiat. Prot. Dos.* **99**(1–4), 143–145 (2002)
8. Psonka, K., Brons, S., Heiss, M., Gudowska-Nowak, E., Taucher-Scholz, G.: Induction of DNA damage by heavy ions measured by atomic force microscopy. *J. Phys. Condens. Matter* **17**, 1443–1446 (2005). doi:[10.1088/0953-8984/17/18/002](https://doi.org/10.1088/0953-8984/17/18/002)
9. Psonka, K., Gudowska-Nowak, E., Brons, S., Elsässer, T., Heiss, M., Taucher-Scholz, G.: Ionizing radiation-induced fragmentation of plasmid DNA – atomic force microscopy and biophysical modelling. *Adv. Space Res.* **39**, 1043–1049 (2007). doi:[10.1016/j.asr.2007.02.089](https://doi.org/10.1016/j.asr.2007.02.089)
10. Chapman, J.D., Reuvers, A.P., Borsia, J., Greenstock, C.L.: Chemical radioprotection and radiosensitization of mammalian cells growing in vitro. *Radiat. Res.* **56**, 291–306 (1973). doi:[10.2307/3573667](https://doi.org/10.2307/3573667)
11. Roots, R., Osaka, S.: Estimation of life times and diffusion distances of radicals involved in X-rays induced strand breaks or killing of mammalian cells. *Radiat. Res.* **64**, 306–320 (1975). doi:[10.2307/3574267](https://doi.org/10.2307/3574267)
12. Ward, J.F.: *The Early Effects of Radiation on DNA*. Springer Verlag, New-York (1991)
13. Hall, E.J.: *Radiobiology for the Radiologist*. Lippincott Company, Philadelphia (1994)
14. Goodhead, D.T.: Mechanism for the biological effectiveness of high-LET radiations. *J. Rad. Res.* **40**(Suppl.), 1–13 (1999). doi:[10.1269/jrr.40.1](https://doi.org/10.1269/jrr.40.1)
15. Smith-Ravin, J., Jeggo, P.A.: Use of damaged plasmid to study DNA repair in X-ray sensitive (xrs) strains of Chinese hamster ovary (CHO) cells. *Int. J. Radiat. Biol.* **56**(6), 951–961 (1989). doi:[10.1080/09553008914552411](https://doi.org/10.1080/09553008914552411)
16. Milligan, J.R., Ng, J.Y.-Y., Wu, C.C.L., Aguilera, J.A., Ward, J.F., Kow, Y.W., Wallace, S.S., Cunningham, R.P.: Methylperoxyl radicals as intermediates in the damage to DNA irradiated in aqueous dimethyl sulfoxide with gamma rays. *Radiat. Res.* **146**, 436–443 (1996). doi:[10.2307/3579305](https://doi.org/10.2307/3579305)
17. Milligan, J.R., Wu, C.C.L., Ng, J.Y.-Y., Aguilera, J.A., Ward, J.F.: Characterisation of the reaction rate coefficient of DNA with hydroxyl radical. *Radiat. Res.* **146**, 510–513 (1996). doi:[10.2307/3579551](https://doi.org/10.2307/3579551)
18. Milligan, J.R., Aguilera, J.A., Ward, J.F.: Variation of single-strand break yield with scavenger concentration for plasmid DNA irradiated in aqueous solution. *Radiat. Res.* **133**, 151–157 (1993). doi:[10.2307/3578350](https://doi.org/10.2307/3578350)
19. Hansma, H.G.: Surface biology of DNA by atomic force microscopy. *Annu. Rev. Chem.* **52**, 71–92 (2001). doi:[10.1146/annurev.physchem.52.1.71](https://doi.org/10.1146/annurev.physchem.52.1.71)
20. Bustamante, C., Vesenska, J., Tang, C.L., Rees, W., Guthold, M., Keller, R.: Circular DNA molecules imaged in air by scanning force microscopy. *Biochem.* **31**(1), 22 (1992). doi:[10.1021/bi00116a005](https://doi.org/10.1021/bi00116a005)
21. Lyubchenko, Y.L., Jacobs, B.L., Lindsay, S.M.: Atomic force microscopy of reovirus dsRNA: a routine technique for length measurements. *Nucleic Acids Res.* **20**(15), 3983–3986 (1992). doi:[10.1093/nar/20.15.3983](https://doi.org/10.1093/nar/20.15.3983)
22. Hansma, H.G., Laney, D.E., Bezanilla, M., Sinsheimer, R.L., Hansma, P.K.: Applications for atomic force microscopy of DNA. *Biophys. J.* **68**, 1672–1677 (1995)
23. Shlyakhtenko, L.S., Gall, A.A., Weimer, J.J., Hawn, D.D., Lyubchenko, Y.L.: Atomic force microscopy imaging of DNA covalently immobilized on a functionalized mica substrate. *Biophys. J.* **77**(1), 568–576 (1999)
24. Binnig, G., Quate, C.F., Geber, C.: Atomic force microscope. *Phys. Rev. Lett.* **56**(9), 930–933 (1986). doi:[10.1103/PhysRevLett.56.930](https://doi.org/10.1103/PhysRevLett.56.930)
