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An Attempt to Reduce the Background Free Radicals in Fingernails for Monitoring Accidental Hand Exposure of Medical Workers

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Featured Application: The technique presented here could be effectively applied for retrospective assessment of the accidental radiation hand exposure of medical workers.

Abstract: While it is recognized that some medical workers could receive significantly higher radiation doses to their hands than the routinely monitored personal doses, accurate retrospective dosimetry of their hand exposure is still challenging. To solve this issue, a combination of electron spin resonance (ESR) measurement and fingernails is worth to be investigated. However, the application of fingernail ESR dosimetry requires establishing an effective protocol to eliminate the background signal (BKG) which changes due to mechanical stress and other unclear factors, so that the radiation doses would be precisely evaluated from the radiation-induced signals (RIS) only. Thus, the authors investigated possible applications of antioxidants to remove or reduce the BKG in fingernails. In the present study, the effectiveness of chemical treatment using the dithiothreitol (DTT) reducing reagent was examined in irradiated and unirradiated fingernails. Chemically and non-chemically treated fingernails were subsequently exposed to 20 Gy of 137 Cs γ -rays and the time changes of the BKG and RIS were confirmed in two different storage conditions: vacuum chamber and freezer. The results show that the non-chemically treated fingernails displayed significant intra-individual variations in the peak-to-peak intensities of both BKG and RIS. RIS from chemically and non-chemically treated samples showed correlations after freezer storage; signals were more stable than the samples stored in the vacuum chamber. Moreover, while the BKG of non-chemically treated samples demonstrated higher levels than those chemically treated, the intra-individual variations were further reduced by the DTT treatment. Our results imply that the use of an antioxidant for hand washing of medical workers prior to starting their work could be effective in reducing the pre-existing free radicals in their fingernails. This also suggests a practical application of hand exposure monitoring using fingernails as a part of radiological emergency preparedness in occupations where radiation or radionuclides are used. Research for finding safer and easier-to-handle antioxidants is to be focused on in future studies.

Keywords: medical worker; radiological accident; emergency response; retrospective dosimetry; ESR; EPR; fingernails; antioxidant

1. Introduction

It is recognized that some medical workers involved in interventional radiology/cardiology, diagnostic/therapeutic nuclear medicine, positron emission tomography, brachytherapy, among others, could receive notably high radiation doses to their extremities. Their hand doses tend to be higher than

the personal doses routinely measured with commonly used personal dosimeters; sometimes they may exceed the skin dose limit (500 mSv per year) even though the values from their personal dosimeters are insignificant [1–3]. In addition, it is still difficult to properly and routinely monitor local hand exposure as dose distributions change to a large extent, depending on the type of handling radiation or radionuclide, and the geometric situation between the hands and radiation source. Recent studies also reported that their fingertip doses could be significantly higher than the average hand dose measured with a ring-type dosimeter [3,4]. We thus need to develop a novel and practical measure to solve this issue.

For this aim, a combination of electron spin resonance (ESR) measurement and fingernails is worth to be investigated. The application of "fingernail ESR dosimetry" has a good potential for quantifying the number of radiation-induced free radicals that could stay long after exposure. For many decades, ESR (or electron paramagnetic resonance, EPR) of biomaterials, such as tooth enamel and bone, has been used successfully for retrospective dosimetry in atomic bomb survivors, radiological accident victims, residents around nuclear testing sites, and radiotherapy patients [5–20]. However, in the case of emergency dosimetry, which requires an urgent assessment of absorbed doses to affected individuals, the number of potential applications using ESR is relatively small and the present guidelines are yet to be developed. Indeed, in both short-term and long-term aftereffects of any radiation accident, a systematic protocol for feasible dose assessment, using the available biomaterials is an utmost need. Additionally, in the search for developing potential tools for retrospective dosimetry, most of the attention has been paid to alternative materials that are carried close to the human body.

