

Effects of water on fingernail electron paramagnetic resonance dosimetry

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ABSTRACT

Electron paramagnetic resonance (EPR) is a promising biodosimetric method, and fingernails are sensitive biomaterials to ionizing radiation. Therefore, kinetic energy released per unit mass (kerma) can be estimated by measuring the level of free radicals within fingernails, using EPR. However, to date this dosimetry has been deficient and insufficiently accurate. In the sampling processes and measurements, water plays a significant role. This paper discusses many effects of water on fingernail EPR dosimetry, including disturbance to EPR measurements and two different effects on the production of free radicals. Water that is unable to contact free radicals can promote the production of free radicals due to indirect ionizing effects. Therefore, varying water content within fingernails can lead to varying growth rates in the free radical concentration after irradiation—these two variables have a linear relationship, with a slope of 1.8143. Thus, EPR dosimetry needs to be adjusted according to the water content of the fingernails of an individual. When the free radicals are exposed to water, the eliminating effect will appear. Therefore, soaking fingernail pieces in water before irradiation, as many researchers have previously done, can cause estimation errors. In addition, nails need to be dehydrated before making accurately quantitative EPR measurements.

KEYWORDS: dosimetry, electron paramagnetic resonance, fingernail, ESR

INTRODUCTION

Electron paramagnetic resonance (EPR) dosimetry has been developed over recent years and used on a range of materials [1–3]. Fingernails have drawn more and more of the attention of researchers because of their radiosensitivity and easy collection. In the event that people have been exposed to ionizing radiation, their radiation dose can be estimated by measuring the free radical concentration induced by radiation [4, 5] within their fingernails. It is valuable to be able to do triage among potential patients to improve the use of medical resources and the survival rate of patients. EPR signals of fingernails can be divided into three kinds, based on their origin [6]: five radiation-induced signals (RIS1–5); five mechanically induced signals (MIS1–5); a background signal (BKG). Only BKG and RIS5 can be found after a 10-min soak of the samples [7]. For this phenomenon, some researchers believed that the radicals associated with BKG could be located close to hydrophobic amino acids

or within a polypeptide helix, which prevents the water molecules to eliminate these free radicals. However, BKG and RIS5 have the same *g* factor (2.004). It is difficult to distinguish these two signals based on the EPR spectrum. Researchers from different institutes have developed many methods to determine the relationship between the RIS and the radiation dose. The most common and reasonable method is as follows: (i) collect one individual's fingernails; (ii) divide them into several groups; (iii) irradiate these groups of samples at different doses, starting from 0 Gy; (iv) measure these samples using EPR and obtain the peak-to-peak amplitude (*A_{pp}*) of the EPR signal; (v) assume the EPR signal intensity of the sample irradiated with 0 Gy is the BKG intensity; (vi) subtract the BKG intensity mentioned above from the measurements of other samples irradiated at different doses to obtain the RIS intensity; (vii) establish the relationship between the RIS and the radiation dose [8]. This relationship is the statistical result from many

individuals. The statistical conclusion may be very inaccurate for a particular individual in its present form. So, it is very important to determine which factors play a part in the generation of free radicals that are induced by radiation. Then, based on these factors, the estimated dose can be corrected. Finally, the individual differences will be taken into account, and a relatively accurate radiation dose, not a rough statistical result, will be obtained.

Water plays a significant role not only in life, but also in radiation injury. The effects of ionizing radiation on biological molecules can be divided into two types: direct ionizing effects, which refers to attacks on macromolecules, such as DNA, by photons or charged particles directly; and indirect ionizing effects, which refers to the damaging of biomolecules by reactive oxygen species (ROS) generated from water radiolysis. Indirect damage induced by water radiolysis products is the larger contributor towards biological damage [9]. So, the water content within fingernails is likely to influence the level of free radicals induced by radiation. This article will discuss the effects of water on the generation of free radicals.

