SPECTROSCOPY LETTERS
Vol. 36, Nos. 5 & 6, pp. 487–499, 2003

# Electron Spin Resonance–Native Signal in Thermally Treated Dental Tissue

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## ABSTRACT

Although the dosimetric Electron Spin Resonance (ESR) signal of hard tissues, particularly enamel, has been extensively studied, little attention has been paid to the native signal. This signal is known to be affected by the health of the tissue, as well as by socio–economic factors. In dental applications several clinical procedures, including the use of laser irradiation, can heat the tissue locally with side effects that must be studied. The purpose of the present work is to study the ESR signals in

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DOI: 10.1081/SL-120026614
0038-7010 (Print); 1532-2289 (Online)

enamel and dentin tissues after thermal treatment with temperatures in the range of 100°C–300°C. Non-irradiated permanent bovine teeth were studied. ESR measurements were performed with a Varian E-4 ESR spectrometer operating in the X band range. Progressively larger ESR signals were produced in dentin tissues previously heat treated at and above 100°C. No detectable signals were observed in similarly treated enamel. The signal shows partial decay at four and six months after thermal treatment. The experimental data for dentin show a correlation with the Arrhenius function with an activation energy of $(41 \pm 2)10^3$ J/mol. After six months, the ESR signal shows a higher activation energy $(67 \pm 3)10^3$ J/mol and the decay shows a activation energy of $(38 \pm 2)10^3$ J/mol. A possible assignment of the signal origin in dentin is difficult. The water lost during thermal treatment and reincorporated during the following six months correlates with the signal gain and subsequent decay. The water lost can produce point defects in the hydroxyapatite, or structural changes in the collagen structure. The results observed here are useful for understanding the thermal effects produced in dentin by infrared laser irradiation, and provides a cautionary warning that annealing conditions in ESR studies of biological tissues should be standardized.

*Key Words:* ESR; EPR; Dentin; Enamel; Thermal treatment; Laser irradiation.

## INTRODUCTION

Hard dental tissues present various distinct paramagnetic signals that can be characterized by electron spin resonance (ESR, also called electron paramagnetic resonance, or EPR) for many purposes. In tooth enamel a signal with axial symmetry at $g_\perp = 2.0018$ and $g_\parallel = 1.9985$ was created after exposure to ionizing radiation.[1] Other signals can occur, such as the mechanically induced isotropic signal at $g = 2.0038$[2] and a native signal with g-value at 2.0045 associated with the enamel organic material.[3] In addition, there are signals that appear under particular conditions. An example is the ESR signal at $g = 2.0020$ which occurs when a non-irrigated dental drill is used for sample preparation.[4] Another example is the signals at $g = 2.0110$ and $g = 2.0052$ which are induced by UV light exposure.[5]

The primary thrust of this paper is to study the changes that occur in the native signal of enamel and dentin after heating. The native signal was first described in bone[6] and appears to have two components, a narrow and broad component. Recent studies have shown that the signal depends on the health and socioeconomic condition of the patients.[7,8]



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While an extensive literature exists for the effects of heating on the dosimetric signals from enamels,[9–14] there have not been any studies of the effects of heating on the native signal of dentin or bone.

In this communication, the changes in the ESR native signal induced by thermal treatment is reported. Several dental procedures, including the use of laser radiation, can locally heat the tissue with side effects that must be studied. The temperature maximum at the surface during the laser irradiation can exceed 1280°C, and melt hydroxyapatite,[15] or approach 300°C during tissue ablation while remaining below 40°C at one millimeter beneath the irradiated area.[16]

The usual lasers used in odontological procedures which involve hard dental tissue are neodymium at 1.06 μm (Nd:YAG),[17] erbium with emission at 2.94 μm (Er:YAG)[18] and holmium with emission at 2.06 μm (Ho:YLF).[19] These lasers and others solid-state lasers normally have a Gaussian spatial beam profile.[20]

There are differences between the thermal effects of action from a laser beam and those from conventional thermal treatment. The laser treatment usually lasts only a fraction of a second. Erbium laser irradiation produces a local temperature rise to a maximum value of 300°C on the surface layer[21] and less in the sub-surface regions. In this work, we study the ESR signals in enamel and dentin tissues after thermal treatment at temperatures between ambient and 300°C.

