Detection limit of electron spin resonance for Japanese deciduous tooth enamel and density separation method for enamel–dentine separation

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ABSTRACT

Electron spin resonance (ESR) dosimetry is one of the most powerful tools for radiation dose reconstruction. The detection limit of this technique using human teeth is reported to be 56 mGy or 67 mGy; however, the absorbed dose of Fukushima residents after the Fukushima Daiichi Nuclear Power Plant (FNPP) accident was estimated to be lower than this detection limit. Our aim is to assess the absorbed radiation dose of children in Fukushima Prefecture after the accident; therefore, it is important to estimate the detection limit for their teeth. The detection limit for enamel of deciduous teeth of Japanese children separated by the mechanical method is estimated to be 115.0 mGy. The density separation method can effectively separate enamel from third molars of Japanese people. As we have collected thousands of teeth from children in Fukushima, the present technique may be useful to examine their external absorbed dose after the FNPP accident.

Keywords: electron spin resonance (ESR); electron paramagnetic resonance (EPR); CO²⁻ radical; retrospective dosimetry; Fukushima Daiichi Nuclear Power Plant (FNPP) accident

INTRODUCTION

The Fukushima Daiichi Nuclear Power Plant (FNPP) accident released substantial amounts of radionuclides into the environment. There are serious concerns about the biological effects of radionuclides on humans and animals. From the perspective of the radiation safety, the estimation of the external absorbed dose of Japanese children who lived in Fukushima prefecture during and after the FNPP accident is important. In this work, we utilised electron spin resonance (ESR), also known as electron paramagnetic resonance (EPR) spectroscopy, which is one of the most powerful tools for external absorbed dose estimation.

It is well known that ESR can detect, identify and quantify free radicals. It is commonly used in fields of polymer science [1, 2], dating [3–6], geology [7–9], biology [10–13] and other fields [14–20]. In the field of radiation dosimetry, ESR dosimetry using teeth was applied for the dose reconstructions of the people exposed to the atomic bomb in Hiroshima [21], the nuclear plant accident in Chernobyl [22, 23], nuclear weapons tests, as well as for nuclear industry workers [24–27] and for the FNPP accident in 2011 [28–31], by measuring the long-lived CO²⁻ radical in teeth. Thus, ESR dosimetry has been used to investigate the absorbed dose of individuals suffering from severe radiation exposure. However, the detection limit (i.e. the lowest detectable
value) of the ESR technique using human molar teeth is reported to be 67 mGy–561 mGy (measured in three laboratories) [32] or 56 mGy–649 mGy (measured in 14 laboratories, mean value of the detection limit was 205 mGy) [33]. On the other hand, the Fukushima Health Management Survey reported that the maximum individual external does of residents in the Fukushima area after the FNPP accident was approximately 25 mSv [34]. One of our goals is to assess the absorbed radiation dose of children in Fukushima after the FNPP accident via the ESR technique using the deciduous teeth of children living in the ex-evacuation zones of the accident. In addition, the deciduous teeth could be useful for detecting low doses because those should have background doses lower than permanent teeth. Therefore, the estimation of the detection limit of ESR has been a key issue.

Organic materials in dentine can interfere with ESR measurement and bias the ESR-reconstructed dose [26, 35, 36]; thus, it has been necessary to remove dentine by mechanical or chemical means and prepare dentine-free enamel samples. However, from the point of view of the internal absorbed dose, the measurement of the ingested radionuclides (such as 137Cs and 90Sr) is important because it offers the possibility to measure the dose from these radionuclides deposited in the dentine [26, 37, 38]. Thus, it was essential to utilise an enamel–dentine separation technique whereby the dentine could be preserved. Furthermore, as with epidemiological studies that have examined thousands of teeth from children [38] or animals in Fukushima, we needed to prepare and examine a large number of enamel samples. Therefore, it was also essential for our enamel sampling technique to facilitate the efficient separation of enamel from dentine in a short period of time. In the previous work, we applied this density separation method to the teeth of Japanese macaque and successfully prepared dentine-free enamel sample [31].

The present study was conducted to: (i) estimate the detection limit of Japanese deciduous teeth, and (ii) evaluate whether the density separation method can be applied for the enamel preparation for human teeth by comparing CO2 radical intensities for Japanese molar teeth enamel separated by the density [31] and mechanical separation methods [26, 39].