MATERIALS AND METHODS

A Bruker A300 EPR spectrometer was used in all these experiments. An ER 4119HS-2100 marker accessory (Bruker, Germany) was used as a spin standard. Each sample was measured three times, and then the average of the three measurements was recorded. The following Apps obtained were relative values, i.e. values in figures equaled the signal intensity of the fingernails divided by the signal intensity of the marker accessory (measured simultaneously). EPR measurements were performed at the X-band. Experiments 1–6 and 8 were performed at room temperature and a relative humidity of 25–35%, and the central magnetic field was 3530 G; Experiment 7 was performed at a temperature of 200 K, and the central magnetic field was 3380 G. The sweep width of all eight experiments was 180 G; the receiver gain was 7960; the modulation frequency was 100 KHz; the modulation amplitude was 5 G; the number of scans was 20; the time constant was 81.92 ms; the sweep time was 10.24 s; and the microwave power was 1.01 mW. In Experiments 1–6 and 8, samples were irradiated by ^{137}Cs gamma ray sources (Canada, Gammacell-40). A beta particle source of ^{90}Sr ($3.912 \times 10^3 \text{ min}^{-1} 2\pi \text{ sr}^{-1}$) was also used in Experiment 4. Because the liquid nitrogen container (20 cm tall) could not be placed into the equipment with the gamma source, in Experiment 7, samples were irradiated using the biological X-ray irradiator (RS2000, Rad Source, USA). All samples were collected from researchers and employees who worked at the institute, male and female, ranging in age from 24 to 50. The fingernails were cut into 1–3 mm pieces with a nail clipper. They were then soaked in distilled water for 10 min to eliminate MIS1–5 in Experiment 1–5 and 7. The eight experimental processes were as follows:

Experiment 1

After being humidified, the samples were measured by EPR. They were then dehydrated at 70°C for 180 min. The dehydrated samples were measured by EPR again. Next, the fingernails were soaked in water for 10 min again. After that, re-humidified samples were measured by EPR for the third time.

Experiment 2

The samples were dehydrated at 70°C for 180 min to remove the water content within the fingernails. Finally, the same samples were measured by EPR after three different radiation doses: 1 Gy, 2 Gy or 5 Gy.

Experiment 3

Based on four different drying treatments (at 70°C for 60 min for group C1, at 70°C for 45 min for group C2, at 70°C for 30 min for group C3, and at 200°C for 20 s for group C4), the samples were divided into four groups: C1, C2, C3 and C4. Four samples from one individual were then measured by EPR. Next, they were all irradiated with 5 Gy. Finally, the variations in the free radical concentration of the samples were measured.

Experiment 4

The samples were divided into four groups (D1, D2, D3 and D4). They were dehydrated in drying oven at 70°C for 180 min, and then were measured by EPR. After EPR measurement, the fingernails were soaked in water for 20 min. Next, the water on the sample surfaces was wiped off, and they were irradiated immediately by different radioactive rays at a range of doses: 1 Gy of gamma-rays, 3 Gy of gamma-rays, 5 Gy of gamma rays, and 1 h of beta rays. After irradiation, the four groups of samples were dehydrated at 70°C for 180 min and measured by EPR again. Finally, the sample labeled 'D3' was irradiated with 5 Gy again, and then re-measured. The sample labeled 'D4' was irradiated again for 30 min, and then re-measured.

Experiment 5

The fingernails were dehydrated at 70°C for 180 min and then divided into two groups ('E1' and 'E2'). The sample labeled E1 was measured by EPR, and then irradiated by 5 Gy. After irradiation, it was dried at 70°C for 180 min and measured again. The sample labeled E2 was dehydrated a second time under the same conditions as E1 and measured by EPR. Next, this sample was irradiated at 5 Gy. Finally, the sample was again measured by EPR. The two results were compared, and the difference in the sample mass after a second dehydration was also recorded.

Experiment 6

Before eliminating MIS1–5, i.e. soaking fingernail pieces in water for 10 min, the mass of three groups of samples was measured. Then samples were soaked for 10 min and dehydrated at 70°C for 180 min. After the above treatments, the mass and free radical concentration were measured. Next, all samples were soaked in water for 20 min, and the mass of the samples was measured for the third time. After weighing the samples, they were respectively dehydrated at 70°C for a range of time spans: 0 min for group F1, 15 min for group F2 and 30 min for group F3. After the fourth weighing of the samples, all the fingernails were irradiated at 5 Gy, and then the EPR signal intensity of each group of samples was measured for a second time.