Fingernails are no doubt one of the most promising biomaterials in this context. Its potential use as a dosimetry tool was first suggested in the late 1980s [21]. Fingernails are mainly composed of α -keratin, a protein which consists of three long right-handed α -helical peptide chains twisted into a left-handed coil strengthened by disulfide bridges (S-S) formed from adjacent cysteine groups [22]. Its exposure to ionizing radiation could generate stable free radicals that are the main sources of ESR signals. Subsequent studies on the ESR signals from fingernails revealed that the spectra are composed of three main components: radiation-induced signal (RIS), which is assumed to be correlated with absorbed dose; mechanic-induced signal (MIS), which is attributable to α -radicals formed along the shear edge of the nail through the breakage of S-S bonds caused by cutting/clipping and other mechanical stresses [21–23]; pre-existing native signal (PES), whose origin remains unknown [24]. Despite such complexities in the signal processing, the easier sampling of fingernails compared to to tooth enamel and bone has attracted some researchers who paved the way for notable developments in fingernail ESR dosimetry techniques in the 21st century [25–40]. Comprehensive reviews about the free radical mechanism and characteristics of fingernail ESR signals can also be found in the literature [41–43].

In those studies, many authors noted that one of the most problematic issues regarding fingernail ESR dosimetry is how to identify and quantify the true component of RIS alone, separated from other background signals (BKG). As demonstrated in some previous studies [36,44–46], the presence of MIS, PES, and other potential confounding factors, such as ultraviolet light and high temperature, contributes to the difficulty in the analyses of fingernail ESR signals. Not surprisingly, numerous attempts have been carried out to circumvent BKG, while RIS remains intact. For example, it was found that water treatment worked effectively in erasing the BKG (e.g., [25,29,30,35,38,47–49]). The application of an antioxidant to freshly cut fingernails was also investigated; it was reported that the 0.1 M dithiothreitol (DTT) reducing reagent with 20 min treatment time reduced both MIS and BKG [26]. It is still unclear, however, if the DTT treatment would be effective for fingernail samples that were stored for a long time.

Long storage in an ambient, humid conditions could cause unstable RIS, as well as changing BKG [29,43]. The difficulty in keeping the stability of RIS and BKG has led some authors to investigate other variants, such as storage in the freezer at low (sub-zero) temperature [31], in a vacuum desiccator with 0% air humidity [35,36,38,49], or in olive oil with low water content [39]. Since research on such

storage mediums started very recently, further comparative studies are desirable to clarify the efficacy for improving the signal stability of RIS and BKG in conjunction with possible chemical treatments.

The present study pursues this direction and tries to contribute to the development of a practical protocol of fingernail ESR dosimetry for routine monitoring of the extremity exposures of medical workers handling radiation sources. Here we investigate the effects of chemical treatment using DTT on the changes of the ESR spectra in irradiated and unirradiated fingernails after a long storage time. The efficacy of DTT in the reduction in intra-individual variations of BKG is also examined through comparative experiments using chemically and non-chemically treated samples stored in two different mediums (i.e., vacuum chamber and freezer).

2. Materials and Methods

2.1. Sample Preparation

Fingernail samples were obtained from one adult volunteer during regular hygienic practice at different collection times (usually every two weeks). In every collection, the samples were immediately placed inside a small tightly sealed plastic bag and stored in darkness inside a vacuum chamber (VE-ALL 1-8-989-01, AS ONE, Osaka, Japan) with silica gel (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) at room temperature. A total of 16 sample collections were used in the experiments described below. All the experiments in the present study (except during irradiation) were performed under subdued red lighting conditions to avoid ambient light exposure.

2.2. Experimental Procedures

Two experimental setups were used in the present study, as illustrated in Figure 1. One "with chemical treatment" process involving the application of DTT reducing reagent and the other "without chemical treatment" process without the DTT reducing agent. It should be noted that this study is only limited to fingernail samples collected from one individual to investigate the intra-individual variations along with the two experimental procedures; no additional experiments were performed for the examination of inter-individual variations.



Figure 1. Flowchart of the stages of fingernail sample preparation and electron spin resonance (ESR) measurements. Two experimental setups were employed—one "with chemical treatment" process including application of dithiothreitol (DTT) and the other "without chemical treatment" process without DTT. The part indicated with a dashed line in the above flow chart was omitted in the "without chemical treatment" process.