## MATERIALS AND METHODS

Non-irradiated permanent bovine teeth were studied. The teeth were cut in 2 mm thick slices and the enamel and dentine were carefully separated and powdered. The effect of thermal action on mineralized tissues was studied, using bovine instead of human enamel and dentin because bovine teeth are easier to collect, have larger dimensions and are subjected to less formal health regulations. The crystallographic structure[22] and the chemical composition[23] of bovine and human dental hard tissues are similar, and the conclusion of this investigation regarding the effect of thermal treatment on ESR radicals is likely to be relevant to human enamel and dentin.

ESR measurements were performed in a Varian E-4 ESR spectrometer with a TE-102 rectangular resonant cavity, operating in the X band range. Each sample was introduced in a glass tube (internal diameter = 5 mm) and centered in the cavity. A glass support was inserted at the bottom of the cavity, such that the glass tube position was not changed when the samples were replaced. After optimizing the measurement conditions, the spectra were taken at 50 mW microwave power and 0.1 mT field modulation, scan

width of 4 mT and modulation frequency of 100 kHz. The ESR spectra were acquired with 2 minutes for each scan and a total of 3 scans.

Samples with a grain size between 25 μm and 38 μm were produced. The samples were standardized in aliquots of 50 mg and heated in an oven at ambient atmosphere to temperatures from 100°C to 300°C for 10, 20, 30, 60, 120 and 180 minutes.

A standard $MgO/Mn^{2+}$ powder sample was used for all measurements as the signal intensity reference. The g-value of the sample was determined by scanning the magnetic field in two directions (increase and decrease of the magnetic field). The spectra was normalized according the microwave frequency ($\sim 9.4$ GHz) and a standard DPPH sample ($g_{DPPH} = 2.0037 \pm 0.0002$).

The samples were stored after heat treatment, and measured after 24 hours to allow the decay of transient radicals, and measured again at 4 months and 6 months, thus evaluating the stability of the radicals.

Sample powder may suffer a temperature elevation from grain friction during grinding, an effect that can be observed by ESR spectroscopy.[2,4,24] Our grinding was done manually to minimize undesirable friction effects in the ESR spectra.

The statistical error for each measurement was not evaluated. To estimate a standard deviation we measured the ESR signals from five samples treated equally (200°C and 30 minutes). The standard deviation was determined to be 8%, and this value was used for all the other measurements.

## RESULTS

No paramagnetic signal in the untreated sample was observed. Thermal treatment between 100°C–300°C produces a paramagnetic signal only in the dentin tissue. Also, no paramagnetic signal was observed in enamel treated between 100°C–300°C. Figure 1 shows the spectra for dentin heated for 30 minutes at temperatures between 100°C and 300°C. Although not visible in Figure 1, a small detectable signal appears in the sample heated at 100°C. The signal increases with increasing heat treatment. The signal shape is complex and indicates signals from two or more radicals that are not spectrally discriminated.

In order to evaluate the stability of the observed radicals, the ESR signals were measured 6 months after the thermal treatment which provided the ESR amplitude 1 day and 6 mounths after the thermal treatment was applied. The signals after 6 months allowed an evaluation of the profile of the stable ESR radicals, and indicated the dependence with the temperature of the unstable radicals. Considering that the amplitude after 24 hours



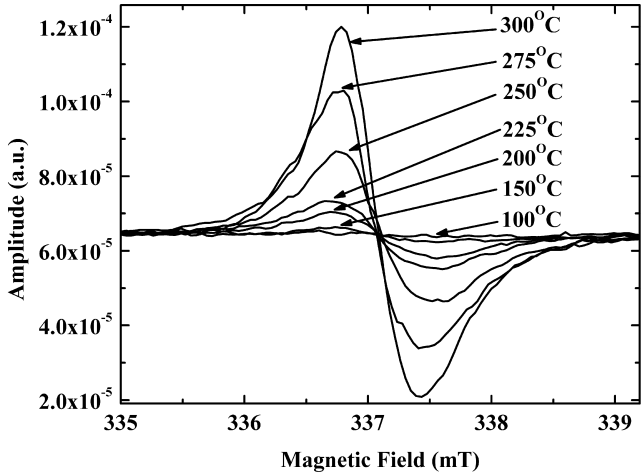


***Figure 1.*** ESR spectra of dentin heated at 30 minutes and temperatures between 100°C–300°C; the amplitude increases with the heat treatment temperature. The non-symmetrical signal shape indicates the combined signal of two or more radicals.