Experiment 7

The samples were dehydrated at 70°C for 180 min and then were soaked in distilled water for 20 min. After wiping the surface water

from the fingernails, they were divided into three groups (G1, G2 and G3) and were measured by EPR at a temperature of 200 K. Next, these three groups of samples were enclosed in small plastic capsules. The sample labeled 'G1' was put in the liquid nitrogen container and floated on the surface of the liquid nitrogen. The irradiation field of the X-ray irradiator was enlarged to cover the whole container. The fingernails were then irradiated at 5 Gy. They were then taken out of the container with tweezers and immediately measured by EPR at 200 K. The sample labeled 'G2' was irradiated at room temperature at 5 Gy, then measured at 200 K. The sample from group 'G3' was processed in a similar way to the sample from group G1, the only difference being that the sample was not measured immediately after the irradiation. Before the final EPR measurement, it was kept at room temperature for 20 min.

Experiment 8

The thumbnails from both hands were collected and divided into two groups. There were equivalent numbers of left and right hand thumbnails in each group. The two groups of fingernails were weighed. Next, a sample from the first group was soaked in distilled water for 10 min, dehydrated at 70°C for 180 min, measured by EPR, and finally weighed again. This EPR signal intensity, normalized by the mass of the first weighing, was regarded as the BKG intensity of the sample. We divided the difference in the mass before and after drying by the mass of the first weighing, and the result was recorded as the mass-loss rate. The sample from the second group was irradiated at 5 Gy, soaked in water for 10 min, dehydrated at 70°C for 180 min, and finally measured by EPR. This EPR signal intensity normalized by its mass was regarded as the intensity of RISS + BKG. Finally, the result of dividing (RISS + BKG) by BKG was recorded as the normalized concentration-change rate.

RESULTS AND DISCUSSION

Dehydration of the samples (Experiments 1 and 2) and the response of the dried samples to radiation

Figure 1, representing the results of Experiment 1, shows the evolution of BKG through the treatment process. After dehydration, the signal intensity increases dramatically. However, dehydration does not create more free radicals, and this can be verified by comparing the signal intensity of re-humidified samples with that of the initial humidified samples. Also, other researchers have demonstrated that BKG is thermally stable [6]. So, growth in the BKG intensity is totally due to the loss of water content. Because water is a polar molecule and can cause non-resonant loss of electromagnetic waves in the resonance cavity, less water content within samples must lead to a higher EPR signal intensity. After the soaking of samples to eliminate the MIS, different samples will have absorbed different amounts of water [10], which will impact the accuracy of the measurements. So, the water content must be removed. According to previous research [11], fingernails have lost most of their water content and maintain a relatively stable sample mass after a dehydration of 70°C for 180 min. Although evaporating water from fingernails in this way is not complete, the effect of the remaining water content on the EPR measurements is limited. Therefore, in this research, in order to compare the different groups of samples quantitatively, they

were dehydrated at 70°C for 180 min to eliminate the measured deviation caused by water. In order to observe the change in signal intensity of the samples after treatment, it was important to keep the sample humidity at the same level before and after the treatment.

Ionizing radiation has two effects on biological molecules: a direct ionizing effect in attacking macromolecules, such as DNA, by photons or charged particles directly; and an indirect ionizing effect in damaging biomolecules through the ROS generated from water radiolysis [12].

Figure 2 shows the results of Experiment 2, which was designed to study the response of dehydrated fingernails to radiation. Figure 2 illustrates the six samples showing a non-linearly increasing EPR signal intensity with increasing radiation dose. Thus, the dried

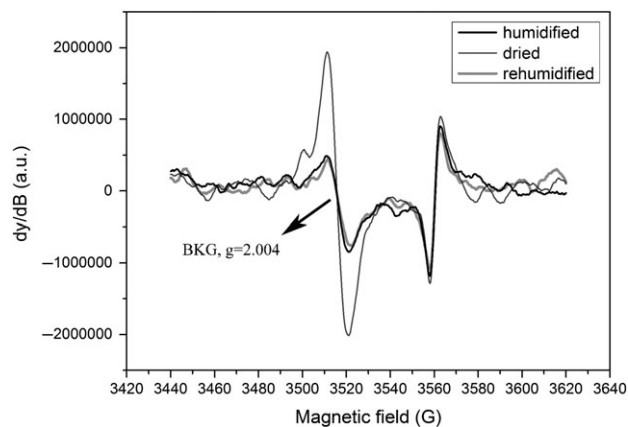


Fig. 1. The EPR spectra of the same sample after soak in distilled water for 10 min, dehydration at 70°C for 180 min, and soak in distilled water for 10 min again successively (results of Experiment 1); signal at 3560 G is from ER 4119HS-2100 marker accessory.

