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Physically-based biodosimetry using in vivo EPR of teeth in patients undergoing total body irradiation

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Abstract

Purpose—The ability to estimate individual exposures to radiation following a large attack or incident has been identified as a necessity for rational and effective emergency medical response. In vivo electron paramagnetic resonance (EPR) spectroscopy of tooth enamel has been developed to meet this need.

Materials and methods—A novel transportable EPR spectrometer, developed to facilitate tooth dosimetry in an emergency response setting, was used to measure upper incisors in a model system, in unirradiated subjects, and in patients who had received total body doses of 2 Gy.

Results—A linear dose response was observed in the model system. A statistically significant increase in the intensity of the radiation-induced EPR signal was observed in irradiated versus unirradiated subjects, with an estimated standard error of dose prediction of 0.9 ± 0.3 Gy.

Conclusions—These results demonstrate the current ability of in vivo EPR tooth dosimetry to distinguish between subjects who have not been irradiated and those who have received exposures that place them at risk for acute radiation syndrome. Procedural and technical developments to further increase the precision of dose estimation and ensure reliable operation in the emergency setting are underway. With these developments EPR tooth dosimetry is likely to be a valuable resource for triage following potential radiation exposure of a large population.

Keywords

electron paramagnetic resonance (EPR); biodosimetry; triage

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Introduction

It is unlikely that there can be an effective medical response for an event in which large numbers of individuals are potentially exposed to clinically significant levels of radiation without a means to determine the exposure level of individuals (International Atomic Energy Agency [IAEA] 2005, Alexander et al. 2007, Flood et al. 2007, Gonzalez 2007, Blakely et al. 2009, Grace et al. 2010). The efficient employment of medical resources, prompt and effective treatment of exposed individuals, and the optimum use of radiation mitigators require knowledge of the dose an individual has received. Accurate dosimetry would help determine which people received doses that did not require acute care, it would classify those patients who need further evaluation into treatment-level categories, and it would help providers and patients anticipate potential long-term consequences of exposures to ionizing radiation. It is very unlikely that individuals will be carrying dosimeters or that their doses can be quickly and accurately calculated based on their location. Therefore, the concept of using biodosimetry has evolved and it is generally agreed to be essential. By definition, biodosimetry employs radiation-induced changes in the person's own tissues to estimate their individual exposure.

In addition to assessment of clinical signs and symptoms (Goans et al. 1997, 2001, Waselenko et al. 2004), a number of very promising radiation biodosimetry approaches have been developed based on biological responses, such as identification of products of DNA damage, gene activation, and metabolic products (Dons and Cervený 1989, Amundson et al. 2003, 2004, Long et al. 2007, Sasaki 2009, Brengues et al. 2010, Britten et al. 2010, Chen et al. 2010, Fenech 2010, Flegal et al. 2010, Garty et al. 2010, Kaspler et al. 2010, Ossetrova et al. 2010, Sharma et al. 2010). While these approaches are sensitive to biological responses to irradiation, which may be directly related to individual morbidity and mortality, there are a number of inherent limitations in their use as independent assays of radiation exposure. It is recognized that the biologically-based biodosimetric techniques have the potential to be confounded by other physiologic and pathophysiologic factors that can affect these parameters both with regard to the magnitude of the response and the timing of the changes (Swartz et al. 2010). These limitations arise from their fundamental nature as biological responses to damage which are not specific to radiation.

Electron Paramagnetic Resonance (EPR) biodosimetry methods are based on the detection of radiation-induced generation of stable radical centers in certain tissues. Many studies in animal and human subjects have characterized the dosimetric properties of tooth enamel and demonstrated that the EPR amplitude varies directly and linearly with absorbed radiation dose, as described thoroughly in a recent review (Fattibene and Callens 2010). The magnitudes of the detected EPR signals are proportional to the total dose of radiation received within the tissue, thereby rendering these tissues endogenous physical dosimeters. This technology is based on the fact that ionizing radiation generates unpaired electrons proportional to dose and that, in tooth enamel, these unpaired electron species are extremely stable, persisting virtually indefinitely (Desrosiers and Schauer 2001). In the natural formation of teeth, carbonate ions (CO_3^{2-}) are incorporated into biological hydroxyapatite [$\text{Ca}_{10}(\text{PO}_4)_6(\text{OH})_2$] during mineralization where they substitute for phosphate and hydroxyl ions. Following irradiation, radiation-induced free electrons are captured and carbonate

radical centers are created. In vivo EPR measurements of tooth enamel have several desirable characteristics that make them especially suitable for estimating radiation dose for triage.