($I_{1day}$) is equal to the sum of the stable ($I_{stable}$) and unstable radicals($I_{instable}$); the amplitude after 6 months ($I_{6months}$) is equal only to the $I_{stable}$. We can subtract the amplitude after 6 months from the initial value ($I_{1day}$) and obtain the amplitude associated with the unstable radicals, i.e., the radicals that are not present after 6 months. The three data sets ($I_{1day}$, $I_{6months}$, $I_{1day}-I_{6months}$) together with the Arrhenius fit are presented in Figure 2.

The ESR signal dependence with temperature was compared with the Arrhenius function. The Arrhenius equation can be wrote as:

$$I_{ESR} = A \exp\left(\frac{E_a}{R}\frac{1}{T}\right)$$

where $I_{ESR}$ is the ESR amplitude, measured peak to peak, A is a constant, $E_a$ is the activation energy and R the gas constant. The Arrhenius function was adjusted to the experimental data, and can be visualized in the Figure 2. The activation energy is extracted from the adjusted experimental data. For the measured data after 1 day the energy is $(41 \pm 2)10^3$ J/mol, after 6 months is $(67 \pm 3)10^3$ J/mol and for the difference between $I_{1day}-I_{6months}$ the fit provided a activation energy of $(38 \pm 2)10^3$ J/mol. The linear fit of the amplitude after 6 months was conducted without the



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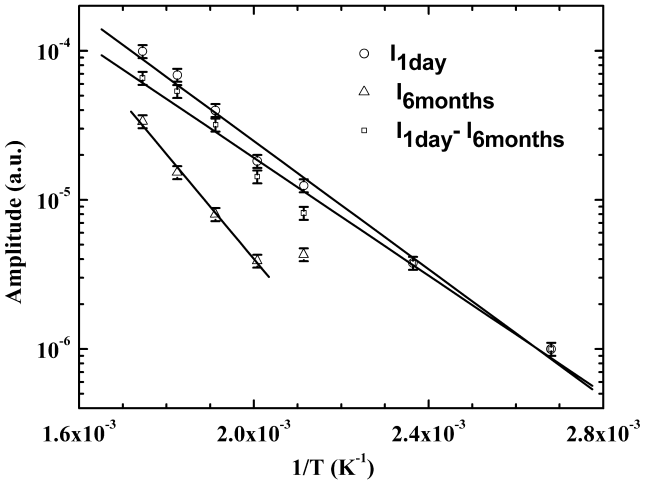


***Figure 2.*** ESR amplitude of dentin heated at 30 minutes and temperatures between 100°C–300°C measured after 1 day and after 6 months. The difference between the amplitude after 1 day and that after 6 months is associated with the instable radicals and the amplitude measured after 6 months with the stable radicals.

amplitude of the sample heated at 200°C, because this amplitude was discrepant from the other amplitude values.

The radicals present after 6 months requires a higher energy ($67 \times 10^3$ J/mol) to be produced while the unstable radicals need a lower energy ($38 \times 10^3$ J/mol). This difference in the activation energies can be associated with two different chemical reactions, or with the same reaction but with reactants in different initial conditions.

Figure 3 compares amplitude values for thermal treatments of 100, 200 and 300°C and different times (1, 2 and 3 hours) measured after 1 day, 4 months and 6 months. The samples treated at 100°C do not show a detectable signal after 4 and 6 months. This observation shows that some of the free radicals identified in this work partially reverted to the natural level in less than 4 months after their formation, and are not dependent on the duration of the heat treatment. The signal shape of samples heat treated at 300°C after 24 hours and 6 months are shown in the Figure 4.

The signal shape of samples heat treated at 300°C after 24 hours and 6 months is shown in the Figure 4. The g-value of the dentin ESR signal was determined, after heating to 200°C for 1 hour, to be g = 2.0043 with a width of 0.44 mT.



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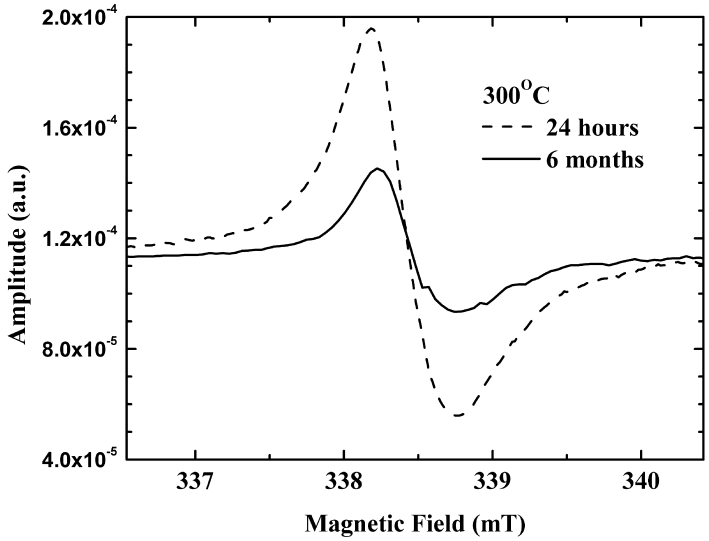


