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Developments in Biodosimetry Methods for Triage, with a Focus on X-band Electron Paramagnetic Resonance In Vivo Fingernail Dosimetry

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Abstract

Instrumentation and application methodologies for rapidly and accurately estimating individual ionizing radiation dose are needed for on-site triage in a radiological/nuclear event. One such methodology is an in vivo X-band electron paramagnetic resonance (EPR) physically-based dosimetry method to directly measure the radiation-induced signal (RIS) in fingernails. The primary components under development are key instrument features, such as resonators with unique geometries that allow for large sampling volumes but limit RIS measurements to the nail plate, and methodological approaches for addressing interfering signals in the nail and calibration of dose from RIS measurements. One resonator development highlighted here is a Surface Array Resonator (SRA) designed to reduce signal detection losses due to the soft tissues underlying the nail plate. Several SRA geometries, along with ergonomic features to stabilize fingernail placement, have been tested in tissue-equivalent nail models and in vivo nail measurements of healthy volunteers, using simulated RIS in their fingernails. These studies demonstrated RIS detection sensitivities and quantitation limits approaching the clinically relevant range of 10 Gy. Studies of the capabilities of the current instrument suggest that a reduction in the variability in RIS measurements can be obtained with refinements to the SRA and ergonomic features of the human interface to the instrument. Additional studies are required before the quantitative limits of the assay can be determined for triage decisions in a field application of dosimetry. These include expanded in vivo nail studies and associated ex vivo nail studies to provide informed approaches to accommodate for a potential interfering native signal in the nails when calculating the RIS from the nail plate spectral measurements, and to provide a method for calibrating dose estimates from the RIS measurements based on quantifying experiments in patients undergoing TBI or total skin electron therapy.

Keywords

Dosimetry; ionizing radiation; electron paramagnetic resonance spectrometry; fingernails; in vivo

Introduction

The possibility of an inadvertent mass exposure to ionizing radiation, due to the release of radioactivity from a nuclear accident or detonation of improvised or strategic nuclear devices, is ever present. This fact has been highlighted by a number of accidents involving nuclear power facilities that resulted in severe reactor core damage and release of radioactive materials into the environment surrounding the power plants at Three Mile Island, Chernobyl and, most recently, the multiple reactor failures at the Fukushima Daiichi nuclear power facility in 2011, and the increased risk of nuclear weapons use as evidenced by the escalation by North Korea in honing its capabilities to launch a nuclear attack.

Although the acute effects of radiation toxicity resulting from power plant accidents were confined predominantly to employees and disaster response personnel who were present during the immediate phase of the disasters, the situation could have been worse if larger amounts of radioactivity had been released into more populated areas. Of even greater concern in regard to the scope and severity of a large-scale exposure to the radiation comes from the threat of an improvised or strategic nuclear weapon detonating within a large city or metropolitan area. Under this scenario the expectation is that up to a million individuals could receive potentially life-threatening exposures, whose clinical significance varies by whether they received whole-body doses or partial body dose, due to shielding from incident radiation and/or greater distance from the blast (Grace et al. 2010; Coleman and Koerner 2016). In all of these scenarios, there will be a need to be able to assure a large population that they have not been exposed to radiation doses that could result in acute effects, and this is very likely to require measurement in the affected population.

Overall goals of dosimetry methods for triage

To address the medical and social needs of these individuals a highly organized and well-prepared response is needed to accommodate assessing and caring for the large number of individuals who will need to be triaged within a few days following the event. The overall goal of the initial triage is to rapidly separate those individuals who can benefit most from medical care from those who do not require medical care or have received radiation doses in excess of what aggressive salvage therapies can offer, so as to avoid overwhelming the capacity of the available medical care system.

Therefore, the initial triage stage of a response effort requires methods that can provide a rapid assessment of the radiation dose received, the distribution of the dose (i.e., whole or partial body) and, if possible, the physiological responses of the individual to the radiation exposure, as well as assessment of other injuries (trauma, burns, etc.) that may also have been sustained. Depending on the technological and staffing complexity of the dosimetry methods, they are either deployed at point-of-care (requiring lower complexity to use) or in distant facilities with more complex, high throughput capabilities (Flood et al. 2016). To

address the needs for rapid dose assessment for individuals arriving at triage centers, on-site widely-distributable point-of-care dosimetry methods will be the first line of dose assessment.

