Toward High Sensitivity ESR Dosimetry of Mammal Teeth: The Effect of Chemical Treatment

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ESR dosimetry/Tooth enamel/Radiation dose/Mammal/Cow.

Investigations were conducted into chemical treatments suitable for concentrating enamel from cow teeth. Cow teeth could be used as alternative to human teeth for retrospective dosimetry when human teeth are not available. It is essential to remove dentin from tooth enamel for low dose radiation dosimetry in order to avoid interference to the ESR signal from organic radicals. Increasing the period of chemical treatment with KOH and NaOH reduced the signal intensity of the organic radicals. The sensitivity of the dosimetric signal from inorganic radicals increased slightly with length of treatment with NaOH, which is consistent with removal of dentin, and rose to a maximum of 20% after 5h with KOH (40°C).

INTRODUCTION

Since it was found that tooth enamel is an excellent material for ESR (electron spin resonance) retrospective dosimetry, many studies have used human teeth to estimate doses from radiation accidents as summarized in some reviews.1,2) Human teeth allow determination of individual doses which are of primary interest. However, human teeth are not always available for retrospective individual dose assessment. In such situations teeth from animals could be used as an alternative to human teeth. Moreover, cow milk and meat play a significant role as sources of radiation exposure for humans. Also doses given to cows could reflect those given to human. In addition, recently, the impact of radiation on non-human species is becoming an important issue.3) It is important to develop the ESR dosimetry technique to measure radiation doses from mammal teeth.

There are a few studies of ESR dose measurements of animals4–6) which have shown that animal teeth are useful for dosimetry. However, further detailed study is needed to establish the method of dosimetry using animal teeth. In the present study, we examine appropriate chemical conditions for sample preparation.

Although mechanical separation has been adopted in some studies,7) the method for chemical treatment with alkaline solutions has been well examined8) for removal of dentin and concentration of enamel from human teeth. Enamel has higher sensitivity for ESR analysis, being composed of 96% of inorganic material, most of which is hydroxypatite and of 3% of organic matter, while dentin contains 70% of inorganic material and 30% of organic matter.9) The enamel and dentin of cow teeth, which are the subject of the present paper, have a similar chemical composition as they are used as substitutes for human teeth in dentistry studies. In a cows molar tooth, a crescent-shaped enamel section is located at the center of the tooth, and enamel also covers the outer surface at the sides. No enamel covers the top of the tooth as it is abraded away by the chewing of grass. On the other hand, a cows front tooth has a structure similar to a human front tooth.

Treatment with alkaline solution will remove both dentin and organic material in enamel, thus reducing the intensity of the interfering organic radical signal and, therefore, increasing the detectability of the dosimetric signal. The best chemical treatment for extracting enamel from cow teeth may be different from that for human teeth. In the present paper, the cow tooth enamel was subjected to various chemical pretreatments so the production efficiency of the ESR signal could be observed and the best procedure arrived at for extracting enamel from cow teeth.

EXPERIMENTAL

Several cow teeth were obtained from a Okayama slaughterhouse. The cow teeth (both front and molar teeth) were first crushed to grains of 0.5 to 1 mm. The aliquots of grains of about 150 mg were treated with NaOH and KOH solutions of 10% to 30% in glass test tubes 10 mm in diameter.
for 1 to 10 hours at 40°C, together with a control sample of grains and deionized water in an ultrasonic bath (Branson 1510J-MT). The temperature was checked every 5–10 minutes and controlled by adding cool water. After rinsing with deionized water and drying at 60°C for 16 hours, the samples were irradiated to 5 Gy by gamma rays from a $^{60}$Co source at the Research Institute for Radiation Biology and Medicine, Hiroshima University, with a dose rate of 100 mGy/min before ESR measurements. The ESR spectra were recorded at room temperature at a microwave power of 5 mW, a modulation frequency of 100 kHz and amplitude of 0.3 mT, a time constant of 0.03 sec, and a scan time of 1 minute, accumulating 40 scans for each measurement, using an ESR spectrometer, JEOL PX-2300, at Okayama University of Science. The standard cylindrical cavity ES-UCX2 with TE$_{011}$ mode was used with the MgO:Mn$^{2+}$ marker installed. The intensity of the marker was set to 700 according to the software controlling the spectrometer so that the third and the fourth lines of Mn$^{2+}$ were shown on both sides of a spectrum using a scan range of 10 mT.

The digitized spectra obtained were converted to text files with 1024 data points for each spectrum, and then processed with a numerical program, called New ER,$^{10}$ to evaluate the intensities of the dosimetric signal and of the interfering background signal from organic radicals. The signal height was divided by the peak-to-peak height of the fourth line of the Mn ESR spectrum to correct possible sensitivity variation of measurements.

**RESULTS**

The mass of the samples was reduced by 15 to 30% by the chemical treatment most probably due to removal of dentin. The errors in signal intensity when repeating measurements were typically 3% for the dosimetric signal and 5% for the organic signal.

The intensities of the interfering signal due to organic radicals in samples without irradiation decreased with increased length of chemical treatment, as shown in Figs. 1a and 1b (KOH treated front teeth and NaOH treated molar teeth, respectively) although some variation in intensities was observed between aliquots as seen in the control samples (treated with deionized water). While the intensity of the organic radical signal was reduced more effectively with a higher concentration of KOH, the effect is almost the same for different concentrations of NaOH. The results obtained for KOH-treated molar teeth were similar to those for KOH-treated front teeth, and the results for NaOH-treated front teeth as those for NaOH-treated molar teeth. The signal intensities of organic radicals observed in tooth enamel irradiated to 5 Gy gave similar results as those for teeth without irradiation.