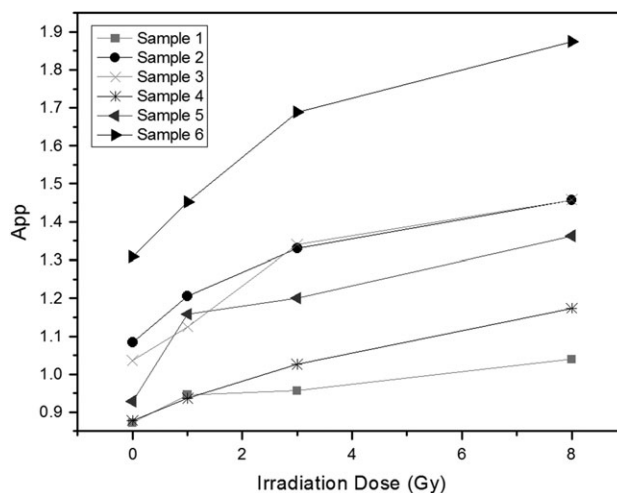


Fig. 2. The variation in EPR signal intensity as a function of cumulative irradiation dose (results of Experiment 2); there are six samples from six different individuals.

samples exposed to radiation also create free radicals. The differences in App between the six samples can be explained because the EPR signal intensity was not normalized by the sample mass. Without water, the photons have a certain probability of directly attacking the keratin forming the fingernails, and thus generating free radicals, which corresponds to the direct ionizing effect of radiation injury in organisms.

In conclusion, experiments 1 and 2 indicated that fingernails need to be heated at 70°C for 180 min to eliminate the effect of different water contents of samples on EPR measurements, and that dehydrated fingernails can create free radicals.

Two different effects of water on the creation of free radicals in fingernails (Experiments 3–6)

The indirect ionizing effect plays an important role in radiation injury. Water radiolysis can create many ROS. When the ROS contact proteins, the unpaired electrons are transferred to the other substances from the ROS. The same free radicals as those produced directly by ionizing macromolecules will be created. So, fingernails with water may create more free radicals than dried samples do. The results of Experiment 3 (as shown in Fig. 3) support this hypothesis.

As discussed above, the different water content in fingernails can lead to different EPR signal intensities because of the non-resonant loss of electromagnetic waves, even if these samples have the same amount of free radicals. Therefore, if the samples have different water content due to different drying times, to compare their signal intensity with each other will be pointless. But the different growth rates of the Apps of the different samples after irradiation at the same dose (divide the App of the sample after irradiation by the App of

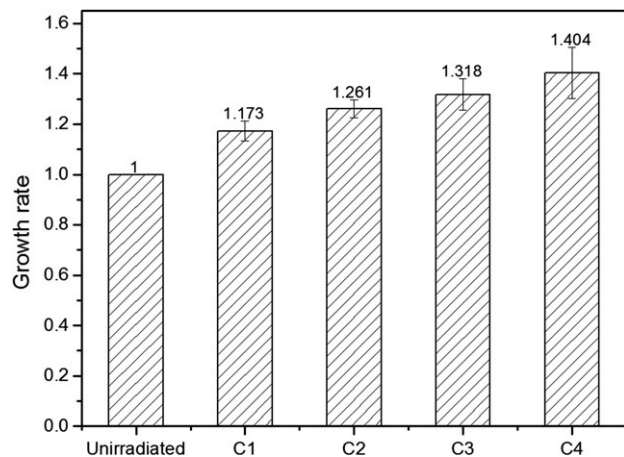


Fig. 3. After varying dehydration treatments (at 70°C for 60 min for group C1, at 70°C for 45 min for group C2, at 70°C for 30 min for group C3, and at 200°C for 20 s for group C4), this graph shows the growth rate of the EPR signal intensity of the four groups of samples through the same irradiation dose of 5 Gy (results of Experiment 3); all the peak-to-peak amplitudes (Apps) of samples that were unirradiated were regarded as a reference standard of '1'. Each group had seven samples.

the same sample before irradiation) can reflect its ability to create free radicals. For this reason, in Fig. 3, the signal intensities of the unirradiated samples are all assumed to be 1, and growth rates, not measurement values, are shown in the figure. As Fig. 3 shows, the samples dehydrated for less time have higher growth rates. It is obvious that fingernails lose more water content through longer drying time. There are two potential reasons why fingernails dried for less time can generate more free radicals after the same irradiation dose. One is that the temperature of 70°C makes the keratin denature. However, the proteins constituting fingernails are made of many layers of dead and flattened cells [13], and those proteins have already denatured. Meanwhile, the sample in part C4 (dehydrated at 200°C for 20 s) had the highest drying temperature and the highest growth rate. Therefore, denaturation induced by heat cannot be the reason. The indirect ionizing effect, as described above, may be the real explanation. Therefore, we conclude that greater water content in fingernails lead to more free radicals after the same irradiation dose.