MATERIALS AND METHODS

Deciduous teeth of Japanese children

This experiment used eight deciduous molar teeth of Japanese children collected before the FNPP accident. Tooth enamel was separated by ‘a mechanical separation method’ without any chemical treatments. Each tooth was cleaned with distilled water and the dentine was ground away with a dental bur until only the hollow shell of the harder enamel was left [26, 39]. The separated enamel was crushed into small grains with nippers. Enamel grains with a diameter between 0.425 mm and 1.4 mm were obtained by passing them through sieves [40, 41]. The pooled enamel was distributed into seven quartz tubes with an inner diameter and outer diameter of 4 mm and 5 mm, respectively, and a weight of ca. 150 mg.

The seven identical enamel samples were irradiated by 60Co gamma rays up to 1000 mGy (unirradiated, 50 mGy, 100 mGy, 150 mGy, 200 mGy, 500 mGy and 1000 mGy) by cumulative irradiation with a dose rate of 130 mGy/h (±1.4%) at the Takasaki Advanced Radiation Research Institute, National Institutes for Quantum and Radiological Science and Technology, Takasaki, Japan. A 3 mm-thick polymethylmethacrylate sheet was placed in front of the samples to provide the build-up of secondary electrons.

Before the first irradiation and after each irradiation, ESR measurements for seven samples were performed utilising a model JES-RE2X ESR spectrometer (JEOL, Tokyo, Japan) with a microwave power of 2 mW, a sweep width of ±5 mT, a modulation width of 0.2 mT, a time constant of 0.03 s and a sweep time of 30 s, for 120 scans [26, 27, 42]. To avoid contamination, all the samples were transferred to new quartz tubes before each measurement. The tube was removed from the cavity and shaken, and then placed again into the cavity before every measurement. All the irradiation and measurements were conducted in air at room temperature. Each sample was measured at least four times for each absorbed dose. Observed spectra were deconvoluted by the EPR Dosimetry program [42] to extract the CO2 radical intensities.

Third molars of Japanese people

To validate the enamel–dentine separation technique (i.e. density separation method), nine third molars extracted from Japanese people for medical reasons were used. Each tooth was cut into two pieces, and the enamel of one piece of each tooth was separated by the mechanical separation method and crushed into grains as described in the above section for deciduous teeth. The other half of each tooth was first crushed into grains using a cryo-press (Microtex Co. Ltd., Japan). Tooth grains with diameters ranging from 0.425 mm to 1.4 mm were collected by sieving, as described above.

The enamel was then separated from dentine by the density separation method [31, 43–45] using sodium metatungstate (Wako Pure Chemical Ind., Tokyo, Japan) [46, 47]. The principle behind this floating method is that when tooth grains including both enamel and dentine particles are put into sodium metatungstate solution with a density of 2.8 g/cm^3, the enamel (i.e. with a density of 2.8 g/cm^3 – 3.0 g/cm^3) sinks, while the dentine (i.e. with a lower density of 2.0 g/cm^3 – 2.1 g/cm^3) floats in the solution (Fig. 1). Thus, the enamel and dentine form distinct layers after centrifugation of the solution.

Enamel could be separated by removing the dentine layer with a pipette, after which the sodium metatungstate solution was discarded. If the tooth grain is from the enamel–dentine interface, some dentine...
may remain in the enamel grain. To make sure to prepare the dentine-free enamel sample or if we observe an irregular ESR signal with the remained dentine [31], treatment of the enamel grains with NaOH solution [31, 32, 48] is effective to remove the remaining small amount of dentine in the tooth grain and to observe an appropriate ESR signal. No irregular signals were observed in this work, we did not treat the samples chemically.

The collected enamel was washed several times with distilled water and dried at 40°C overnight, then they were used for ESR measurements. Each enamel sample was placed in a quartz tube with an inner and outer diameter of 4 mm and 5 mm, respectively, and a weight of ca. 100 mg, then measured at least five times to estimate the CO$_2^-$ radical intensities.

**RESULTS AND DISCUSSION**

Typical tooth ESR spectrum and spectrum deconvolution

Figure 2(a) shows the typical ESR spectrum of a 500 mGy-irradiated deciduous tooth from a Japanese child (green), fitted spectrum (black) and deconvoluted spectra of radiation induced radicals which are CO$_2^-$ radical (red) and native radical (blue). The ESR spectrum consists of at least five components, including background components, radiation induced components and some unknown component [31, 42]. The major components are the radiation induced components shown as red and blue signals in the figure. One can see that the summation of several functions (fitted spectrum) is similar to the original spectrum. Figure 2(b) shows the ESR spectra for the same deciduous tooth with different absorbed dose. With increasing the absorbed dose, the sharp peak due to CO$_2^-$ radical appears on the shoulder of the ESR spectrum, therefore, it is important to reduce the native radical component due to dentine to extract the CO$_2^-$ radical intensity.

