FACTORS AFFECTING THE QUALITY OF TOOTH ENAMEL FOR *IN VIVO* EPR-BASED RETROSPECTIVE BIODOSIMETRY

Céline M. Desmet, Philippe Levêque and Bernard Gallez*

Biomedical Magnetic Resonance Research Group, Louvain Drug Research Institute, Université Catholique de Louvain, Avenue Mounier 73 – B1.73.08, B-1200 Brussels, Belgium

*Corresponding author: Bernard.gallez@uclouvain.be

***In vivo* electron paramagnetic resonance biodosimetry on tooth enamel is likely to be an important technology for triage of overexposed individuals after a major radiological incident. The accuracy and robustness of the technique relies on various properties of the enamel such as the geometry of the tooth, the presence of restorations, whitening treatments or exposition to sunlight. Those factors are reviewed, and their influence on dosimetry specifically for triage purposes is discussed.**

INTRODUCTION

Retrospective biodosimetry is a specific aspect of ionizing radiation dosimetry that is specially required when individuals not wearing conventional dosimeters are overexposed to ionizing radiations⁽¹⁾. In such circumstances, an evaluation of the absorbed dose is needed to provide appropriate medical care^(2, 3).

Various biodosimetry methods, either biologically based or physically based techniques, have been described and successfully used to ascertain an accurate dose reconstruction (to name only a few of them, dicentric assay, micronuclei assay, FISH painting, electron paramagnetic resonance (EPR), optically stimulated luminescence (OSL) or numerical simulations; for review, see refs.^(4–6)). When the dosimetry is required for only a limited number of people, a combination of techniques is also possible in order to get the most accurate results, because the capacities of both dosimetric and medical services are not saturated. Furthermore, medical care can be initiated based on a rapid estimation of the dose, and the strategy can be adapted later when a more accurate dose estimation becomes available, requiring more time-consuming dosimetric techniques.

Retrospective dosimetry using EPR and samples of tooth enamel, thus *ex vivo* enamel dosimetry, is now the most advanced physical method, and this well-established technique is recommended by international organizations such as the International Atomic Energy Agency (IAEA). Although quite accurate, it requires invasive collection of enamel samples⁽⁷⁾.

On the contrary, when a very large number of individuals is expected to be exposed to life-threatening doses, for instance, after the detonation of an improvised nuclear device, both medical and dosimetric services using conventional techniques are likely to be rapidly overwhelmed. Those scenarios are

described by competent authorities that have also published guidelines describing appropriate management of those events^(8–10).

A robust triage strategy, relying on adequate biodosimetry tools, must be available in order to efficiently match the capacities of the medical offer and the needs of treatment and help decision makers in their strategy⁽¹¹⁾.

Under these circumstances, the desired characteristics of biodosimetric tools are somewhat different from the conventional ones.

Those characteristics have been extensively reviewed and discussed in the literature and specialized meetings such as the Biodose Conference^(6, 11). The most important features that should be met for initial triage are the capacity to identify victims likely to have been exposed to doses above 2–3 Gy, desirably in the field, very quickly and with a high throughput. *In vivo* EPR biodosimetry on tooth enamel is a suggested technique meeting those features.

The presence of stable free radicals after irradiation of calcified tissues was reported as early as 1955 by Gordy *et al.* in skull bone⁽¹²⁾. They remarkably demonstrated that ionizing radiations induced the formation of radicals in mineralized tissue in a dose-dependent manner. Later, Brady and Swartz suggested the use of tooth enamel as natural dosimeter, combined with the additive re-irradiation method and EPR detection, to assess the dose after accidental overexposure⁽¹³⁾. Ikeya and Ishii extended the approach to *in vivo* measurements⁽¹⁴⁾.

It is worthwhile to mention that Swartz and his group at the Dartmouth Geisel School of Medicine have, since then, considerably developed both the technique and the instrumentation, and have recently proposed an EPR spectrometer for *in vivo* measurements of tooth enamel, directly in the mouth of the overexposed individual. This system, in the field

deployable, has been extensively described and tested^(15–19).

The basis of tooth dosimetry is the dose-dependent formation of stable CO_2^- radicals by ionizing radiations in the hydroxyapatite matrix of the tooth enamel (for comprehensive review, see Fattibene and Callens⁽²⁰⁾).