- They are based on physical processes that are not confounded by other types of trauma and stress that would be likely to occur in a major radiation event.
- The radiation-induced signal requires no time to evolve and is stable indefinitely, so the measurements can be made at any time after the event.
- The measurements are non-destructive so that repeated measurements can be made for increased precision and verification.
- Measurements are not affected by individual variations in physiology or pathophysiology.
- The measurements can be made at the site of the event with immediate readout.
- The measurements are provided specifically for the tooth enamel, which could aid in determining the homogeneity of the exposure given additional dose estimates for other sites or the whole-body average dose provided by complementary methods.
- The measurements have the potential to be made with highly automated devices operated by minimally trained personnel with throughput times of less than 5 minutes per measurement.
- EPR measurements are not affected by dose-rate.
- EPR in vivo measurements are non-invasive.
- The dose-response is linear up to at least 30 Gy.
- The measured responses are similar for all types of low linear energy transfer radiation.

Rationale for pursuing technical development in human subjects

Because of the ultimate need for application in human subjects and the ready availability of isolated human teeth, the majority of the studies carried out to characterize the fundamental dosimetric properties of tooth enamel have been made directly in human tissues. In vivo studies with human subjects have been performed using a number of different groups and protocols, including measurements with non-irradiated subjects with normal teeth, unirradiated volunteers who had irradiated teeth placed in gaps present in their dentition, and patients who received radiation to their teeth as part of treatment for head and neck cancer (Iwasaki et al. 2005a, 2005b, Swartz et al. 2005, 2006, 2007, Williams et al. 2007, 2010, Demidenko et al. 2007).

While these studies have been very productive, further development and refinement of the technology to optimize performance and to enable reliable and effective use in the intended emergency setting will benefit from the use of more refined experimental models. This includes studies with patients who have been treated with total body irradiation (TBI) and the use of anthropomorphic models of the human mouth that incorporate human teeth and

reflect the natural anatomical and microwave characteristics. Patients receiving TBI prior to hematopoietic stem cell transplant (HSCT) are arguably the most appropriate population for continued refinement and validation of the EPR biodosimetry technology. Specific advantages of this model include: (i) TBI treatment mimics the expected field conditions for irradiation in contrast to other radiation therapies which are highly localized; (ii) TBI may involve administration of several fractions, making it is possible to measure the same person prior to radiation and then at several times during treatment so that inpatient dose response can be assessed; and (iii) the measured dose estimates can be compared to the prescribed dose, as opposed to analyses involving victims of radiation accidents where true doses are not well defined.

While there are some significant problems with the use of TBI patients as primary test subjects for the development of biologically-based biodosimetry assays, these concerns do not apply for physical biodosimetry. The physical changes in teeth detected by EPR are not impacted by injury, disease status or biological reactions, and the physical changes occur immediately, are stable, and reflect total dose irrespective of rate of exposure or episodes of exposure. In contrast, the significant perturbations to biological responses engendered by illness, concomitant chemotherapy, and supportive care treatments administered to transplant patients would markedly perturb the effects of TBI in biological dosimetry measurements. Additionally, in the case of fractionated TBI, the temporal dependencies and variability of active biological responses add significant complexity to the interpretation of biodosimetry measurements, effectively preventing repetitive doses from being treated as additive. In contrast, the immediate generation and robust stability of the radical centers quantified using the physically-based EPR tooth biodosimetry approach makes the effect of serial irradiation procedures strictly additive and amenable to characterization of the partial and integrated dose response.

