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Dose response of hydrazine – Deproteinated tooth enamel under blue light stimulation

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ABSTRACT

The beta dose response and Optically Stimulated Luminescence (OSL) signal stability characteristics of human tooth enamel deproteinated by hydrazine reagent under blue photon stimulation are reported. Removal of the protein organic component of tooth enamel resulted in a higher OSL sensitivity and slower fading of OSL signals. The effect of chemical sample preparation on the enamel sample sensitivity is discussed and further steps to make this deproteinization treatment suitable for in vitro dose reconstruction studies are suggested.

1. Introduction

The optically stimulated luminescence (OSL) technique can determine the absorbed dose of crystalline materials previously exposed to ionizing radiation by using blue, green or infrared (IR) stimulation (Botter-Jensen et al., 2003). OSL was firstly used for individual retrospective dose assessment on human tooth enamel by Godfrey-Smith and Pass (1997) who observed dose-dependent IR and green stimulated luminescence signals from tooth enamel deproteinated by NaOH (sodium hydroxide). Un-deproteinated teeth showed a greater dose-dependent infrared stimulated luminescence (IRSL) sensitivity, however, and measurable signals were obtained only from doses as high as 120 Gy. IRSL properties of anthropological human bone and remains of pig teeth using acid treatment (e.g., hydrochloric acid and hydrogen peroxide) were also investigated by Meriç et al. (2008) and the IRSL dose responses of these materials were found compatible with feldspar and quartz compounds commonly used in dating of archaeological and geological samples.

With improvements in OSL readout technology, tests were performed under blue photon stimulation. It was demonstrated that blue photon stimulation was more efficient in depleting the OSL signal than green and IR stimulation on untreated tooth enamel (Godfrey-Smith, 2008; Yukihara et al., 2007). Although both of the above mentioned studies utilized the same readout technology and blue light stimulation on untreated tooth enamel, Yukihara et al. (2007) observed minimum measurable doses of the order of tens of Grays (20–30 Gy) and designed a new high-sensitivity OSL (HS-OSL) system specifically for this investigation. But Godfrey-Smith (2008) observed significant OSL signals for beta doses starting from 1.4 Gy, and pointed out that the results obtained were encouraging for the future design of a portable *in vivo* OSL dosimeter for tooth enamel dosimetry.

The goal of these recent OSL studies is the development of an *in vivo* method for dose reconstruction. However, in vitro experiments with extracted teeth are still necessary to make further improvement in the sample sensitivity and deal with the stability problem of the OSL signal. In this context, investigation of the effect of deproteinization on tooth enamel would be useful in OSL measurements if extracted teeth is available.

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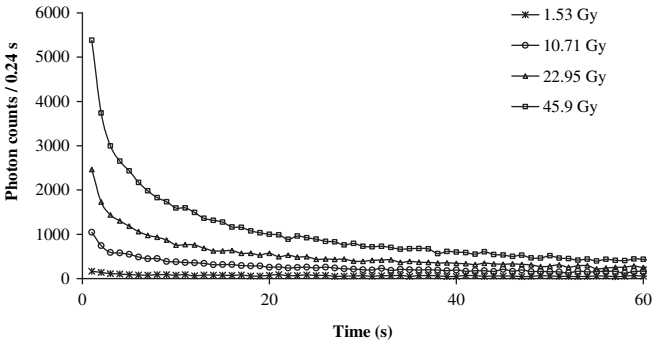

Fig. 1. OSL signals of hydrazine deproteinated tooth enamel after doses of 1.53–45.9 Gy. The OSL signal of an undosed enamel sample defined the background. Within measurement error, the OSL dose response of the 0.76 Gy equals the background of 53 ± 5 counts/0.24 s plus three times its standard deviation. Therefore the starting curve was selected as 1.53 Gy. The aliquot size was 4.6 mg.

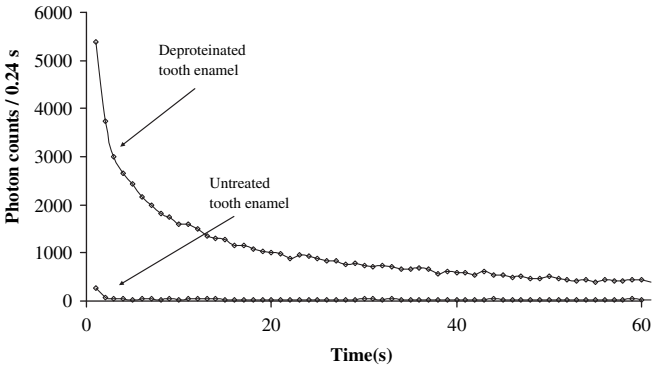

Fig. 2. OSL decay curves for tooth enamel irradiated with 46 Gy before and after hydrazine treatment. The aliquot sizes were 4.6 mg and 4.71 mg for deproteinated and untreated enamel samples, respectively.