Because enamel is used as natural dosimeter, much attention has been given to the different factors affecting the quality of the enamel and potentially the results of the dosimetry. In this article, several important aspects are pointed out and discussed, namely the geometry of the tooth, the role of restorations, the influence of sunlight and the influence of whitening treatments.

GEOMETRY OF THE TOOTH

One main parameter that can influence the dose determination is the size and geometry of the measured tooth. As the amount of free radicals created in the enamel will be dependent on the mass of enamel, the size of the tooth used for the measurements will influence the results. It was demonstrated that a better reproducibility in measurements were obtained after normalization of the results by the tooth size⁽²¹⁾. Also, several works^(22, 23) evidenced that the geometry of the tooth as well as the geometry of the coil and the positioning of the tooth regarding to the surface coil were particularly important for dose estimation by EPR. In this context, works are also made to image and quantify tooth enamel using a magnetic resonance imaging technique (MRI) (zero echo time MRI) in order to evaluate relationship between enamel variations (in terms of size and defects) and dose estimations based on EPR⁽²³⁾.

ROLE OF TOOTH RESTORATIONS

Another important factor that has to be taken into account when using teeth as dosimeter is the presence of dental restorations.

Tooth restorations are quite common, as ~91% of US adults aged 20–64 have dental caries in permanent teeth⁽²⁴⁾.

They can affect dosimetric results in several ways, which are reviewed in this section.

Amalgam fillings

Regarding the historical amalgam fillings in teeth, consisting of liquid (elemental) mercury and a powdered alloy composed of silver, tin and copper, it was demonstrated that the presence of such restorations did not influence the measured EPR signal^(25, 26).

The situation is quite different with the new generation of polymeric fillings, which have largely replaced amalgam fillings.

Composite fillings

Dimethacrylate-based composites are now commonly used for restoration of teeth in dentistry. They are composed of an organic matrix (20–50% w/w) and inorganic fillers (50–80% w/w)⁽²⁷⁾. The organic matrix contains dimethacrylate monomers, photo- and co-initiator of the polymerization. These composite materials are photopolymerized *in situ* using visible light. During the polymerization process, free radicals are created and normally recombined at the end of the polymerization process. But, due to the vitrification that occurs during the polymerization, the mobility of free radicals is hardly decreased and these stabilized free radicals remain trapped in the polymerized matrix⁽²⁸⁾. They are easily detectable using EPR. It was demonstrated that two types of free radicals are created during the polymerization process. The superimposition of the spectrum of these two radicals give a complex nine-line spectrum⁽²⁹⁾.

The EPR signal recorded in dental composites, hereafter the ‘polymerization signal’, possesses a similar resonance frequency compared to the dosimetric signal of tooth enamel (Figure 1) and can consequently potentially interfere in the dose determination based on the EPR dosimetric signal.

Also, fillers that composed the inorganic part of composites mainly consist of silica particles or barium glasses⁽²⁷⁾ in which stable free radicals are generated following ionizing radiations exposure that can be observed by EPR spectroscopy^(30–32). This signal, named ‘radiation-induced’ signal in this article, could also potentially affect the dose determination.

The influence of the different EPR signals of composites on the dosimetric signal of teeth is discussed in the following.

Figure 1. EPR dosimetric signal of tooth enamel (black line) and EPR polymerization signal recorded in commercial dimethacrylate-based composite (gray line). Intensity is expressed in normalized units (n.u.) to DPPH signal.

Nineteen of the dental composites most widely used for restorations of incisors on the US market were screened for the polymerization signal, and its stability monitored. In X-band, a signal was measured in all commercial composites tested with a variation in the signal intensity that can represent 16.5–106.4 times the intensity of the signal recorded in irradiated tooth enamel at 3 Gy in the same measurements conditions during the first hour after the polymerization⁽³³⁾. Signal intensity variations were mainly due to the composition of the composites tested (type and proportion of monomers used in the composites).

Using a L-band spectrometer similar to those used for *in vivo* dosimetry, the signal can also be observed and can be confounded with the dosimetric signal of teeth, when the EPR spectrum is recorded a few hours after the polymerization of the composite.