This experiment used eight sample collections with two different storage conditions: four for vacuum chamber and four for freezer. A more detailed description of the storage conditions is given in Section 2.4. In each collection, the fingernails from the right/left hands of the volunteer (~90 mg) were pooled and divided into four portions. Each portion contained 5-6 aliquots, totaling 20 mg. It must be noted that the first measurement of BKG from all the samples was performed before the chemical treatment process. Three portions were treated for 30 min with 500 µL aqueous solutions of 0.1 M DTT reducing reagent (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan), while the remaining portion was not treated or irradiated and kept as the control. In total, each storage condition consisted of 12 portions of treated samples and 4 portions of control samples. Following the treatment, all the samples were rinsed with 500 µL ultrapure water separated through microfiltration, treated with 500 µL of ethanol (FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) for 3 min and rinsed again with 500 μ L ultrapure water separated through microfiltration. The samples were dried inside the dry heat sterilizer (MOV-112 S-PJ, PHC Holdings Co., Ltd., Tokyo, Japan) at 25 °C for 1 h (hereafter we will call them as chemically treated samples.) After drying the chemically treated samples, the second ESR measurement was immediately conducted to examine the efficacy of chemical treatment using DTT to the ESR spectra of fingernails. Additionally, soon after the second ESR measurement, all the samples were stored inside the vacuum chamber (12 portions of chemically treated, 4 portions of control) for 24 h and the third ESR measurement was performed the next day. Shortly after the third ESR measurement, three portions from each collection of the chemically treated samples were irradiated to 20 Gy of 137 Cs γ -rays. Further technical details of the irradiation are provided in the next section.

2.2.2. Without Chemical Treatment

Similar to the experiment mentioned above, eight sample collections with two different storage conditions (i.e., 4 sample collections for each storage condition) were also made. In each collection, the fingernails from the right/left hands of the volunteer (~90 mg) were also pooled and divided into four portions (20 mg each). The samples used in this experiment were not given any chemical treatment (hereafter we will call them as non-chemically treated samples). Three portions from each collection of the non-chemically treated samples were irradiated to 20 Gy of ¹³⁷Cs γ -rays while the other one was left unirradiated and kept as the control. The first measurement of BKG from all the samples was performed before the irradiation process. In total, each storage condition used in this experiment consisted of 12 portions of non-chemically treated samples and 4 portions of control samples. A summary of the experimental work on non-chemically treated samples is also shown in Figure 1. It is important to note that the storage conditions and irradiations utilized in non-chemically treated samples were also similar to the chemically treated samples, as described in the following Sections 2.3 and 2.4.

2.3. Irradiation

Irradiation of the samples was administered using a γ -ray irradiator (Gammacell40 Exactor Low Dose Rate Research Irradiator, Best Theratronics Ltd., Ottawa, Canada) with a dose rate equal to 0.80 Gy min⁻¹. It has dual ¹³⁷Cs γ -ray sources with a total radiation activity of 178 TBq. All the samples were irradiated to a dose of 20 Gy with consideration of the clinical dose level in radiotherapy (tens of Gy). Though we recognized that this dose level was higher than the reported skin doses (0.5 to 2 Gy) of the hands in recent radiological incidents that occurred in medical facilities (e.g., [50–52]), we envisaged that the local doses to fingertips could be much higher than the average skin dose in such an incident. Thus, we have intended to assess the three-dimensional dose distribution of a hand based on measured fingernail doses.

Three portions of the samples from each collection were positioned at the center of the container ($\varphi 260 \times 100$ mm) perpendicular to the radiation sources, as shown in Figure 2. The dose uniformity

inside the whole area of the sample container was \pm 7%. During irradiation, the room lighting inside the facility was turned off to limit the ambient light exposure of the samples. Following exposure of the samples to radiation, ESR measurements were immediately performed.



Figure 2. Experimental setup for the irradiation of the fingernail samples with the γ -ray irradiator (**left**) and the geometry of irradiated samples placed in between two ¹³⁷Cs γ -ray sources (**right**).