Materials and methods

Measurements of incisor teeth using the transportable EPR dosimeter

Detailed descriptions of the technical development of the EPR spectrometers used for in vivo measurements are given in previously published reports (Salikhov et al. 2003, Swartz et al. 2004, 2005, 2006, Salikhov et al. 2005, Walczak et al. 2005, Williams et al. 2007, 2010, Hirata et al. 2000). These continuous wave (CW) EPR spectrometers operate at L-Band frequencies near 1.2 GHz and with main magnetic fields near 420 mT. The current configuration of the transportable EPR tooth dosimetry system is based upon a 30 kg dipole permanent magnet (Resonance Research Inc., Billerica, MA, USA). This magnet has a 17 cm pole spacing for measurements of the human head and is particularly well-suited for measurements of the central incisor teeth (Figure 1). This complete system can presently be deployed within two $1 \times 1 \times 1.2 \text{ m}^3$ shock-resistant boxes (Rackmount Solutions, Ltd, Piano, TX, USA), including a custom-built compact platform for placement on an existing table or with a separate custom-built stand for the magnet. As currently configured, the spectrometer can be transported in a suitable vehicle, easily rolled to the desired location, and put into operation in ~ 20 min. The spectrometer can be operated using power from either a single 120 V outlet or portable generator. The deployment capabilities of the system

have been assessed and verified in a series of emergency management simulations (Nicolalde et al. 2010), including recent field exercises which took place at a local firehouse and schoolyard and in demonstrations of the dosimeter at EPR conferences in San Juan, Puerto Rico, and at the Medical College of Wisconsin.

Proper positioning of subjects' heads within the magnet, with the teeth of interest located inside the central volume of homogeneous magnetic field, is established and maintained using a custom-built platform which is fixed to the magnet (Figure 2). A U-shaped tray is mounted to the underside of the platform and effectively guides the subjects as they position themselves and assists them to remain immobile during the measurements. Non-invasive measurements of the teeth in situ are made using custom-built external surface loop resonators that have been adapted specifically for intra-oral measurements (Swartz et al. 2005, Williams et al. 2007, 2010). These resonators are equipped with automatic tuning and coupling control circuitry to minimize effects of minor motion of subjects and to facilitate fully automated operation of the spectrometer (Hirata et al. 2000, Salikhov et al. 2003). The resonator used for incisor measurements has a detection loop with an internal diameter of 7 mm, which covers a single incisor tooth surface, and is configured to enable measurements on either the front or back surface of the tooth. At the current stage of development, the resonator is positioned by a suitably trained operator using a non-magnetic and lockable articulating arm (MJR Medical Supply Inc., Huntington, NY, USA). Developments are under way to mechanically automate this process to increase reliability and remove the need for operator expertise or discretion. For each measurement, the resonator is covered with a hygienic plastic barrier (Henry Schein, Melville, NY, USA), as is accepted in common dental practice. A reference standard containing perdeuterated Tempone (^{15}N -PDT) (Sigma-Aldrich, St Louis, MO, USA) is fixed to the resonator as a quality control measure, enabling direct verification that the proper instrumental parameters are set and that instrument performance is within tolerances. Measurements are performed according to a standard operating procedure, which includes the collection of three serially-acquired sets of spectra with independent placements of the resonator. Instrumental parameters include 4 G Zeeman modulation at 20 kHz, scan width of 25 G, and scan time of 3 sec. Following positioning of the subject in the magnet, the entire data collection process from initial tuning to the completion of data collection is completed in 5 min. The spectra for each of the three serially-acquired sets are analyzed using spectral fitting to estimate the average amplitude of the radiation-induced signal (Demidenko et al. 2007, Williams et al. 2010).

Anthropomorphic mouth model development

In order to further facilitate rapid development of instrumental and measurement techniques to improve dosimetric precision, we have designed and constructed a series of anthropomorphic mouth models that accurately simulate the conditions of in vivo measurements. These models incorporate sets of neighboring, and opposing, natural teeth set in an alginate dental casting material with high water content (KromaFaze, DUX Dental., Oxnard, CA, USA). The teeth used in these models are natural, healthy, whole human teeth, which have been donated following extraction and made available through the National Disease Research Interchange (NDRI, Philadelphia, PA, USA). The dental cast simulates the anatomy and reproduces the radio-frequency (RF) characteristics of the oral cavity. In

addition to the high water content of the casting material, free saline is applied to the model to mimic the presence of saliva. An example mouth model and its use are shown in Figure 2d.