Fortunately, this polymerization signal is known to be unstable, due to a hydroperoxydation phenomenon⁽³⁴⁾, with a decay influenced by the composition of the surrounding medium.

In a large sample of commercial composites tested, the decay kinetics were compatible with a bi-exponential model, with a fast first compartment ($t_{1/2}$ below 48 hours) and a slower second one ($t_{1/2}$ ranging from 200–650 hours) (Figure 2)⁽³³⁾. Importantly, from an operational standpoint, using L-band spectrometers adapted for *in vivo* measurements, polymerization signal was under the limit of detection as of 18 d after initiation of the polymerization. Nevertheless, it must be mentioned that in a few cases (IPS Empress Direct, Tetric EvoCeram, GrandioSo, Ceram-X), a stable nitroxide-like signal, of low intensity, was detectable in the long term⁽³³⁾.

While restorations are not easily detected by untrained operators, as many composite resins fluoresce under ultraviolet (UV) light, they could be

detected with the help of a UV lamp. One strategy could consist in excluding restored teeth from measurements and measuring an adjacent tooth, although it is not granted that they would not bear any restoration. So the systematic exclusion of restored teeth would significantly impact the global efficacy of the technique. On the contrary, a procedure designed to include the measurement of restored teeth, including a correction of the signal if necessary, would offer a better adaptability of the technique.

Influence of irradiated restorations

Dental composites contain a large fraction of inorganic fillers (~50–80% wt), such as silica particles or barium glasses⁽²⁷⁾, materials which are known to give an EPR signal when exposed to ionizing radiations^(30–32). Free radicals are also likely to be induced in the organic matrix itself, where they can remain stable for an undetermined period of time. Commercial composites were systematically screened to search for a possible radiation-induced signal in the restoration⁽³⁵⁾.

EPR signals were indeed observed in all the tested composites after irradiation at very high doses (100 Gy). The shape of the observed radiation-induced signal was complex and differed in function of the composites studied (Figure 3).

The intensity of the measured signals was also different from one composite to another, with a signal-dose relationship approaching linearity in most of cases. At lower dose level, i.e. 10 Gy, the radiation-induced signal in the composite was detectable in only 3 of the 14 composites. The signal in this case had an intensity of 0.29–0.33 normalized units (n.u.) compared to 0.73 n.u. of dental enamel irradiated at the same dose. Below 10 Gy, the signal was not detectable in X-band conditions. Kinetics studies realized in X-band on irradiated composites at 100 Gy demonstrated that the radiation-induced signal in the composite was unstable in time. Indeed, the signal was only detected the first 24 hours for 7 out of 14 composites. Only one composite had a signal detectable for >1 week, which was still present 1 month after irradiation.

Interference of the irradiated composites on the dosimetric signal in teeth

In order to study the influence of irradiated composites on the dosimetric signal of the enamel, standardized barrels of composites were placed in an irradiated tooth, and measured *ex vivo* using a L-band spectrometer designed for *in vivo* measurement. As anticipated from results obtained in X-band, the radiation-induced signal in the composites did not influence the dosimetric signal of the

Figure 2. Decay kinetics curves of the polymerization signal recorded for two different dimethacrylate-based composites (Synergy D6 in gray, Filtek Supreme Ultra in black). Intensity is expressed in normalized units (n.u.) to DPPH.

Figure 3. Radiation-induced signal recorded in X-band in two different commercial composites 1 hour after irradiation at 100 Gy (top: TPH3, bottom: N'Durance).

Figure 4. EPR signal recorded in L-band in a tooth irradiated at 15 Gy (A) and the same tooth containing a composite (IPS Empress Direct) also irradiated at 15 Gy (B). Peaks at low and high field are due to the ^{15}N -PDT standard (gray arrows). Each spectrum shows original signal (black) and fitted signal (red).

enamel when irradiated at a dose of 15 Gy⁽³⁵⁾. A statistically significant influence of the signal was observed for only one composite, but it appeared that this interference was rather due to the stable nitroxide-like signal instead of the radiation-induced signal (Figure 4).

UV AND SUNLIGHT

UV and sunlight can also induce an EPR signal in the enamel, very similar to the dosimetric signal, confounding the interpretation of the dosimetric signal. That is why, for *ex vivo* tooth dosimetry, the IAEA recommends to use enamel from molars, which are more protected from direct sunlight, because of the shielding of the mouth⁽⁷⁾.