Strengths and limitations of dosimetry based on biological and physical responses to dose

Dosimetry methods can be biologically based, i.e., measuring dose dependent perturbations in cellular structures (e.g., chromosomal aberrations), processes (e.g., gene expression), loss of cells (e.g., decreased lymphocyte counts), or systemic responses (e.g., cytokine levels) (Swartz et al. 2014). In regard to biologically-based dosimetry methods, there are several well established, “gold standard” methods including the dicentric assay (DCA), cytokinesis block micronucleus assay (CBMN) or the lymphocyte depletion kinetics assay (Sullivan et al. 2013). Although these are well established assays for performing retrospective dosimetry in small events, they are not as amenable to performing rapid dosimetry for the purposes of initial triage of large numbers, largely due to the delay of 1–2 days to complete the assay or to the logistical difficulties related to multiple blood draws (Flood et al. 2014). Other biodosimetry methods are under development that can provide more rapid assessment of dose, such as those that rely on dose dependent levels of DNA double strand break damage (gamma H2Ax assay), or changes in gene or protein expression (Marchetti et al. 2006, Paul and Amundson 2008, Rothkamm et al. 2013a, 2013b, Xu et al. 2013 Brenner et al. 2015). These gene or protein expression assays also have the potential advantage of assessing physiological or systemic responses to radiation that could further inform medical care decisions, leading to improved survivability or response to treatments. Nonetheless, in regard to meeting the demands of rapid radiation dosimetry, many biologically based assays have limited applicability due to their inherent time-dependent relationship to biological responses to any type of damage, including that induced by ionizing radiation, which includes: (1) an initial latency period, followed next by (2) a rapid period of response during which the biomarker matures, followed by (3) a relatively short, stable response level which is then followed by, (4) a return toward initial levels (Fig. 1 illustrates the general case for biodosimetry assays that are upregulated by exposure; the periods are similar for downregulated bioassays, albeit in the opposite direction of change). Unlike the nearly instantaneous, stable and permanent physical response to dose exposure, the dynamic processes inherent in the biological parameters’ response restrict the timeframe available to obtain valid samples and impact the overall timeframe to obtain valid results. Because these changes may also be influenced by other factors such as concomitant injury, the optimal time to assess the biomarker and/or how and when to interpret biomarkers when sampled ‘at convenience’ due to logistical necessity further compromise the interpretation of the assay’s results for triage decisions.

An alternate approach to dosimetry uses methods that are physically based, i.e., they are based on assaying the dose-dependent chemical/physical responses in calcified or hardened tissues such as in bone, teeth or nails (finger- and toenails) or in solid materials (cell phone components, salts, glass, etc.) (Ainsbury et al. 2016, Bailiff et al. 2016, McKeever and Sholom 2016, Romanyukha et al. 2014a, Swartz et al. 2014). Physically-based biodosimetry methods rely on measuring dose dependent chemical changes that occur in biological

materials (typically hardened or calciferous biomaterial such as teeth or nails) as a means of estimating radiation dose to an individual (Swartz et al. 2012). This typically leads to a rapid onset in the chemical response to the incident radiation exposure and to a long-term, stable response (Fig. 1). Because of this, physical dosimetry offers some potential advantages over biologically based dosimetry methods, i.e., physical dosimetry: 1) usually does not require removal of a sample, 2) does not require time for changes to occur, 3) is unlikely to be affected by other perturbations associated with an acute event, e.g., stress, wounds, and burns, 4) is unlikely to be affected by pre-existing medical conditions, 5) provides dose at spatially defined sites, and 6) the assays do not require considerable expertise to perform or evaluate. There are also some limitations: physical biodosimetry methods may not be as sensitive as some biological parameters; may not be applicable to some individuals; provide dose only at a very specific site on the individual; and do not reflect the clinical implications of the radiation exposure (e.g., do not show damage to vital organs). Even so, the physically based methods do offer a rapid method for estimating dose in a triage situation.

In vivo EPR dosimetry methods

One of the most well-known and highly reliable physically-based biodosimetry methods is based on the measurement of the carbonate radical anions formed when tooth enamel is exposed to ionizing radiation using electron paramagnetic resonance spectrometry. This dosimetry method has long been used in retrospective dosimetry to accurately estimate acute and chronic exposure doses of radiation to an individual because of the permanence of the radiation-induced signal in the tooth and the extremely low inter-individual variation in the dose-response of the signal (Fattibene and Callens 2010). Recent innovations in EPR instrumentation now provide a means to conduct minimally invasive, in vivo measurements of the radiation induced signal directly on the teeth in situ (Swartz et al. 2014, Miyake et al. 2016) in a portable instrumentation platform (Flood et al. 2014, Williams et al. 2014). It is these features which make this biodosimetry method an excellent choice as a point-of-care dosimetry method for the purposes of rapid, dose-based medical care screening during the triage of individuals in a mass radiation exposure event.