The ability of the mouth model to simulate the RF characteristics were initially assessed by comparing electrical properties of a resonator when installed in the model and on the intact teeth of three volunteer subjects, including measurement of the quality factor (Q) and RF frequency (ν_{RF}) of the resonance. Following these tests, the equivalence of the EPR signals in the model and in vivo was directly evaluated in a series of measurements performed using in vivo subjects and the mouth model. For these measurements a thin layer of paramagnetic generic artist's charcoal was temporarily affixed to the tooth surfaces prior to measurement. This charcoal material contains carbon radicals in high densities, which provide a large EPR signal and enables precise measurements of variability in signal amplitude resulting from variations in the RF environment or in the positioning of the detector loop. The same charcoal sample was used for measurements on the labial and lingual incisor surfaces for both the in vivo and model systems, such that equal signal amplitudes would be observed when the detection loop is placed accurately on the tooth surfaces and the RF characteristics are similar. Measurements were repeated a total of four times in the mouth model and eight times with the human volunteer.

In vitro characterization of dose response

The dose response for measurements of the labial and lingual surfaces of upper incisor teeth was investigated using natural human teeth, which were positioned in the mouth model and measured using procedures identical to those used for in vivo measurements. Three different teeth were measured at five different doses, including 0, 2, 5, 7.5, and 10 Gy. For each tooth at each dose, three separate measurements were made, with independent placements of the resonator on the tooth and 60 sec of data collection for each (total acquisition time per tooth = 180 sec). The spectra for each tooth at each dose were analyzed using spectral fitting to provide independent estimates of the amplitude of the radiation-induced signal, which were then averaged together for dose estimation. The results of these analyses were used to characterize the observed dose responses for both tooth surfaces and estimate the standard error of dose prediction (SEP), as defined below.

In vivo dose estimation

Initial assessment of the use of upper incisor measurements for screening was performed with six TBI patients and 10 unirradiated subjects using the deployable dipole dosimetry system and the standard EPR acquisition parameters. This research was approved by the Committee for the Protection of Human Subjects, the Institutional Review Board (IRB) at Dartmouth College. TBI patients received non-myeloablative treatment at the Dartmouth Hitchcock Medical Center (DHMC) with a prescribed dose of 2 Gy to the midline at the umbilicus. Measurements were performed in each subject with the detection loop placed alternately on the lingual and labial surfaces, for a total of three repetitions on each surface. The three independent EPR amplitudes estimated for each surface were averaged to provide a single value for each tooth surface. The complete set of averaged measurements for each surface was then used to estimate the dose response relationship and the SEP. Additionally,

the dose response relationship derived *a priori* using the mouth model was applied to the in vivo data.

Expression of dosimetric precision

Standard error of dose prediction (SEP) is defined as $\sqrt{\frac{\sum_{i=1}^N (D_i - \hat{D}_i)^2}{N - 2}}$, where N is the total number of measurements within the calibration set, D_i is the given dose for the i^{th} measurement, and $\hat{D}_i$ is the predicted dose from the calibration curve computed as

$\hat{D}_i = \frac{y_i - a}{b}$, where y_i is the measured EPR signal amplitude and a and b are parameters of the calibration curve. SEP provides a quantitative representation of the precision of the dose assessment using the method of inverse regression (Draper and Smith 1998). In the homoscedastic case, SEP provides an estimate of the underlying standard deviation of estimated doses for samples with a given true dose. Accordingly, it can be used to characterize the intrinsic error of dose prediction. As the SEP estimates the intrinsic error of dose prediction based on a limited number of samples drawn from a population, there is an associated uncertainty which can be defined for this estimate. In order to more clearly identify meaningful differences in SEP values across calibration sets we include estimates of these uncertainties based on the delta method.

Results

Assessment of anthropomorphic mouth model

For a standard resonator with a 10 mm detection loop, the free-space Q and ν_{RF} were 520 and 1.189 GHz, respectively. Based on repeated measurements in the mouth model these values dropped to 242 ± 3 and 1.1806 ± 0.0002 GHz due to the presence of dielectric losses. Averaging across measurements in human subjects ($n = 3$), these values were measured to be 240 ± 10 and 1.185 ± 0.001 GHz. The similarity of these values is indicative of consistent RF characteristics of the model and in vivo systems, which lead to comparable losses and sensitivities of the EPR measurements.

For in vivo measurements of incisors with affixed charcoal, the Q ranged from 210–220 (5% variation) across both surfaces and both systems. For these experiments, extra time was devoted and experienced volunteers were used to ensure that the resonator detection loop was positioned accurately on the tooth surface and the influence of resonator positioning on signal amplitude was minimized. The overall variation in the observed average EPR amplitudes were small (0.24–0.25 V; <5%), indicating that for the accurately positioned resonator detection loop the RF environments were similar (Figure 3). These results reaffirm the equivalence of the in vivo and mouth model systems.