For incisors, the sunlight contribution can be as high as 500 mGy in 50 y⁽³⁶⁾. Consequently, it has been shown that the dose obtained from the enamel located on the outside surface of the tooth could be twice the one obtained if enamel is sampled from the inner side.

Several methods have been proposed to take into account the contribution of the sunlight signal, especially at low doses^(36–38), but the transposition to *in vivo* measurements in L-band is not yet documented.

This influence should also be investigated when performing dosimetry *in vivo* on the external side of incisors. Given the present sensitivity of detection of the dosimetric signal, it is unlikely that this UV-induced signal can be detected. However, it could contribute to a small extent to the dosimetric signal recorded for teeth irradiated at low doses.

TOOTH WITHENING TREATMENTS

Tooth whitening is becoming increasingly popular, as it is a simple technique that can be performed at home with a proven clinical efficacy⁽³⁹⁾.

Tooth bleaching can be performed using oxidizing agents such as hydrogen peroxide or carbamide peroxide. Those agents also form free radicals and reactive oxygen molecules⁽⁴⁰⁾. Hydrogen peroxide, under neutral condition of pH, was shown neither to induce changes in the enamel organic nor inorganic contents⁽⁴¹⁾. On the contrary, carbamide peroxide gels lowered the mineral content, as evaluated by energy-dispersive X-ray spectroscopy⁽⁴²⁾.

Because they both generate free radicals, reactions with the enamel leading to the formation of stable radicals in the matrix could not be theoretically excluded.

The presence of an EPR signal in the enamel after bleaching of the teeth using various concentrations of H_2O_2 or carbamide peroxide gel and different contact times up to 5 weeks was investigated.

Preliminary results showed that under the most aggressive conditions (30% H_2O_2 , carbamide peroxide 16%) and bleaching up to several days, no EPR signal could be measured, both in L-band and in X-band (Figure 5).

As light and heat increase the reactivity of hydrogen peroxide, lasers have been used in external bleaching therapy^(43, 44).

Figure 5. EPR spectra in teeth after a bleaching treatment. Top panel: spectra recorded in L-band in a tooth exposed to 30% H_2O_2 during 1 hour (gray line) and 6 d (dashed gray line) and in a tooth exposed to carbamide peroxide 16% during 6 d (dotted gray line). Black line represents EPR spectrum of a non-treated tooth. Bottom panel: spectra recorded in X-band in a tooth exposed to 30% H_2O_2 during 1 minute (gray line) and in a tooth exposed to carbamide peroxide 16% during 5 weeks (dotted gray line) compared to a non-treated tooth (black line).

This activation is due to a rise in temperature at the surface of the enamel (thermocatalysis) or to a direct excitation by light (photolysis) resulting in an increased release of hydroxyl radicals. The results of several studies focusing on the effect of different types of laser lamps on the properties of the enamel were summarized by De Moor recently⁽⁴⁴⁾. Overall, no significant influence on the mineral content could be evidenced, compared to the control bleaching procedure, without the use of the laser.

Nevertheless, a more thorough investigation of these aspects should be initiated. The group of Fattibene in Rome (Istituto Superiore di Sanita) is currently investigating those aspects, and complete results should be published in the very near future.

CONCLUSION

After a major accident due to a nuclear device and involving a large number of overexposed victims, triage will be a mandatory and essential step in the early hours after the incident. Among the available

retrospective dosimetry techniques, *in vivo* EPR measurement of the dose in the enamel will play a key role, alone or in combination with other methodologies, because it allows for the measurement of the dose very quickly (a few minutes), with a high throughput, and with a satisfactory accuracy in the dose range required for initial triage. It is furthermore deployable in the field. Nevertheless, several factors are known to potentially affect the robustness of the technique, among the factors related to the properties of the enamel. Within the framework and with the support of several programs from the NIAID and the BARDA, several studies have been conducted to elucidate their incidence on the dosimetry. Tooth whitening treatments are still under investigation, but first results are encouraging and suggest no or mild influence. We have demonstrated that tooth restorations can give an interfering signal, but only in the few days after the polymerization; for triage purposes, in most cases, it can be ignored.

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