Experiment 4 was designed to verify the conclusions from Experiment 3. In Experiment 4, the drying process was performed twice. Because the EPR signal intensities of the samples with different water content are different, each sample was always dehydrated at 70°C for 180 min before EPR measurements for the sake of maintaining the same humidity. Without the effect of the water content on the signal intensity, the App is able to represent the concentration of the free radicals. As Fig. 4 shows, the samples that have been irradiated do not generate free radicals. These results contradict the above conclusion. After the samples are soaked, the fingernails must have a greater water content than the samples dehydrated for a few minutes. Therefore, based on the conclusion of Experiment 3, the sample in Experiment 4

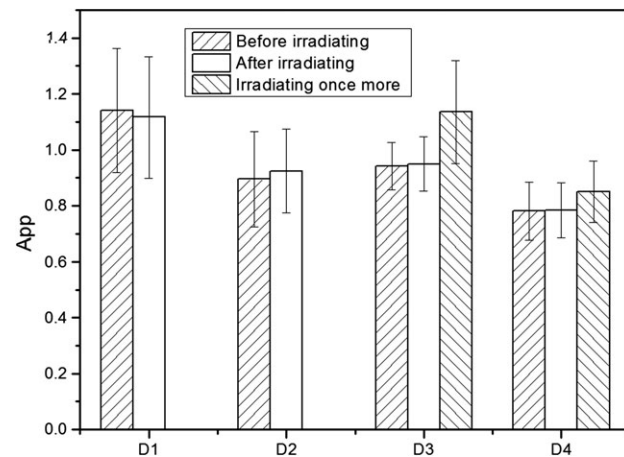


Fig. 4. The EPR signal intensity of four groups of samples before and after various amounts of irradiation: 1 Gy of gamma-rays for D1, 3 Gy of gamma-rays for D2, 5 Gy of gamma rays for D3, and 1 h of beta rays for D4 (results of Experiment 4); before irradiation, these samples were soaked in water for 20 min; the surface water was wiped away, and then the samples were irradiated immediately; the samples labeled 'D3' and 'D4' were irradiated once more. The samples were collected from six individuals. 'App' refers to the peak-to-peak amplitude of a sample.

should have a higher EPR signal intensity after irradiation, instead of an unchanged signal intensity. The interaction of photons with materials is probabilistic. However, beta-rays, which are charged particles (unlike photons), must interact with materials. The similar result for the samples in group D3 means that samples *do* generate free radicals after irradiation, but that these free radicals are eliminated immediately. Meanwhile, it has been demonstrated that samples in which the previous free radical concentration remains the same after irradiation are able to generate free radicals. Thus, maintaining the same EPR signal intensity after irradiation was not due to a change in fingernail property, and that may be due to the water content absorbed from the outside. In Experiment 3, the water content evaporated was mainly from the sample itself. However, in Experiment 4, after a different sample treatment process from Experiment 3, the fingernails lost all their original water and absorbed the external water.

In Experiment 4, the fingernails were dehydrated twice, with a drying time of 6 h in total. The drying time of the samples differed from that in Experiment 3. Thus, Experiment 5 was designed to investigate the impact of drying over a long period on the production of free radicals. Irradiation was performed for the two groups of samples at (i) the middle of the 6 h and (ii) after 6 h, respectively. The experimental results are presented in Fig. 5. Both groups of fingernails have a higher EPR signal intensity than when unirradiated. Meanwhile, the mass of the samples after the first dehydration was decreased after the second dehydration (as shown in Table 1). The fact that the samples in Group E1 have a higher growth rate than the samples in Group E2 can be ascribed to the same reason as mentioned in Fig. 3, i.e. indirect ionizing effect. Annealing of the signals by heating is another possible explanation. In conclusion, after 6 h of drying the samples are able to generate free radicals through irradiation.