It has been shown that the strong reducing reagent hydrazine completely deproteinates and slightly dehydrates compact bones under nearly anhydrous conditions with only moderate heating, and this induces no alteration in structural properties of the inorganic phase (Termine et al., 1972). It has also been demonstrated (Ivannikov et al., 2001) that treatment of enamel powder with the highly active reduction reagent hydrazine eliminates the background EPR signal (which is assumed to originate from the organic component) in tooth enamel. The radiation-induced EPR signal in enamel is practically not changed after treatment by hydrazine (Ivannikov et al., 2001).

There are many advantages, as described by Yukihara et al. (2007) and Godfrey-Smith (2008), of OSL over EPR in retrospective dose assessment. Therefore, the present study investigated the effect of chemical sample preparation with hydrazine reagent on tooth enamel sample sensitivity, when state-of-the-art OSL readout technology.

Tooth enamel is composed of hydroxyapatite $\{Ca_{10}(PO_4)_6(OH)_2\}$ needle crystallites, a solid-state integrating dosimeter, about 0.6–0.9 μm long that are dispersed in an aqueous organic gel (IAEA-TECDOC- 1331, 2002). The minimum measurable dose and signal stability hitherto obtained with current readout technology were not sufficient for retrospective dose assessment on untreated tooth enamel. Therefore, the motivation of this study was to eliminate the aqueous organic gel surrounding the needle crystals in order to investigate the effect of the protein organic component on minimum detectable dose and OSL signal stability in tooth enamel.

2. Laboratory investigation

Firstly, enamel samples were separated from human teeth by a dental drill. The enamel size fractions separated from several teeth were 20–150 μ. Tooth samples were provided by the National Institutes of Health in Turkey. Teeth from donors with different age and gender do not exhibit a difference in chemical structure. The change of age and sex affects only the relative ratio of enamel and dentine (Kaushal et al., 2003; Hillson, 2005; Schwartz and Dean, 2005). Therefore, the effect of different age and sex was not investigated.

Hydrazine molecules form strong hydrogen bonds with water molecules, which decrease the reactivity of hydrazine for the deproteinization process. This is why hydrazine was made anhydrous first, and then used in deproteinization. Anhydrous hydrazine was prepared from 99.9% Hydrazine hydrate ($N_2H_4 \cdot H_2O$ Merck). In a poly tetrafluoroethylene flask, 200 ml $N_2H_4 \cdot H_2O$ was mixed with 125 g NaOH (Merck). The mixture was left to stand for 24 h, and after this period was filtered using Whatman 42 filter paper. The filtrate was distilled in a semi-micro distillation system. The first 10 ml of the fractions was discarded and the remainder, about 110 ml, was stored in a dark polyethylene-lidded container for use in the deproteinization experiments.

Hydrazine deproteinization steps were performed as described by Ivannikov et al. (2001) except the ultrasonic bath step. Enamel samples (about 150 mg) were placed into 10 ml glass flasks, which can be sealed with covers. In the first step defatting was performed in a 1:1 mixture of ethanol with ethyl ether for 1 h. After the defatting phase, the solution was decanted and the samples dried. These dried samples were exposed to 3 ml hydrazine for about 24 h at 55 °C in an ultrasonic bath. After this step, the reagent was decanted. Samples were subsequently washed with 50% ethanol–water solution and with pure ethanol during one day. After the samples were dried in open air, aliquots were prepared with silicon spray and weighed. The same aliquot (4.6 mg) was used to obtain the results shown for deproteinated sample in Figs. 1, 2 and 4 and the aliquot size of untreated tooth enamel in Fig. 2 was 4.71 mg and that shown in Fig. 3, 5.4 mg.

All OSL measurements were performed with a Risø TL/OSL reader (TL/OSL-DA-20, Risø National Laboratory). The samples were stimulated with either blue LEDs (470 ± 20 nm), resulting in an OSL signal during a total period of 60 s or an IR laser diode (830 ± 10 nm), resulting in an IRSL signal during a total period of 100 s. The irradiations were performed using the Riso irradiator containing a $^{90}Sr/^{90}Y$ beta source (40 mCi) with a calibrated dose rate of 0.153 Gy s^{-1}.

Light detection was carried out with a photomultiplier tube (PMT) bi alkali EMI 9235QA which has an extended UV response with maximum detection efficiency between 300 and 400 nm. To prevent scattered stimulation light from reaching the PMT, a 7.5 mm Hoya U-340 detection filter, which has a peak transmission around 340 nm is employed. Since it was reported by Godfrey-Smith and Pass (1997) that heating the enamel resulted in a decrease in green stimulated luminescence sensitivity, the deproteinated samples were not preheated.

3. Results and discussion

Untreated enamel samples were investigated using blue light stimulation to obtain a clearer picture of dose response changes after the deproteinization treatment. The OSL curves obtained for untreated enamel (20–46 Gy) were similar to those obtained by Yukihara et al. (2007) using a dose of 50 Gy. No significant OSL signal was observed for doses lower than 46 Gy from untreated tooth enamel.