Dose response of incisor teeth, using the mouth model

The EPR amplitudes for the radiation induced signals (V_{RIS}) observed for each tooth at each dose and for both surfaces are shown in Figure 4. Based on the measurements in the mouth model, dose calibrations of $V_{\text{RIS}} = (0.018 \pm 0.001) \text{ V/Gy} \times \text{Dose} + (0.035 \pm 0.007) \text{ V}$ and $V_{\text{RIS}} = (0.012 \pm 0.001) \text{ V/Gy} \times \text{Dose} + (0.025 \pm 0.008) \text{ V}$ were observed for the labial and

lingual surfaces, respectively. The corresponding SEP values were 1.1 ± 0.2 Gy and 1.8 ± 0.4 Gy. In these estimates the relatively larger SEP value and associated uncertainty observed for the lingual surfaces reflect the fact that the dose response is less sensitive for this surface, potentially due to a smaller volume of enamel within the sensitive region defined by the resonator detection loop.

In vivo tooth dosimetry: Discrimination between unirradiated subjects and TBI patients

A clear distinction between the measurements of the labial incisor surfaces in the two in vivo populations was observed, with a single outlier from the unirradiated population within the range observed for the TBI patients (Figure 5). A statistically significant 75% increase in mean signal amplitude was observed for the TBI subjects, relative to the measurements in normal subjects of the same surface ($p = 0.001$, one-tailed Student's t -test with equal variance). These data indicate a dose response with calibration $V_{RIS} = (0.011 \pm 0.003) \text{ V/Gy} \times \text{Dose} + (0.028 \pm 0.004) \text{ V}$ with a SEP value of 0.9 ± 0.3 Gy.

When the data from the mouth model and in vivo measurements are compared, reasonable correspondence between the observed values at each dose is observed given the level of variance. However, when the *a priori* dose response calibration based on the mouth model measurement is applied to the in vivo data a general underestimate of the dose is observed. This effect is also apparent when the slopes of the dose responses derived independently from each dataset are compared, where the slope of the response observed for the in vivo data is 40–50% smaller than that observed in the mouth model, though data are limited for the in vivo data to measurements at two doses. The root of this discrepancy requires further study including additional data collection, but would be consistent with a systematic decrease in the signals recorded in vivo potentially due to increased separation between the detection loop and the tooth surface following movement by the subject.

Discussion

Utilizing the existing apparatus, expert operators, and averages of three independent measurements per subject requiring a total data acquisition time of <5 min, the ability to discriminate between unirradiated subjects and patients who received 2 Gy was confirmed. Using EPR measurement of the labial incisor surfaces and a simple amplitude threshold, 15 out of 16 (94%) subjects were assigned into the proper exposure group.

The ease and reliability of these measurements was aided by the use of the upper incisor teeth, which are easily accessible for interrogation, have relatively flat surfaces, and are more generally free of restorative modification. Despite these advantages, there has been a concern that radical centers generated by routine ultraviolet (UV) exposure from sunlight could confound the ability to perform accurate dosimetry in naturally exposed incisor teeth (Liidja et al. 1996, Ivannikov et al. 1997, Nakamura et al. 1998, Nilsson et al. 2001, IAEA 2002, Fattibene and Callens 2010, Sholom et al. 2010). Among the studies of the effects of UV on EPR tooth dosimetry, the data presented by Ivannikov et al. (1997) most clearly describes the existence and impact of UV generated signals in naturally exposed teeth. In a set of measurements, including 136 individual teeth with 14 upper central incisors collected from a Russian population with no history of significant radiation, molar teeth had on

average estimated dose of approximately 0.1 Gy and the more exposed front incisor teeth had an average estimated dose of 0.28 ± 0.03 Gy. Similar tooth position-dependent variations in estimated doses were measured by Sholom et al. (2000). While these doses and the differences across teeth may be relevant for epidemiological studies, their relevance for emergency screening is markedly reduced. For emergency uses, a commonly expressed goal is discrimination of exposure at a 2 Gy threshold, and for this purpose, the use of incisor teeth may be highly effective. In addition, while there are detailed accounts in the literature of UV-induced EPR signals, quantitative data and assessment of significance are not yet available for whole teeth irradiated naturally in vivo and measured at L-band with surface loop resonators.