2.4. Storage Conditions

In experiments with chemically and non-chemically treated samples, two different storage conditions (vacuum chamber and freezer) were used. The samples were initially placed inside a stainless-steel vacuum fresh box (E-305, Asahi Light Metal Industry Co. Ltd., Osaka, Japan) with silica gel (192-18285, FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan) before storage in the vacuum chamber or freezer. The humidity logger (LR5001, Hioki, Nagano, Japan) with a built-in sensor (LR9504, Hioki, Nagano, Japan) and a communication adapter (LR5091, Hioki, Nagano, Japan) were used to record the air humidity inside the two storage conditions. The recorded air humidity and temperature inside the vacuum chamber were 2% and 20°C, respectively. In the case of freezer storage, the recorded temperature was -20 °C, and approximately a 3% air humidity level was achieved with the use of silica gel.

2.5. Instrumental Settings

ESR measurements were conducted at room temperature on an X-band (~9.4 GHz) spectrometer JES-FA 100 (JEOL Inc., Chiba, Japan) with an ES-UCX2 standard cavity using a 5 mm sample tube. Spectra acquisition parameters were as follows: 1 mW microwave power; 10 mT sweep width; 30 s sweep time; 0.5 mT modulation width; time constant 0.03 s; number of scans 10. During spectra acquisition, the MgO: Mn²⁺ internal reference sample was used for calibration. The ESR signal intensities were measured as the peak-to-peak amplitude (App) of the main "singlet" of the ESR spectra (i.e., BKG-singlet, RIS-singlet), as described by Sholom and McKeever [36]. The ESR spectra were adjusted in linear baseline correction within the analytical software of the spectrometer. The ESR spectra were also repeated at different times to test the stability of the signal after radiation exposure.

3. Results and Discussion

Figure 3 shows the evolution of the ESR spectra obtained from (a) non-chemically and (b) chemically treated fingernail samples before and after irradiation with 20 Gy of ¹³⁷Cs γ -rays following storage in the vacuum chamber. All spectra were recorded soon after the corresponding chemical treatment or irradiation of the samples and continuously monitored for up to 7 days. Each spectrum is an average of 12 spectra from 4 sample collections (3 portions of chemically/non-chemically treated samples per collection). Solid lines correspond to the ESR signals measured after exposure to radiation,

while dotted lines are the background signal before irradiation (referred to as "NT–BKG". Dashed lines show the background signal after chemical treatment of the samples (referred to as "T–BKG").

As can be seen, the ESR signals of both non-chemically and chemically treated samples increased during the first day of storage in the vacuum chamber and continuously increased up to 7 days after γ -ray irradiation. This behavior was very similar to most of the irradiated vacuum-stored samples with water treatment [35,36,38]. It seemed, notwithstanding, that the increase rate in chemically treated samples (30% signal increase over the first 24 h after irradiation) was slower than those in the non-chemically treated samples (39%). Moreover, it was found that the non-zero, quasi-stable background signal substantially reduced its ESR intensity when subjected to DTT, compared to the non-chemically treated background signal (NT–BKG) demonstrated in Figure 3a. It should also be noted that the background signal with chemical treatment (T–BKG) was quite stable: there was no signal growth in its ESR intensity for 1 day after storage in the vacuum chamber at room temperature.



Figure 3. Evolution of the ESR spectra recorded at different times in (**a**) non-chemically and (**b**) chemically treated fingernail samples exposed to 20 Gy of 137 Cs γ -rays following storage in the vacuum chamber (2% air humidity level, 20 °C temperature). The notations NT–BKG and T–BKG correspond to the background signal before irradiation for non-chemically treated samples and after chemical treatment for those chemically treated, respectively. IR represents γ -ray irradiation.

Comparative plots on the evolution of ESR spectra observed in the fingernail samples exposed to 20 Gy of 137 Cs γ -rays, following storage in the freezer, are shown in Figure 4. Each of the spectra shown here is the mean of 12 spectra obtained from four sample collections (three portions of chemically/non-chemically treated samples per collection). It is worth mentioning that the chemically treated samples presented in Figure 4b were also kept inside the vacuum chamber for 24 h in between chemical treatment and irradiation. All notations used in Figure 4 are the same as those described in Figure 3.