Another very promising use of physical dosimetry biodosimetry method is based on a dose dependent radiation-induced signal (RIS) in finger- and toenails as a means for estimating radiation dose, offering the additional advantage of accessing dose distribution in an individual. A large effort has been devoted to the analysis of the RIS in finger and/or toenail clippings (Trompier et al. 2007, Romanyukha et al. 2010, Choi et al. 2014, He et al. 2014, Romanyukha et al. 2014b, Trompier et al. 2014, Khailov et al. 2015, Wang et al. 2015, Marciniak and Ciesielski 2016, Tipikin et al. 2016, Sholom and McKeever 2017). The ability of the clipped nail dosimetry method to estimate dose has been demonstrated in individuals receiving a high radiation dose due to accidental exposure (>10 Gy) (Romanyukha et al. 2014b, Trompier et al. 2014b). Advancing these efforts into a rapid dosimetry method will require improvements in nail sample processing and instrumental innovations (Choi et al. 2014, Wang et al. 2015, Elajaili et al. 2016, Marciniak and Ciesielski 2016) along with implementation of portable X-band EPR instrument platforms (Suzuki et al. 2010, Romanyukha et al. 2014b).

An even more attractive approach would be to make the measurements of the RIS in vivo, directly on the finger- or toenail. This approach would be applicable for all individuals regardless of the availability of sufficient nail length and would avoid the need to process nails to remove the interfering mechanically induced signal in nail clippings which occur when the nail is clipped to collect it from a subject (Reyes et al. 2008, Black and Swarts 2010, Wilcox et al. 2010, He et al. 2011, Trompier et al. 2014a, Marciniak and Ciesielski 2016). Configurations for in vivo measurements using X-band EPR techniques and specially designed resonators to localize the 9 GHz microwave field from the resonator to interact only with the keratinized fingernails and not the underlying soft tissues on a portable instrument platform for very convenient use in triage are readily envisioned (Sidabras et al. 2014, Grinberg et al. 2016). Herein is a description of the current status of our in vivo EPR nail dosimetry instrument and the steps needed to move this forward towards a field ready application.

Materials and Methods

This study was performed in strict accordance with a Dartmouth College Institutional Review Board (IRB) protocol. Use of a questionnaire to collect demographic information, nail health, and use of select dietary supplements and nail measure procedures for making in vivo nail measurements in healthy volunteers were approved by the Dartmouth College IRB.

In vitro finger/fingernail models

Surface array resonators and finger positioning techniques were initially developed through the use of in vitro models to replicate the in vivo situation. Measurements of the native background signal and the dose-response of the RIS in fingernails were measured in the nail plates isolated from six cadaver fingers (index or middle fingers). Nail plates isolated from cadaver fingers were soaked in deionized water for 15 min, and then dried overnight in equilibrium with the ambient humidity prior to irradiation to doses of 0–10 Gy. Following irradiation, the plates were mounted on a block of 35% polyacrylamide gel wrapped in plastic wrap using thin strips of cellophane tape. The polyacrylamide gel (PAA) provides a good model of the soft tissue that underlies the nail plate (Sidabras et al. 2014). Once mounted, the nail plate/polyacrylamide assembly was inserted into the resonator housing and affixed so that the nail plate is oriented directly under the SRA and in contact with a thin Teflon sheet covering the SRA elements. The final spectra used in the analysis of nail background or RIS signals was obtained from an average of 50×7 s scans

For studies of native background and RIS dose-response in intact cadaver fingers, the six index or middle fingers were thawed to room temperature in plastic bags to retain water content of the nail plate. Cadaver fingers were serially irradiated (0, 5, 10, 15 or 20 Gy) at room temperature within plastic bags. Following each radiation dose, the cadaver fingers were removed briefly from the plastic bags and inserted into the finger mounting platform within the resonator housing, oriented directly under, and in contact with, a thin Teflon sheet covering the SRA element. The final spectra used in the analysis of nail background or RIS signals was obtained from an average of 50×7 s scans.

Nail plate water content in isolated and intact nail plates, and the nail plates of live subjects, was determined using a near-infrared (NIR) Spectrometer (Model NIRS-T5-02-1100-2200-SMA, 1100-2200 nm, BaySpec, Inc.) through the method described by Egawa *et al* (2003). Water content in the nails of live subjects and the intact cadaver nail plates was within the range of 17–23%.