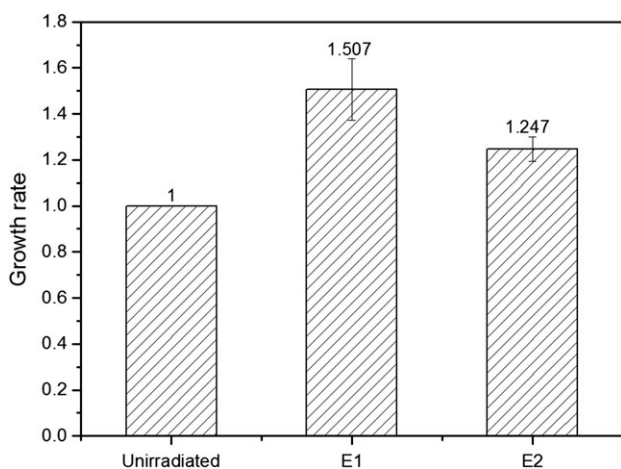


Fig. 5. The growth rate of the EPR signal intensity of samples after irradiation (results of Experiment 5). The sample in group E1 was dehydrated for 180 min, irradiated at 5 Gy, and then dehydrated again. The sample in group E2 was dehydrated for 360 min, and then irradiated at 5 Gy. Each group has five samples.

After eliminating the drying time difference, the re-humidification of samples (the only other difference between Experiments 3 and 4) must be the cause of the different results.

In Experiment 3, the evaporated water from the fingernails was mainly the original water content of the fingernails. But in Experiment 4, after dehydrating and soaking the samples, much of the water in the fingernails was from the external environment. The results of Experiment 4 indicate that the absorbed water may have a different effect on generating free radicals to that of the original water content of the fingernails. The results of Experiment 6, shown in Fig. 6, demonstrate this. The change in the water content of the fingernails at different stages is shown in Table 2a and 2b. This data demonstrates that fingernails are able to absorb the external water, and this can be verified by comparing the sample mass after soaking for 20 min with the initial mass. Also, after dehydration for 15 or 30 min, there was still some external water in the fingernails. Figure 6 and Table 2 indicate that the samples with less external water can generate more free radicals after irradiation. From these experimental results, we can see that water has two different effects on free radicals.

In conclusion, the original water in fingernails can promote the creation of free radicals, but water absorbed from outside stops the creation of free radicals.

Exploring the reason why water has two different effects on the creation of free radicals (Experiment 7)

Based on the indirect ionizing effect, it is reasonable that water content can promote the production of free radicals. However, many previous researchers have reported that water can also eliminate the free radicals within fingernails [7, 10, 14, 15]. Both viewpoints about water seem to be validated by our data and previous research. However, it is notable that water absorbed from the external environment is free water, but the water originally contained in the fingernails is not. Experiment 7 has proved that the different physical states of water can lead to different results. As Fig. 7 shows, the samples irradiated at the temperature of liquid nitrogen can generate free radicals, but the samples irradiated at room temperature cannot. The water content in the samples in Experiment 7 was all from the external environment. When the irradiation performed at ~ 77 K, the free water became crystallized water. Therefore, the

Table 1. The differences in sample mass after two dehydrations; samples in this experiment were from five individuals

Sample no.	First dehydration (mg)	Second dehydration (mg)	Difference (mg)
1	14.6	14.4	0.2
2	12.6	12.3	0.3
3	13	12.8	0.2
4	14.5	14.3	0.2
5	7	6.9	0.1

Table 2a. The mass of the sample labeled 'F2' through various treatments; all samples were dehydrated at 70°C

Sample no.	Initial mass (mg)	After dehydration for 180 min (mg)	After soaking for 20 min (mg)	After dehydration for 15 min (mg)
1	13.6	12.2	13.8	12.8
2	13.2	12.2	13.3	12.8
3	13.2	11.9	13.5	12.2
4	12.8	11.6	13.1	12.1
5	10.8	9.5	11.1	10.2
6	10.1	9.2	10.4	9.6

Table 2b. The mass of the sample labeled 'F3' through various treatments; all samples were dehydrated at 70°C