As seen in this figure, the freezer storage resulted in better stability of ESR signals for both chemically and non-chemically treated samples following γ -ray irradiation, in contrast with the vacuum-stored samples. The recorded ESR signals in freezer-stored non-chemically treated samples increased by about 15% after 1 day of exposure and further increased up to 25% after a week (Figure 4a). As with chemically treated samples, there was only about a 9% increase in the ESR intensity after 1 day and 17% after 7 days (Figure 4b). Furthermore, the use of DTT for chemical treatment prior to irradiation was found to have significantly reduced the intensity of the ESR spectra in irradiated fingernail samples (i.e., RIS). These observations are in accordance with previously reported work from Romanyukha et al. [26]. It was confirmed that the background signal from unirradiated freezer-stored samples with chemical treatment (T–BKG) also showed no significant increase within 24 h of storage time inside the vacuum chamber (Figure 4b), as seen with the vacuum-stored samples (Figure 3b).



Figure 4. Evolution of the ESR spectra recorded at different times in (**a**) non-chemically and (**b**) chemically treated samples exposed to 20 Gy of 137 Cs γ -rays following storage in the freezer. The NT–BKG, T–BKG, and IR have the same notations as in Figure 3.

Results shown in Figure 5 indicate the efficacy of DTT on the stability of RIS in chemically and non-chemically treated samples at two different storage conditions (vacuum chamber and freezer). ESR signals were measured as the peak-to-peak intensity in the magnetic field range from 328.9 mT to 329.7 mT. All experimental data points were normalized to the first ESR signal of the samples measured soon after exposure to 20 Gy (this corresponds to the fourth and second ESR measurement for chemically and non-chemically treated samples, respectively, as illustrated in Figure 1. Measurements of the RIS were also taken up to 7 days in irradiated fingernail samples collected from the same individual. The dotted lines with unfilled and filled box markers correspond to non-chemically treated samples stored in the vacuum chamber and freezer, respectively. Solid lines with unfilled and filled circle markers represent chemically treated samples stored in the vacuum chamber and freezer, respectively.



Figure 5. ESR signal peak-to-peak amplitude (App) changes with time in chemically and non-chemically treated samples stored at two different conditions (vacuum chamber and freezer). All experimental points from each experiment were normalized with regard to the first measurement data soon after the exposure to 20 Gy of 137 Cs γ -rays.

As shown in Figure 5, it can be argued that the effect of DTT is more pronounced in fingernail samples stored in the freezer than those in the vacuum chamber. It may be observed that the signal intensities in chemically treated samples stored in the freezer increased after 24 h but remained almost unchanged for up to 7 days after irradiation, while the corresponding signals in the vacuum-stored chemically treated samples continued to increase for the same measurement times. Another interesting observation is that the behavior of the signals obtained in non-chemically treated samples correlated well with the measured signals in chemically treated samples for both storage conditions and

hence the freezer-stored samples (with 3% air humidity at -20 °C temperature) were deemed to be significantly more stable than the vacuum-stored samples (with 2% air humidity at 20 °C temperature). This observation broadly supports the finding of Reyes et al. [31], that persistent ESR signals can be achieved if samples are kept at low (sub-zero) temperature, although the air humidity level was not mentioned in their experimental work.

Next, a comparison was made for background signals measured from non-chemically treated unirradiated fingernail (control) samples stored in the vacuum chamber and freezer, as presented in Figure 6. ESR signals were measured as the peak-to-peak amplitude (App) of the main background-singlet of the spectra from the (a) vacuum-stored and (b) freezer-stored samples. Each storage medium in this figure consisted of eight portions of non-chemically treated unirradiated samples and measured in the same way as the irradiated samples, as described in Figure 5. It may be observed that the median intensity of the background signals in both storage conditions were all at the same level, but the data sets between the two mediums showed a very different distribution view. One can also notice from the plots in Figure 6 that the background signals obtained from the freezer-stored samples span much the same range of values and were more variable than in the vacuum-stored samples. Moreover, some individual outlying data points can be seen in the vacuum-stored samples which may be caused by intra-individual variations within the group of samples. Nevertheless, the stability of the background signals observed in storage conditions with either low humidity at room temperature or low humidity at sub-zero temperature can be regarded as good overall, and conclusions regarding the effect of low or lack of oxygen to the signal stability were equivocal and formed the needs of further scrutiny.