In vivo fingernail measurements

IRB approved protocols for human use were developed to take measurements on unirradiated nails of volunteer subjects. The protocols established methods for subject eligibility, ergonomic positioning of the subject for measurements, proper placement of the finger and fingernail relative to the resonator for effective data acquisition, and standard methods for data acquisition and analysis. For each measurement session, subjects were instructed to sit facing the magnet with their right or left arm extending into the magnet, placing the index finger into the resonator housing that lies between the magnet pole faces, until the finger is closely-fixed within the resonator, with the nail plate oriented under and in direct contact with the Teflon sheet protecting the SRA. The wrist and arm were braced, using custom made supports to provide optimal comfort.

EPR spectra were acquired, with the subject positioned for in vivo measurements of the index finger, using two approximately 3 minute measurement sessions consisting of 25×7 s scans per session, with a 3 minute rest period with the subject outside of the device between scan sessions. The final spectra used in the analysis of nail background or simulated RIS signals was obtained from averages based on all 50 scans.

For each subject, measurements of three different signals were made: the first was on the uncovered nail plate (to measure the background signal in the nail). The remaining two were made using two different types of adhesive plastic film covering the nails which had inherent EPR signals that appropriately simulate the singlet line shape of the RIS spectrum.

The plastic films used to simulate the RIS signal in nails were Kapton® (Dupont) polyamide films, in two thicknesses (0.025 and 0.05 mm) with a 0.04 mm acrylic adhesive for a total thickness of 0.06 and 0.09 mm, heretofore referred to as the Kapton25 and Kapton35 films, respectively. The signal intensities of the Kapton25 and Kapton35 films have a dose equivalent signal intensity of 15 and 35 Gy, respectively, as measured from the average dose response in intact nail plates on cadaver fingers, irradiated to doses of 15 or 35 Gy (the water content of the intact nail plates on cadaver fingers (19–23% by weight) which approximate that of nail plates in living subjects (17–23% by weight)). These two films provide an EPR signal that simulates well the singlet line shape of the RIS spectrum. Prior to the positioning of the subject's finger in the instrument, a section of film was sized and cut to the dimensions of the nail plate of the index finger, and then affixed to the nail plate.

EPR instrument and operating parameters

EPR spectra are acquired using a Bruker EMX X-band EPR spectrometer with ER041X microwave bridge, and EMX080 magnet power supply and dipole electromagnet (10-inch diameter pole face). Magnetic field modulation (100 kHz frequency) and signal acquisition were controlled by connecting the modulation reference out from the EMX spectrometer to a

Stanford Research Systems model SR810 DSP lock-in amplifier (time constant 6dB [3 ms], 500 mV sensitivity, low noise mode). Field modulation amplitude was regulated by amplifying the 100 kHz signal output from the lock-in amplifier through a custom-built amplifier, providing a 0.5 mT modulation amplitude within the SRA housing. A slide-screw tuner was used to adjust the coupling of the resonator and microwave bridge. Spectral acquisition parameters were the following: 15.0 mT sweep width, 5.12 s scan time, 10.49 ms time constant, 5.12 ms conversion time, 10.01 output microwave power, receiver gain 5×10^4 , and averages of 25 or 50, 7 s (5.12 s field scan time plus 1.88 s system reset and equilibrium time) scans.

Results and Discussion

Surface array resonator for nail dosimetry

At the operating frequency of nominally 9.3–9.5 GHz (X-band), the underlying soft tissue of the finger posed a challenge to achieve optimum detection sensitivity and consistency in the measurements of the radiation-induced signal (RIS) in the nails. To minimize the effects that the soft tissues have on RIS measurements made in vivo, several SRA geometries have been designed and tested. As described by Sidabras *et al.* (2014), these SRA geometries use distributions of currents to create a near-field magnetic pattern that limits the field to the nail plate and minimize microwave losses into the soft tissue bed underlying the nail plate. In making modifications to the cross section of the arrays, the electric field component of the microwave radiation incident at the nail plate is greatly minimized, thus resulting in further gains in resonator efficiency (Fig. 2).

In addition to the SRA development, a design for a shield housing was needed to provide a firm anchor of the SRA. It included modulation coils to provide a means to modulate magnetic field near the SRA (Fig. 3). The shield housing also functioned to provide a support platform for optimizing placement of a finger and nail plate under the SRA (Fig. 4). The combination of these design features was tested in in vitro finger models and in vivo fingernail measurements on volunteer subjects, with the results as described below.