Sample no.	Initial mass (mg)	After dehydration for 180 min (mg)	After soaking for 20 min (mg)	After dehydration for 30 min (mg)
1	13.8	12.1	13.8	12.3
2	13.4	11.7	13.5	12.1
3	12	10.9	12.1	11.1
4	11.4	10	11.5	10.4
5	12.5	11.6	12.4	11.7
6	14.2	12.8	14.3	13.1

dominant factor here was the physical state of the water. Also, the samples irradiated at the temperature of liquid nitrogen, then kept at room temperature for 30 min before EPR measurement could generate fewer free radicals. The physical state of the water can influence the amount of free radicals produced. As Fig. 7 shows, the relatively high growth rate, 1.550, indicates that the indirect ionizing effect still works at 77 K [16]. When the samples irradiated at the temperature of liquid nitrogen were kept at room temperature for 30 min, instead of being measured by EPR at 200 K immediately, the crystallized water would have melted and become free water again due to the room temperature. Then the eliminating effect of water will appear. As for the reason why water cannot eliminate the BKG, i.e. the intrinsic free radicals, some researchers believe that the radicals associated with BKG could be located close to hydrophobic amino acids or within the polypeptide helix, which would prevent the water molecules from reacting with these radicals.

In conclusion, two different effects of water on the creation of free radicals was ascribed to the physical states of water, i.e. solid water can promote the creation of free radicals, but liquid water cannot, regardless of whether the water was originally contained in the fingernails or absorbed from the external environment.

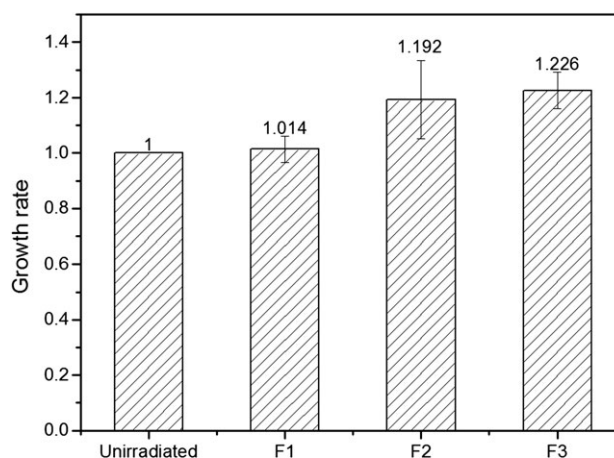


Fig. 6. The growth rate of the EPR signal intensity of the samples after irradiation (results of Experiment 6). After dehydration at 70°C for 180 min, three groups of samples were soaked in distilled water for 20 min. They were then dehydrated at 70°C for different length (0 min for F1, 15 min for F2, and 30 min for F3). Finally, they were all irradiated at 5 Gy. Their initial EPR signal intensities were assumed to be 1. Each group had six samples.

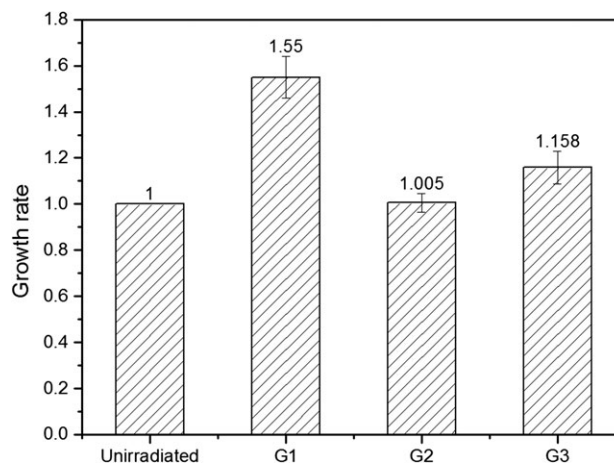


Fig. 7. The growth rate of the EPR signal intensity of samples after irradiation (results of Experiment 7). These samples were dehydrated at 70°C for 180 min, and then were soaked in distilled water for 20 min. After wiping the surface water from the fingernails, they were irradiated at 5 Gy at different temperatures (~77 K for G1, room temperature for G2, and ~77 K for G3). In contrast to the process for G1, the samples in G3 were kept at room temperature for 20 min after irradiation, instead of being measured by EPR immediately. All of the EPR measurements in this experiment were performed at 200 K. All the peak-to-peak amplitudes (Apps) of unirradiated samples were assumed to be 1. These samples were collected from seven individuals.