*Figure 3.* ESR amplitude of treatments for 1, 2, and 3 hours for 100°C, 200°C, and 300°C. The samples heat treatment at 100°C give no ESR signal after four and six months.

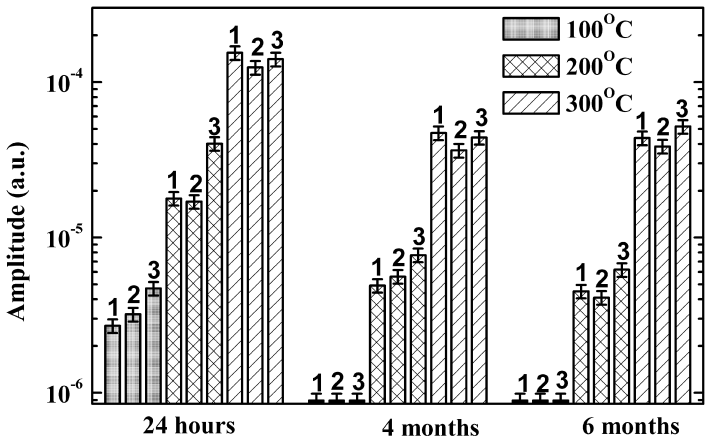


*Figure 4.* ESR spectra of the dentin heat treated at 300°C for 2 hours; measured 1 day and 6 months after the treatment.



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Measurements were also made for samples thermally treated at a fixed temperature and for different times (10 minutes up to 3 hours). Despite a difference in amplitude between samples heat treated for 10 minutes and for 3 hours, the values do not show a strong dependence on the heat treatment time. This result suggests that the ESR radicals are formed at the beginning of the thermal treatment. The reaction mechanism responsible for the radical production reaches equilibrium quickly as thermal equilibrium of the sample is established.

## DISCUSSION

The non detectable ESR signal in our bovine enamel and dentin samples is in contrast with the results in the literature for human teeth. Broad and narrow signals in native human samples are observed.[7,8] They associated the native signal to many different origins, such as pathological processes, diet, age, environment pollution, sunlight and other ionizing radiation, i.e., individual peculiarities that are usually not present in bovine teeth. Our experiment was conducted with teeth from bovine specimens near 2 years old, free of dental diseases and not exposed to ionizing radiation and probably with an exposure to sunlight lower than human teeth in the interval of only 2 years.

The presence of point defects and imperfections in solids may trap electrons and give rise to an entity with unpaired electrons. The release of structural water or other radicals from the hydroxyapatite, such as the carbonate radical, can produce point defects in the hydroxyapatite, and its recombination in the lattice may be one of the mechanisms that accounts for the ESR amplitude reduction after 4 and 6 months.

During thermal treatment, the first chemical compound released from the enamel is water, followed by organic material and carbonate radicals.[25–27] Analyzing the thermal stability of the chemical compounds present in the tissue, the three mentioned compounds (water, organic material and carbonate radicals) are unstable and probably are responsible for the ESR signals that we observed. Despite the fact that the tissue is composed of several systems, which can provide unpaired electrons, we can begin discussing some possible mechanisms that are presented in the literature.

The presence of unpaired electrons in biological tissues can be in free radicals, point defects in solids, transition-metals ions and rare-earth ions,[28,29] after fracture and cutting.[30,31] Bonded water, F-center electrons at the position of hydroxyl ions, and conduction electrons have also



been attributed to observed ESR signals in bone tissue.[28] With some restrictions, it is possible to compare the results of soft tissue and hard tissue and improve the discussion about the origin of the ESR signal in hard tissues. The collagen molecule has the same structure in soft tissues, dentin and bone. Free radicals were detectable in the amino acids lysine, arginine, histidine, tryptophan and cysteine. It's signals show central singlet lines, attributed to carbon-centered radicals, with $g = 2.004$.[32] This g-value and the value of a broad signal observed in natural enamel ($g = 2.0045$)[3] are very close to the observed g-value in thermally treated dentin ($g = 2.0043$).