25. Muir, T., Morales, M., Root, J., Kumar, I., Garcia, B., Vellandi, C., Jenigian, D., Marsh, T., Henderson, E., Vesenska, J.: The morphology of duplex and quadruplex DNA on mica. *J. Vac. Sci. Technol. A* **16**(3), 1172–1177 (1998). doi:[10.1116/1.581254](https://doi.org/10.1116/1.581254)
26. Bezanilla, M., Manne, S., Laney, D.E., Lyubchenko, Y.L., Hansma, H.G.: Absorption of DNA to mica, silylated mica, and minerals: characterization by atomic force microscopy. *Langmuir* **11**, 655–659 (1995). doi:[10.1021/la00002a050](https://doi.org/10.1021/la00002a050)
27. Severin, N., Barner, J., Kalachev, A.A., Rabe, J.P.: Manipulation and overstretching of genes on solid substrates. *Nano Lett.* **4**(4), 577–579 (2004). doi:[10.1021/nl035147d](https://doi.org/10.1021/nl035147d)
28. Rief, M., Oesterhelte, B., Heymann, B., Gaub, H.E.: Reversible unfolding of individual titin Ig-domains by AFM. *Science* **275**, 1295–1297 (1997). doi:[10.1126/science.275.5304.1295](https://doi.org/10.1126/science.275.5304.1295)
29. Porath, D., Bezryadin, A., de Vries, S., Dekker, C.: Direct measurement of electrical transport through DNA molecules. *Nature* **403**, 635–638 (2000). doi:[10.1038/35001029](https://doi.org/10.1038/35001029)
30. Gutiérrez, R., Mandal, S., Cuniberti, L.: Dissipative effects in the electronic transport through DNA molecular wires. *Phys. Rev. B* **71**, 235116 (2005). doi:[10.1103/PhysRevB.71.235116](https://doi.org/10.1103/PhysRevB.71.235116)
31. Gervasio, F.L., Carloni, P., Parrinello, M.: Electronic structure of wet DNA. *Phys. Rev. Lett.* **89**(10), 108102 (2002). doi:[10.1103/PhysRevLett.89.108102](https://doi.org/10.1103/PhysRevLett.89.108102)

32. Taucher-Scholz, G., Kraft, G.: Influence of radiation quality on the yield of DNA strand breaks in SV40 DNA irradiated in solution. *Radiat. Res.* **151**(5), 595–604 (1999). doi:[10.2307/3580036](https://doi.org/10.2307/3580036)
33. ImageJ. <http://rsb.info.nih.gov/ij/>
34. Murakami, J., Hirokawa, H., Hayata, I.: Analysis of radiation damage DNA by atomic force microscopy in comparison with gel electrophoresis studies. *Biochem. Biophys. Methods.* **44**, 31–40 (2000). doi:[10.1016/S0165-022X\(00\)00049-X](https://doi.org/10.1016/S0165-022X(00)00049-X)
35. Pang, D., Berman, B.L., Chasovskikh, S., Rodgers, E.J., Dritschilo, A.: Investigation of neutron-induced damage in DNA by atomic force microscopy: evidence of clustered DNA lesions. *Radiat. Res.* **150**, 612–618 (1998). doi:[10.2307/3579883](https://doi.org/10.2307/3579883)
36. Jones, G.D.D., Milligan, J.R., Ward, J.F., Calabro-Jones, P.M., Aquilera, J.A.: Yield of strand breaks as a function of scavenger concentration and LET for SV40 irradiated with He ions. *Radiat. Res.* **136**, 190–196 (1993). doi:[10.2307/3578610](https://doi.org/10.2307/3578610)
37. Terato, H., Ide, H.: Clustered DNA damage induced by heavy ion particles. *Biol. Sci. Space* **18**(4), 206–215 (2004). doi:[10.2187/bss.18.206](https://doi.org/10.2187/bss.18.206)
38. Newman, H.C., Prise, K.M., Folkard, M., Michael, B.D.: DNA double-strand break distribution in X-rays and α -particle irradiated V79 cells: evidence for non-random breakeage. *Int. J. Radiat. Biol.* **71**, 347–363 (1997). doi:[10.1080/095530097143978](https://doi.org/10.1080/095530097143978)
39. Wong, R.S.L., Dynlacht, J.R., Cedervall, B., Dewey, W.C.: Analysis by pulsed-field gel electrophoresis of DNA double stand breaks induced by heat and/or X-irradiation in bulk and replicating DNA of CHO cells. *Int. J. Radiat. Biol.* **68**(2), 141–152 (1995). doi:[10.1080/09553009514551041](https://doi.org/10.1080/09553009514551041)
40. Sutherland, B.M., Bennett, P.V., Sidorkina, O., Laval, J.: Clustered DNA damages induced in isolated DNA and human cells by low doses of ionising radiation. *Proc. Natl. Acad. Sci. USA* **97**, 103–108 (2000). doi:[10.1073/pnas.97.1.103](https://doi.org/10.1073/pnas.97.1.103)