For these measurements in unirradiated subjects and patients receiving exposures from 2 Gy TBI, the standard error of dose prediction was measured to be 0.9 ± 0.3 Gy. While precision at this level could be useful for some screening applications, efforts are under way to increase the dosimetric precision to provide further improved specificity and sensitivity. Efforts focus on mitigation of several identified sources of noise and error, including variability in the positioning of the resonator detection loop on the tooth of interest, the effects of patient movement during the measurement process, and drift of the spectral baseline which leads to distortion of the measured EPR signal. The sensitivity profile of the resonator detection loop is inhomogeneous and has significant fall-off with increased distance from the loop (Pollock 2010). In order to eliminate the possibility of separation between the subject and loop from arising during measurements, a fixed resonator positioning device which incorporates spring loading to maintain uniform pressure of the loop on the tooth has been designed and is being tested. This device also incorporates a three-dimensional staging device which facilitates more precise positioning of the loop and record of its position. Baseline distortion is a spectral manifestation of coupling between the electrical and mechanical components of the instrument, specifically including vibrations and eddy currents related to field modulation (Sealy et al. 1985). These distortions are not reduced by simple averaging of repeated scans, but can be ameliorated through averaging of independent measurements where the instrumental geometry has been adjusted. This type of averaging is part of our standard operating procedure, and efforts to further reduce the amplitude of this noise source are underway, including advanced bridge designs incorporating baseline offset compensation circuitry, multi-frequency data acquisition, and the use of faster sweep rates to reduce the potential for magnetic coupling.

With these technical advances, reduction of the SEP to values below 0.5 Gy appears to be fully feasible. Additional refinements of the instrument to facilitate deployment of the instruments and reliable operation by untrained personnel and emergency responders are also under way. These include complete automation of instrumental electrical settings, the development of ergonomic and self-correcting devices and processes for subject and resonator positioning, and optimization of the complete instrument in terms of size and weight. In addition to increased reliability and utility, these advances are also expected to increase overall throughput. With this combination of performance characteristics, in vivo EPR tooth dosimetry appears to be capable of meeting the need for screening for clinically significant radiation exposures in the wake of an incident where large numbers of people may have been exposed.

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Figure 1.
The existing transportable EPR tooth dosimeter incorporates a 30 kg dipole magnet and a set of integrated detection, control, and power supply instruments which are housed in a rugged deployable case.

Figure 2.

Measurements can be performed routinely in the transportable dosimeter with 17 cm magnet for both human subjects and the mouth model. (a, b) Subjects positioned for in vivo measurements, (c) mouth model placed in between poles of magnet with resonator positioned for measurement of the front surface of the upper left central incisor, (d) magnification of placement of resonator detector loop on the surface of the tooth. For visual clarity resonators are shown here without the sterile plastic barrier, which usually covers the resonator.

Figure 3.

Reproducibility of resonator positioning on upper central incisor teeth was evaluated by measuring the EPR amplitude for a layer of paramagnetic char affixed to their back and front surfaces in human subjects and in the mouth model. No significant differences in EPR signal amplitude were observed, which is indicative of accurate resonator positioning and the equivalence of measurements in the mouth model and the in vivo human mouth. Error bars represent the standard error of the mean ($n = 8$ in vivo, $n = 4$ mouth model).

Figure 4.

The dose responses of upper incisor teeth in the mouth model for both labial (left) and lingual (right) surfaces are shown. The open circles in each panel represent independent EPR measurements of three distinct teeth irradiated to the indicated doses.

Figure 5.

The radiation-induced EPR signal amplitudes measured for the labial (left) and lingual (right) surfaces of upper incisor teeth in groups of unirradiated subjects ($n = 6$) and patients that had received total body irradiation with a prescribed dose of 2 Gy ($n = 6$ for labial, $n = 5$ for lingual). The minimum values, the lower quartiles, medians, upper quartiles, and maximum values are shown, along with the mean. Single outliers, included in box-plots, were identified in the labial-unirradiated and the lingual-TBI datasets, where deviations beyond the quartile amplitudes greater than $1.5 \times$ the inter-quartile range were observed. A statistically significant difference was observed between the signals recorded from the normal and TBI subjects on labial surfaces ($p = 0.001$) based on the one-tailed Student's t -test with uniform variance. For the lingual surfaces the similarly derived p -values was found to be 0.013.