During heat treatment, the first chemical compound released from enamel is water. The literature shows that adsorbed water is totally released from the tissue at 200°C[25] and the water bonded to the tissue structure is totally released when heated to 400°C. The adsorbed water is reversible and can be incorporated again after dehydration, while the water released between 200°C and 400°C is characterized as irreversible. In the temperature range of 100–400°C, organic material of the dentin is released with a maximum loss around 320°C.[26] Another compound that is released from the enamel is the carbonate radical. The loss of this radical starts at 100°C, is more rapid at 700°C, and around 1000°C it is totally released from the tissue.[27] A similar release profile is expected in dentin because of the similarity of dentin and enamel mineral matrix. When the specimens are hydrated the loosely bound water is restored, but not the tightly bound water, which is also named ''structural water'' and which is eliminated between 200–400°C. Thus, samples heated up to 200°C are restored to their natural condition, but this does not happen for samples heat treated above 200°C.

The two kinds of water are in accordance with the observed ESR amplitude (Figure 1). Those samples thermally treated under 200°C produce ESR signals that are unstable and reversible to the initial condition, without ESR radicals, owing to the behavior of adsorbed water. The ESR radicals observed for samples thermally treated above 200°C show an amplitude reduction and are stable owing to the irreversible loss of structural water.

Thermal treatment at temperatures between 100°C–300°C, act mainly on the organic matrix, carbonate, and the water present in the tissues. Enamel has a low weight fraction of water (3 wt%) and organic material (1 wt%). Dentin has a greater fraction of water (20 wt%) and organic material (10 wt%).[33] This difference in weight fraction of water and organic material is probably the major reason for the differences observed in the two tissues.

The chemical composition of the enamel and dentin mineral matrix is similar, composed of non-stoichiometric hydroxyapatite with incorporation of carbonate radicals. A difference in the crystal size in the two tissues is



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observed. Enamel crystals are larger than dentin crystals.[33] The main components in the organic matrix of the enamel are non collagen proteins and, in the dentin matrix, 90 wt% collagen protein. We conclude that the observed signals do not originate from the mineral matrix because the two tissues have the same mineral chemical composition. If the paramagnetic signal had a mineral origin, ESR spectra similar to those in dentin would be observed in enamel after similar thermal treatment.

Despite the predicted loss of organic material from heated dentin at high temperatures,[26] changes in the collagen structure are observed after thermal treatment or after erbium irradiation.[34] The structural change in dentin, which is thermally treated at low temperatures, is a degradation of the collagen molecule by disrupting the amino acids that form the hydrogen bridges with water molecules. The loss of water after thermal treatment breaks these hydrogen bridges, and changes the collagen structure. Subsequent incorporation of water re-introduces the hydrogen bridges, and restores the collagen structure before thermal treatment.

Is difficult to assign an origin to the ESR signals in dentin because the tissue is a complex system composed of inorganic and organic matrix, water and small quantities of transition-metals that are potential radicals with unpaired electrons. Despite these difficulties, a general discussion can be conducted together with an interpretation of the activation energy. The energy associated with the stable radicals, those persisting after 6 months, is higher than the energy associated with the unstable radicals (those that decay). If we consider that the water lost can produce structural changes in the organic material, or point defects in the hydroxyapatite, the stable radicals with the higher energy activation can be associated with a loss of tightly bonded, structural, water and unstable radicals with a lower energy activation can be associated with the loss of loosely bound (adsorbed) water.

The results observed are useful in research on laser interaction with dental hard tissues, and can help explain effects observed after high intensity laser radiation, such as fluoride uptake and acid resistance of the irradiated tissue.[19,35,36]

## CONCLUSIONS

ESR signals are observed in dentin tissue after thermal treatment at temperatures between 100°C–300°C and no detectable signals were observed in enamel treated in the same way. The radical responsible for the signals has a possible partial decay 6 months after the thermal treatment, and the differing activation energies are indicative of two different chemical reactions.



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The ESR signal can be associated with water lost after thermal treatment. The loss of structural water can produce point defects in the hydroxyapatite lattice, or the loss of the water that participates in the ''hydrogen bridge'' can produce changes in the collagen structure.

## ACKNOWLEDGMENT

This work was supported by grants from FAPESP, CNPq and CAPES.

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Received February 4, 2003
Accepted August 4, 2003



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