**The relationship between the CO$_2^-$ radical intensity and the absorbed dose for deciduous tooth enamel of Japanese children separated by the mechanical separation method**

The dose response curve of the CO$_2^-$ radical intensity is shown in Fig. 3. Plots represent the mean value of the CO$_2^-$ radical intensity for each sample, and the error bars represent the standard deviation for each sample. The CO$_2^-$ radical intensity increases with increasing dose. The coefficient of determination, $R^2$, for CO$_2^-$ radical vs dose represented by a solid line is estimated to be 0.989, indicative of the clear linear relationship between the CO$_2^-$ radical intensity and the absorbed dose. According to the definitions [32, 33, 49–51], the detection limit of this work (represented by dashed lines in the figure) is estimated to be 115.0 mGy.

The detection limit of 115.0 mGy is higher than the lowest reported values of 67 mGy [32] and 56 mGy [33] for human molar teeth. This is maybe due to the larger sample-to-sample variation in deciduous teeth [52]. We estimated the absorbed dose for about 10 deciduous teeth, however, we could not find teeth which show the absorbed dose obviously higher than the detection limit. Further study is required to improve the detection limit for deciduous tooth enamel separated by the density separation method with appropriate chemical treatments to accurately quantify small amounts of radicals generated by doses less than 100 mGy.

**Comparison of the CO$_2^-$ radical intensities for Japanese molar teeth enamel separated by the density and mechanical separation methods**

Tooth enamel consists of ~96% inorganic matter (mostly hydroxyapatite), 3% water and less than 1% organic matter (protein matrix). In contrast, dentine contains 70% inorganic matter, and 30% organic matter and water [26]. As mentioned previously, it is essential to collect the enamel free from dentine, because the organic materials in dentine interfere with the ESR measurement and biases the ESR-reconstructed dose. Although a chemical method using NaOH/KOH can easily separate enamel [26, 32, 33, 48], the dentine is lost in this method. We are also interested in the internal absorbed dose due to $^{137}$Cs [53] and $^{90}$Sr [54–56], so it is necessary to keep the dentine. Therefore, neither the mechanical separation method nor the chemical method is suitable for our purposes. Furthermore, these methods, especially the mechanical separation method, could not be applied for such epidemiological studies whereby a large number of enamel samples are examined, because it...
would have been extremely time-consuming to mechanically separate enamel from dentine.

In the present work, we applied the density separation method to separate enamel that preserves dentine and requires little time for sample preparation. Figure 4 shows the results of the comparison test; the vertical axis represents the CO$_2^-$ radical intensities of enamel separated by the floating method (red) along with those separated by the traditional mechanical separation method (blue) for nine teeth. The observed CO$_2^-$ radical intensities for enamel of Japanese molar teeth separated by the density and the traditional mechanical separation methods were consistent within the standard deviation of the remeasurements (error bars) as shown in Fig. 4, suggesting that we can obtain the enamel sample by the density separation method equivalent to the mechanical separation method without losing dentine. Although we were concerned about possible chemical contamination of the teeth due to sodium metatungstate, the results indicate that no contamination was observed.

The advantages of the density separation method are the following: (i) no special skill is required, (ii) it is operator independent, (iii) it can separate enamel in parallel with dentine, high quality enamel samples with less organic materials can be obtained by simply increasing the sodium metatungstate density accordingly, (v) it takes little time for sample preparation (working hours reduces to 1/10 compare to the mechanical separation method), and (vi) moreover, this method can be applied to teeth of animals such as macaques [31], raccoons [57] and mice, which are difficult to process with the mechanical separation method because of the small size and structural complexity of their teeth.

In subsequent research, we will attempt to estimate the absorbed dose of wild raccoons in Fukushima prefecture and discuss the relationship between the biological effects and the absorbed doses. In addition, preparing more precise dose response curves for Japanese deciduous teeth and estimating the absorbed dose less than 100 mGy of the children in Fukushima prefecture is our next important work.

**SUMMARY**

We have estimated the detection limit of Japanese deciduous teeth is 115.0 mGy, which is higher than the lowest reported values of 67 mGy [31] and 56 mGy [33] for human molar teeth, maybe due to the larger sample-to-sample variation in deciduous teeth. We successfully demonstrated that the separation technique using sodium metatungstate is moderately useful for enamel–dentine separation for Japanese molar teeth, and has advantages such as ease of separation, operator independence, and the ability to separate large amounts of enamel in parallel and obtain enamel. We are going to apply the density separation method for Japanese deciduous teeth to prepare more precise dose response curve and estimate the absorbed dose of children in Fukushima in an epidemiological context.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

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