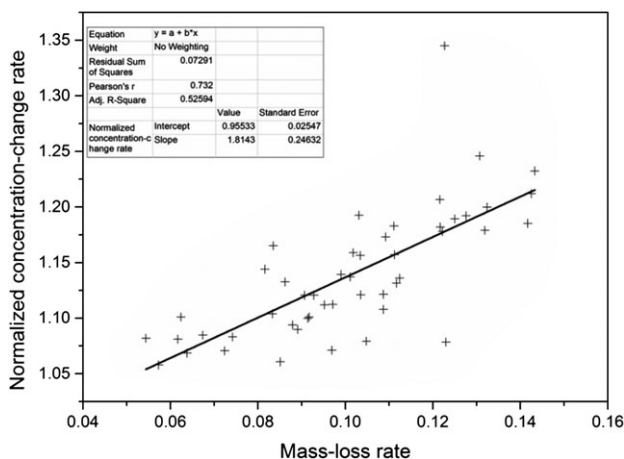


Fig. 8. The growth rate of the EPR signal intensities of samples after irradiation at 5 Gy as a function of mass-loss rate through dehydration at 70°C for 180 min (results of Experiment 8). The App was normalized by using the sample mass from the first weighing. We divided the difference in the mass before and after dehydration by the sample mass from the first weighing, and the result was recorded as the mass-loss rate. A total of 49 samples were used in this experiment.

Matters needing attention and the correction factor for water content within fingernails (Experiment 8)

In considering these conclusions, two aspects need to be noticed. The first thing to notice is the sample treatment process. As known, the major component of fingernails is alpha-keratin, which can form sulphur radicals by cutting [17]. It is found that water can eliminate these free radicals, which are associated with MIS. Therefore, many researchers have assumed that after soaking fingernail pieces in distilled water for 10 min, they can be regarded as untreated *in vivo* samples, because in the event of a radiation accident, the fingernails of potential victims are *in vivo*, not cut into pieces. Then, these 'untreated' fingernails are irradiated at different doses in order to conduct dose reconstruction research. Although these samples do not have those free radicals induced by mechanical stress after cutting, fingernails that have absorbed water cannot respond to radiation in the same way as fingernails *in vivo*. This is critical for dose reconstruction, so researchers should pay attention to the design of experimental procedures to avoid inadvertent errors.

Another thing to query is whether the measured results of the EPR reflect the real radiation dose. The water content of fingernails can influence the production of free radicals. If fingernails from two individuals have different water content, after being exposed to the same radiation dose, their measured results for EPR will be different. Based on the current method, the person whose fingernails have more original water will probably be considered to have been exposed to higher level of radiation dose than the other person. All of the above was verified in Experiment 8. Also, a correction factor was obtained. In Experiment 8, because the BKG intensities of the different fingernails of one individual were different [18], and a

single fingernail was too small to meet the experimental requirements, the thumbnails only were collected (because there were no statistical differences between the BKG intensities of the two hands). Also, to further reduce the difference, the fingernails from each hand were equally divided into two groups as far as possible, and these were then gathered into two groups (of equivalent BKG intensity) for Experiment 8. Figure 8 shows that the mass-loss rate of the fingernails had a linear relationship with the normalized concentration-change rate. This demonstrated that samples with more internal water can produce more free radicals after irradiation. However, it cannot be ignored that many points are distant from the fitting line. As researchers know, there are many kinds of keratins within fingernails, and these are the raw materials that can generate free radicals. Because the interaction of photons with materials is probabilistic, more keratins could generate more free radicals through radiation. Therefore, the different keratin content of the fingernails of different individuals could also influence the amount of free radicals. A more accurate method would be to generate a bivariate correction based on different water and keratin content. However, the free radical labeled 'RIS5', which is used to estimate radiation dose has not been identified, and the amino acid that generates RIS5 has not been identified either. Therefore, the bivariate correction is impracticable for now.

CONCLUSION

Water either promotes or eliminates the generation of free radicals within fingernails, depending on the form of the water. Promoting the production of free radicals can be ascribed to the indirect ionizing effect. Also, different levels of water content within fingernails can lead to different growth rates in the free radical concentration induced by irradiation, and these two variables have a linear relationship with a slope of 1.8143. The eliminating effect has been known about for some time, but the chemical reaction mechanism remains unclear. This mechanism and the quantitative measuring method for the particular keratin that generates RIS need to be investigated further. One thing to note is that soaking fingernail pieces in water before irradiation can cause estimation errors. In addition, the nail samples need to be dehydrated before EPR measurements to obtain maximum accuracy.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

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