X-band EPR SRA resonator testing in in vitro nail models

Three array configurations were designed and tested, a 7, 9 and 11 element SRA (Sidabras *et al.* 2014). These configurations provided extensions (90% field output) of 0.85, 0.6 and 0.45 mm from the face of the array elements. Early SRA development work focused on the 7-element SRA, which had good signal detection sensitivities and dose response down to 2.5 Gy in various irradiated finger models involving simulated nail plates (alanine-polystyrene films) and isolated cadaver nail plates mounted on polyacrylamide gel (PAA). However, when measuring the RIS dose-response in the isolated nail plates without the PAA backing, the slopes of the dose-response curve were higher than those measured in the simulated nail plate and isolated cadaver nail plates mounted on the PAA backing. This is likely due to losses of microwave signal due to extension of the microwave field beyond the thickness of the nail plate and into the underlying soft tissue PAA model.

In response to the test results of the 7-element SRA, an 11-element SRA was designed and tested for detection sensitivity and precision in an in vitro fingernail model. The 11-element array concentrates the detection volume of the resonator to well within the nail plate and greatly minimizes losses in detection sensitivity due to the lossy PAA gel. Six new nail plates were isolated from cadaver fingers and equilibrated to the ambient humidity to ensure a dose response of the RIS that is consistent with the 7-element study. These plates were serially irradiated to doses of 0–10 Gy and mounted on tissue equivalent polyacrylamide (35%) gels (PAA) to simulate the soft tissue of the finger.

The dose-response of the RIS measured in these nail plates demonstrated resolution down to 2 Gy (Fig. 5). The 2 Gy RIS was clearly differentiated from the background signal in the nails in all six cadaver nail plates (Fig. 6A). Also, there was good reproducibility in the quintuple measurements of the signal intensity of the RIS (from a 20 Gy dose), made following five independent placements of a nail at the SRA, performed on each of the six isolated cadaver nails, as shown in Figure 6B.

The final experiment examined the dose response of the RIS in intact cadaver nail plates. With intact cadaver nail plates there was a decrease in the overall intensity of the native background and RIS signals (Fig. 7) compared to the isolated nail plate model (Fig. 6A). This decrease is due to losses in microwave signal caused by the high water content of the cadaver nail plates (19–23% by weight) compared to isolated nails (<6% by weight). The high water content of the intact nails also contributed to a 2.7-fold reduction in the slope of the RIS dose response compared to that found for the isolated nail plates, which effectively reduces the detection threshold of the assay from approximately 2 Gy (isolated nail plate model) to approximately 6 Gy in the intact cadaver nail plate model. This reduction in the dose-response is likely the result of the loss of the RIS2 in the intact nail due to the higher water content of a cadaver nail plate. It is known that of the two major components of the RIS, the stability of the more intense RIS2 signal is affected by the water content of the nails, whereas the less intense RIS5 is stable even under conditions of high nail water content and elevated temperatures (Trompier et al. 2009, Trompier et al. 2014a). It is hypothesized that due to the elevated water content of the irradiated nail plate intact on the cadaver finger, the RIS2 signal had primarily decayed prior to measurement, leaving the RIS5 as the remaining measurable radiation-induced signal. Thus, the dose response curves of the nail plates on PAA (containing both the RIS2 and RIS5) and intact cadaver nail plates (RIS5) provide the upper and lower dose responses, respectively, in physically-based dosimetry using in vivo EPR techniques.

Under the least favorable conditions (high water content) of the cadaver nail plate, a signal-to-noise ratio of 1.5–2 was obtained for the native background, whereas ratios of 2–8 for the nails irradiated to doses of 5–20 Gy were obtained. Under the current operational parameters and system configuration of the X-band EPR nail dosimetry instrument, and the cadaver fingernail model used in this study, a dose of 10 Gy or greater was discernable from the native background signal in the nails.

X-Band EPR SRA resonator testing in in vivo nail models

Extension of the 11-element SRA to in vivo fingernail measurements was found to be too conservative in depth sensitivity, resulting in reduced detection sensitivity. Although the losses in resonator efficiency were greatly minimized with the 11-element SRA, the extension of the microwave field was only 0.45–0.5 mm from the face of the array elements. Therefore, only about 2/3 of the nail plate thickness was being sampled when making in vivo measurements of signals in the nail plates. An intermediate array in the form of a 9-element SRA was employed. Computer modeling of the 9-element SRA using Ansys HFSS indicated that extension of the microwave field would be on the order of 0.6–0.7 mm from the face of the array elements (Sidabras et al. 2014). This 9-element SRA field profile matches more closely to the typical thickness of fingernails. Based on experimental detection sensitivity and precision measurements in in vivo testing on fingernails of volunteer subjects, the 9-element SRA was found to be more sensitive for in vivo measurements of nail signals.