Figure 6. Distribution of the ESR signal peak-to-peak amplitude (App) from non-chemically treated unirradiated fingernail samples stored inside the (**a**) vacuum chamber with 2% air humidity at 20 °C and (**b**) freezer with 3% air humidity at -20 °C recorded at different times.

Figure 7 compares the intra-individual variations of the ESR signal peak-to-peak amplitude (App) measured before and after treatment with DTT in unirradiated fingernail samples stored in the (a) vacuum chamber and (b) freezer. Each storage consisted of four sample collections and the time interval between sample collections was 2 weeks. Each experimental data point shown in this plot is an average of spectral measurements on three portions of unirradiated fingernail samples obtained from a single donor, and the reported errors are the standard deviation. The background signals of the samples were measured as the peak-to-peak intensity from the main singlet of the ESR spectra. Measurements were taken prior to chemical treatment, soon after the corresponding drying conditions in the chemical treatment process, and at 1, 2, 3, and 7 days after chemical treatment.



Figure 7. Changes in the background ESR signal (peak-to-peak amplitude, App) in unirradiated fingernail samples stored in the (**a**) vacuum chamber and (**b**) freezer measured before and after (soon, 1, 2, 3, and 7 days) the DTT treatment.

As seen in this figure, there was a significant intra-individual variation in the recorded initial background signals between the samples from the same individual, whereupon no pre-treatment was applied. After application of DTT treatment, however, the background signals were observed to be broadly consistent with each other, giving rise to the opportunity to correct for such uncertainties in the intra-individual variations within the same group of samples. Furthermore, the recorded App of the native background signals have been reduced and are quite stable over the 24 h time delay between chemical treatment and storage of the samples: no substantial gain in the peak-to-peak intensity was encountered in both storage conditions. This finding is interesting and could be useful as many of the previous studies advanced differing views that pre-existing native background signal tends to grow after drying in ambient air conditions and thus cannot be entirely removed in the spectra [42]. Further investigations into this notable observation should be made with applications of other antioxidant materials that could have similar effects.

4. Conclusions

In the present work, we confirmed that the BKG (non-RIS) of fingernails can be significantly reduced by applying the DTT reducing reagent. After the DTT treatment, the fingernail samples provided good signal stability for both background and RIS signals with less intra-individual variations. Additionally, we demonstrated that the freezer storage would be more effective to maintain the stability of RIS after γ -ray irradiation than vacuum storage; it is thus desirable that collected fingernail samples be stored below the freezing point for performing more accurate dosimetry.

These findings are preferably to be applied in the monitoring of hand exposure of medical workers. More concretely, workers who might face high hand dose exposure from radiation sources are proposed to take the following steps to carry out precise dose assessments: (1) dip or wipe one's fingers with a reducing reagent before the relevant work; (2) get a small portion of the fingernail (e.g., from the little finger) as a control sample and store it in a freezer (<0 °C); (3) perform the work; (4) cut the fingernails soon after completing the work in the same way as with the control sample; (5) store the collected samples in the freezer; (6) measure the ESR signals of all the samples, including the control at the same time; (7) evaluate the finger dose by subtracting the MIS measured with the control sample from the ESR signal of the respective sample; (8) assess the three-dimensional dose distribution of the hand.

While the efficacy of DTT for chemical treatment was particularly good for BKG reduction and RIS stabilization in the fingernail ESR dosimetry, this procedure has some weaknesses. One is that the DTT treatment substantially reduced the peak-to-peak intensities of both BKG and RIS. This fact implies that the chemical treatment would reduce the capacity of trapping free radicals, which could induce an unfavorable saturation response at a lower dose level. Further, the samples used in this study were

obtained from the fingernails of only one individual and that possible inter-individual variations have not been identified. It must be critical for practical application of fingernail ESR dosimetry to verify that the findings of the current study can be universally applied through comprehensive analyses of the fingernail samples taken from different individuals. Another technical drawback is the toxicity of DTT, which allows only trained professionals to use hazardous chemicals. Considering these issues, we are currently focusing on research to find a better antioxidant material that is safer and easier to handle for any workers posing occupational risks of hand exposure from radiation sources.

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