With NaOH treatment, the intensity of the dosimetric signal observed both in front and molar teeth after irradiation to 5 Gy were, as shown in Figs. 2a, slightly increased over time and the intensities were about 25 to 30% larger in front teeth than in molar teeth. A similar trend was also observed in front teeth treated with KOH with the intensities peaking at specific durations of KOH treatment in the case of molar teeth: 7 hours in a 10% solution, 5 hours in 15 and 20% solutions, and 3 hours in a 30% solution, as shown in Fig. 2b, except for the points for 10 hours in 15 and 30% solutions. Of those peak intensities in the figure, 5 hours of treatment in the 20% solution gave the maximum intensity.

**Fig. 1.** The intensities of the organic radical signal, normalized by mass after the treatment, observed (a) in KOH treated front teeth and those (b) in NaOH treated molar teeth, without irradiation. The concentrations of the solutions were 15, 15, 20, and 30% with a control (0% = deionized water).
sities were also generally about 25 to 30% larger in front teeth than in molar teeth.

**DISCUSSION**

The organic radical signal was reduced to 15 to 50% of the initial intensity by KOH and NaOH treatment, and about 15 to 30% of the total mass was removed by both KOH and NaOH treatment of cow teeth in the present experiments. This means that the effect of KOH and NaOH is not only to remove dentin but also to remove organic material contained in the enamel. The NaOH solution reached maximum effect at 5 hours and 15% as shown in Fig. 1b. On the other hand, higher concentrations gave better results in the case of KOH, and the effect of a 30% KOH solution is about the same as those of similar NaOH solutions, as shown in Fig. 1a.

The fact that the treatments remove 15–30% of mass would explain the slight increase in the intensity of the dosimetric signal with duration of treatment, Fig. 2a. The treatment probably removes dentin more effectively than enamel, and, therefore, the intensity, shown in the figure, which is normalized by the remaining mass, increased because most of the dosimetric signal is coming from enamel rather than from dentin.

However, this does not explain why the intensity of the dosimetric signal of the untreated front tooth sample is larger than those in treated samples, as shown in Fig. 2a. This tendency is clearer in molar teeth as shown in Fig. 2b. As this was also observed in the control samples (tooth material plus deionized water without KOH or NaOH), it would probably be the effect of water rather than alkaline solutions. We currently have no reasonable explanation for this phenomenon, but note that ultrasound plus water could reduce the sensitivity of the dosimetric signal at least in cow teeth.

The signal intensities shown in Figs 2a and 2b correspond to the sensitivities of the dosimetric signal to radiation dose, as these signal intensities were measured after gamma ray irradiation to 5 Gy. As shown in Fig. 2b, it is possible that KOH sensitizes the dosimetric signal as the increase in sensitivity is more than the loss of mass by KOH treatment. The decrease of the sensitivity (intensity in the figure) at durations longer than the peak condition may be because KOH partly destroys the crystal structure of hydroxyapatite or removes too many impurities, such as carbonate which is the source of the dosimetric signal (CO$_2^-$). However, further study is necessary to confirm this effect because the above discussion ignores the points at 10 hours of treatment in 15 and 30% solutions. It is also possible that KOH treatment simply increases the variation and scattering of the signal intensities.

In the practical procedures of dosimetry, the ratio of the dosimetric signal intensity to interfering signal needs to be high and the chemical treatment simple. When NaOH solution is used, a 5 hour treatment in 10 to 15% concentration is enough for this purpose; the organic signal decreases sufficiently and the signal sensitivity of the dosimetric signal stays about the same. In the case of KOH, the sensitivity reaches a maximum at 5 hours of treatment in a 20% solution, but the organic radical signal has not decreased sufficiently by this time. A 30% solution decreases the organic radical signal well for 5 hours treatment, but the sensitivity of the dosimetric signal is not very elevated, making it difficult to tell which treatment is better.

When front teeth are used for dosimetry, mechanical sep-
ation, instead of chemical extraction, could be considered in cases in which it is not so difficult to remove dentin from simply structured teeth because, as discussed above, there is possible decrease of the sensitivity of the dosimetric signal due to the effect of water and ultrasound in chemical treatment. However, we need to use a chemical method to concentrate enamel from molar teeth with a complex structure. Where there is some effect of ultraviolet from sunlight\textsuperscript{11} on the front teeth of cows, molar teeth should be used instead.

The present study examined the effect of chemical treatment of teeth samples on the intensity of the interfering organic radical signal and on the sensitivity of the dosimetric signal. It is necessary to confirm further that the chemical treatment does not affect the existing dosimetric signal and to monitor the correct dose with control experiments.

Further development of this cow-teeth methodology is important as an alternative to human teeth for retrospective dosimetry.\textsuperscript{12,13} Such an approach will be suitable for estimation of local doses, while taking into account the essential heterogeneity of radioactive fallout following nuclear tests in SNTS.\textsuperscript{14}

\section*{CONCLUSION}

The effects of chemical treatment on cow teeth were studied in the present study. Both KOH and NaOH reduced the interfering organic signal. A 10 to 15\% NaOH solution reduced the signal intensity to a plateau value at duration of 5 hours at 40°C with ultrasound. With NaOH and with KOH treatment for front teeth the sensitivity of the dosimetric signal increased slightly over the period of treatment. This can be explained by the removal of dentin from the teeth. With KOH and molar teeth on the other hand, there are sensitivity peaks at specific points on the treatment timescale.

\section*{ACKNOWLEDGEMENTS}

This work was supported by Grant-in-Aid for Promotion of Science provided by Japan Society of Promotion of Science (15405006) and in part by the Promotion Program for Creation of a Start-up Business from Universities (2004) provided by the Okayama Prefecture Industrial Promotion Foundation.

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