Figure 8 demonstrates this using the Kapton25 and Kapton35 polyimide films, affixed to the in vivo nail to simulate the measurement of 15 Gy and 35 Gy equivalent RIS signal intensities, respectively, of four healthy volunteer in comparison to nail background signals. Peak-to-peak signal intensities of the background and Kapton25 signals were normalized to the signal intensity of an internal standard (Bruker proprietary field marker accessory, $g = 1.99$). The precision of multiple, independent Kapton25 measurements (Table 1) made on the same individual (intra-individual) after correction for the underlying background signal intensity, was typically under 14%. However, variability between volunteers (inter-individual) was as high as 20%, due to differences in size and curvature of nail plates. The mean signal intensity of the native background measured in 4 healthy volunteers was equivalent to a 3.6 Gy equivalent RIS signal intensity.

These results represent the first in vivo EPR fingernail studies to detect the presence of the native background signal in nail plates, similar to that observed in clipped nails (Reyes et al. 2008, He et al. 2014, Romanyukha et al. 2014b, Trompier et al. 2014a, 2014b). The data suggests that the background signal measured both in vivo and in clipped nails is a native signal and not necessarily formed in clipped nails as a consequence of the nail cutting process. There was greater intra- and inter-individual variability in the background signal intensity compared to the in vivo Kapton25 film measurements. This may be due to differences in the curvatures of the nail plates between fingers within the same and different individuals (Murdan 2011) and distribution of free radical centers in the nail plates that give rise to the native background signal. For example, the exposure of nails to UV light gives rise to a signal in nails that is virtually indistinguishable from the RIS (Sahiner et al. 2015, Tipikin et al. 2016), which may lead to differences in the depth distribution of the free radicals formed in the nail.

Future development of in vivo EPR fingernail dosimetry

The results obtained from the measurement of the RIS in in vitro fingernail models and of the simulated RIS measured in vivo in fingernails have demonstrated the proof-of-principle capability of the current EPR instrument system. The system consists of a commercial EPR

spectrometer with a custom-made SRA resonator. It has the potential to detect the RIS at doses that are relevant for the purposes of estimating dose in field triage situations. In the in vitro nail models used in this work, the RIS is easily detected in isolated cadaver nail plates irradiated to 2 Gy and discernable from the background signal in an unoptimized instrument system. However, under conditions that are representative of the state of nails (water content and temperature) in live subjects, the current capabilities of the unoptimized EPR instrument are sufficient for detection of the RIS (mainly the RIS5) in high dose exposure events (10 Gy and higher) as suggested by the results of the irradiated intact cadaver nail plate model, especially in situations where partial body exposure has occurred, for example the hand, and a native signal in nails can be assessed in the unexposed hand. This approach has been shown to be successful when using clipped nails for estimating dose in accidental radiation exposures to the hand (Romanyukha et al. 2014b, Trompier et al. 2014b).

Improvements in the EPR instrumentation are expected to improve the detection limits and the precision of the RIS measurements in vivo. As highlighted by the variability in the results of the in vivo measurements of the background signal and the signal from the Kapton25 film affixed to the nail plates, additional design modifications of the SRA are required to gain greater conformity of the nail size and curvatures of the nail plate for better matching of the resonator and nail plate interface. These modifications would not only reduce the variations in the nail measurements but also increase the signal-to-noise by at least a factor of 2–3. Other measures to further reduce measurement variability include improvements in ergonomic features of the human interface to the instrument, such as finger, hand, arm and body positioning and stabilization, in addition to moisture and temperature control at the resonator and nail plate interface.

In addition to the resonator design modifications and ergonomic considerations, there are gains to be realized in the instrument components and spectral acquisition and data processing systems. Resonator coupling designs have yet to be optimized to maximize the efficiency of the resonator and detection sensitivity. Incorporation of a newly available, custom-built microwave bridge is expected to increase stability of measurements using low Q-value resonators, such as the SRA, and conducting rapid scan spectra acquisition to increase signal-to-noise (Stoner et al. 2004, Eaton et al. 2014, Elajaili et al. 2016). The combination of the coupling optimization and integration of the custom microwave bridge, along with the design modifications in the SRA resonator as described above, are expected to make gains in signal-to-noise of at least 20, which will easily offset some losses in resonator efficiencies due to the moisture content of the nail plates in vivo. Additionally, rapid scan is performed at a rate faster than physiological cofounders, such as heartbeat, that contribute to noise and baseline, further improving the quality of the data.

Despite the improvements in instrumentation that are expected to provide advancements in RIS detection and measurement capabilities, there are other factors that may limit the application of the in vivo EPR nail dosimetry method for retrospective dosimetry on individuals suspected of exposure to ionizing radiation within the range of clinically relevant doses. The most important factors are the native background signal in nails and calibration of the RIS measured in vivo. As has been demonstrated in this work, a native background signal is detected in the nail plates of cadaver fingers and, more importantly, in the nail

plates of live subjects. This native signal overlaps with the RIS signal and potentially confounds the measurement of the RIS. Given the magnitude of the native signal, it may be difficult to clearly measure the RIS *in vivo* at doses that are important to initial dose triage. For example, estimation of dose at the 2 Gy threshold required for initial triage cannot be accomplished unless the background can be measured directly for each subject, or can be corrected based on an estimated signal intensity that is derived from population norms for the native signal. Estimating the native background from population norms, as derived from surveys of the native background measured *in vivo* in volunteer subjects, is possible but the accuracy of a dose estimate calculated from an RIS will depend on the precision of the background correction. Additional study of the native background is needed to more fully define the variability of the native background signal and refine the estimates of the signal based on demographic classifications, for example gender and race, as is suggested from unpublished results from native background measurements in clipped nails.

In addition to the complexity of correcting for the native background in *in vivo* measurements in irradiated nails, there is the need to develop a calibration method to calculate a dose estimate from RIS measured in nail plates. Given the instability of the RIS2, especially under the water content and temperature of nail plates in live subjects, the calibration of dose estimates are expected to be based on the stable, RIS5 signal in nails. For this to be practical it is important to determine at what point the RIS2 is fully decayed in nails *in vivo*, thus providing a time line from which the stable RIS5 can be measured. Calibration of the RIS5 signal intensity to dose is possible within human subjects based on measurements of the RIS in nail plates of patients undergoing: (1) total body irradiation (TBI) in preparation for bone marrow transplants for such cancers as lymphoma or leukemia, or (2) patients receiving total skin electron therapy (TSET) to treat skin-based T-cell lymphomas (mycosis fungoides) and conditions such as Kaposi's sarcoma. Because of the stability of the RIS5 in nails (Trompier et al. 2014a), a calibration function can be generated for this signal based on the additive signal intensity of the RIS5 produced in the nail plates of patients as they receive successive fractions of radiation dose. Also, there may be a low variability in the dose response of the RIS5 *in vivo* due to the limited range of water content of the nail plate *in vivo* (Egawa et al. 2003). The probability of success of this calibration approach can be maximized by fully correcting for the native background signal, although this is not necessary as incremental changes in total nail plate signal (RIS5 + background signal) with added dose in the same nail plate can be measured that will reflect the dose dependent changes in the RIS5 signal. While a dose response for the RIS5 can be generated for calibrating signal intensity to a dose estimate, it remains to be seen whether a universal calibration function can be generated for the RIS5. Further efforts to advance the *in vivo* EPR nail dosimetry method will depend on achieving sufficient detection sensitivity and measurement precision of the RIS5 signal in nails, and the success of accounting for the native background signal in the irradiated nail plate signal.

Conclusion

The *in vivo* fingernail EPR instrument being developed shows promise as a portable dosimetry platform for the purpose of triage in the event of a nuclear disaster. The assay method takes advantage of the virtually instant production of the irradiation-induced, dose

dependent signal and the extended lifetime of the signal that are typical of physical dosimetry methods. We have developed a first-generation resonator that has sufficient sensitivity for initial development of instrumentation as tested in in vitro cadaver nail plate models, in vivo cadaver fingers, and in vivo on volunteers. We found that the current limitations are due to detection sensitivity, and the stability of the holder and contouring of the resonator to the curvature of the nail plate. Further gains in sensitivity are expected to offset losses in resonator efficiency due to moisture in the nail plate with the implementation of currently available advanced microwave bridge and spectral acquisition technologies. With these gains in the in vivo fingernail EPR instrumentation performance, reliable estimates are expected to approach the 2 Gy threshold dose needed for the initial screening of victims of a large scale nuclear event. However, key to this objective is the successful ability to fully correct for the native background in nails and establish a calibration method to convert RIS5 intensity measurements to dose estimates. These will be addressed in further in vivo nail developmental efforts and from information gained from ex vivo studies in clipped nails.

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Figure 1.

Time course illustration of dose dependent assays for dosimetry: physical and biologically-based assays for dosimetry. At (1: time of exposure), the person's baseline value varies with the individual in both cases. At (2: end of latency period), there is no delay in response for physical assays but there may be a latency period before biological radiation-induced parameters begin to evolve. At (3: plateau) physical changes essentially occur instantaneously while most biological parameters evolve relatively slowly (from hours to days or weeks) until they reach a plateau. At (4: end of plateau), the diagnostic period during which valid samples can be obtained is essentially limitless for physical changes but ends for biological assays when the values begin to change back to baseline. (5: return to baseline) does not occur in physical changes (e.g., the dose-dependent changes in tooth enamel are stable for millions of years); however, biological parameters return toward normal values as the response cycle ends.

Figure 2.

Cross-sections of two SRA designs, showing the electric field simulated by HFSS with the sample (fingernail) and the underlying finger being measured in vivo. Note: The darker the gray of the cross-section, the lower the electric field strength. Comparing the electric fields in (A) vs (B): Design (B) has 4 times less electric field deposited in the nail and finger compared to Design (A), which results in Design (B) having 2 times greater resonator efficiency and 2 times greater EPR signal strength at a constant field, as compared to Design A.

Figure 3. The shield houses modulation coils and anchors the SRA. The shield also functions to anchor the finger positioning support to stabilize position of fingertip and nail plate under the SRA.

Figure 4.

Support structures are designed and built to stabilize fingernail positioning at the SRA resonator. Additional instrument interface features (not shown) include hand, wrist and arm supports to help in maximizing comfort of the volunteer, thus providing further stabilization of the finger and nail positioning at the SRA resonator.

Figure 5.

EPR X-band spectra taken within an in vitro nail model consisting of a fingernail isolated from a cadaver finger irradiated to 0 and 2 Gy gamma ray dose, and mounted on a block of 35% acrylamide gel (soft tissue model). The overlapping spectra show the spectral difference between the background signal (black) and RIS signal from the 2 Gy dose (grey). Spectra were acquired using the 11-element SRA with a 337.5 mT magnetic field center, a 15.0 mT scan width (5.0 mT scan width shown), and microwave frequency of 9.48 GHz.

Figure 6.

A. Dose-response of RIS intensity in six isolated cadaver fingernails mounted on a 35% polyacrylamide gel (PAA) finger model using an 11-element. A SEIP of 2.07 Gy is calculated. B. Nail repeatability measurements using the six nails in the study. The points in the figure represent the mean $\pm$ SEM of quintuple measurements of RIS intensities in each nail (irradiated to a 20 Gy dose), each made following independent placement of the nail under the resonator.

Figure 7.

Dose-response of RIS intensity in six nail plates, intact on cadaver fingers, as measured using a 9-element SRA. The points in the figure represent the mean $\pm$ SEM of the RIS intensities measured in the six cadaver fingernail plates irradiated serially to doses of 0, 5, 10, 15 or 20 Gy dose.

Figure 8.

EPR X-band spectra acquired in vivo from a fingernail of a volunteer, either uncovered (solid line) showing the native signal in the nail plate, or covered with the Kapton35 (medium dash) or Kapton25 (small dash) signal standard films. The Kapton35 and Kapton25 spectra shown in the figure have been corrected for the native signal. The amplitudes of the Kapton35 and Kapton25 spectra approximate that expected for RIS formed in nail plates intact on cadaver fingers irradiated to a dose of 35 or 15 Gy, respectively. Spectra were acquired using a 9-element SRA with a 331.5 mT magnetic field center, a 15.0 mT scan width (5.0 mT scan width shown), and a microwave frequency of 9.31 GHz.

Table 1

Comparison of in vivo EPR fingernail measurements of the native background and a simulated RIS signal

Parameter	Signal Source	
	Native Background Signal	Kapton25 Film Standard
Mean Signal Ratio ¹	0.0964	0.397
Intra-individual RSD ^{2,3}	0.209	0.138
Inter-individual RSD ^{2,4}	0.267	0.194
RIS Intensity (Gy-equivalent)	3.6	15

¹ Mean signal ratio - ratio of the peak-to-peak intensities of the background or Kapton25 signals over the peak-to-peak intensity of the field reference marker (internal standard) measured in the fingernail spectra acquired from four volunteer subjects

² RSD – relative standard deviation

³ Intra-individual variation – calculated from five separate in vivo measurements, randomly acquired from left and right index fingers within the same individual, with (simulated RIS) or without (background) Kapton25 film affixed to nail plate

⁴ Inter-individual variation – calculated from in vivo fingernail measures acquired from the index fingers of four volunteer subjects, with (simulated RIS) or without (background) Kapton25 film affixed to nail plate