Development of an innovative 9 GHz EPR surface detection method and its application to non-invasive human fingers and nails investigation

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Abstract

We developed an innovative surface-type cavity for 9 GHz electron paramagnetic resonance (EPR) and used it to non-invasively measure human fingers and nails. This surface-type cavity measures a sample on the top of the cavity instead of a sample inserted into the cavity. To verify the performance of this method, 5–10 μL of 0.1 mM 4-hydroxy-2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPOL) aqueous solution in a 1.0-mm (i. d.) glass capillary was used. Although the detectable radical concentration of the surface-type cavity is lower than that of a commercial cavity, the surface-type cavity can measure biomedical samples. In addition, the cavity is capable of measuring human fingers and nails. The dynamics of a topically applied 1.0-mM TEMPOL solution with a commercial lotion (5:1 by weight) on a human finger and nail were investigated. The present EPR results suggest that TEMPOL in solution may not easily permeate into the finger and nail during the measurements. Therefore, 9 GHz surface-type detection exhibits the good potential to study paramagnetic species in bulky biomedical samples.

Introduction

Electron paramagnetic resonance (EPR) or electron spin resonance (ESR) spectroscopy utilizes electron spin resonance and measures the resonant microwave power absorption (transition) of unpaired electrons subjected to a constant magnetic field in an atom, a molecule, or a compound. Although 9 GHz EPR is a useful technique for measuring free radicals, the sample sizes and quantities are limited by the microwave frequency and cavity size [1, 2]. Ordinary sample measurements are performed by inserting a sample in a resonance cavity. In addition to the limitation of sample size and quantity, there is a large dielectric loss for aqueous samples.

An alternative method to measure various samples is by utilizing the microwave leakage from the cavity. A TE_{111}-mode cylindrical cavity was developed to obtain better sensitivity and resolution for human tooth dosimetry [3]. Nakagawa et al. further modified and developed the surface-type cavity [4], and surface-type detection was recently improved for human finger and nail measurements [5]. The new EPR surface technique could provide confirmatory information about the dynamics of free radicals for bulky biomedical samples at a molecular level.

We investigated human fingers and nails using our innovative 9 GHz EPR surface-type detection. First, we developed the innovative 9 GHz EPR surface-type cavity. Second, the performance of the cavity was compared with that of a JEOL commercial cavity. Third, we non-invasively investigated the spin-probe dynamics of in human fingers and nails. Moreover, we discussed the application of EPR surface-type detection to various biomedical samples.

Material and methods

Innovative 9 GHz EPR cavity (TE_{111} mode) and samples

A commercially available JEOL RE-3X 9 GHz EPR spectrometer was used for various measurements using the innovative surface-type cavity (TE_{111}) and a JEOL commercial cavity (TE_{011}). A surface-type cavity containing a sample aperture with 8-mm diameter was fabricated for biomedical samples. The field modulation located on both sides just above the cavity (e.g. Figs. 1 and 4). The diameter of a one-turn coil was 39 mm. The basic concepts and mathematical calculation of the cavity have been described in a previous study [4].

The ethical principles for non-clinical biomedical studies involving human participants in the Declaration of Helsinki Principles were adhered to in every aspect. A volunteer understood the concepts of the experimental procedures and the importance of the research before the measurements were performed. The spin probe, 4-hydroxy-2,2,6,6-tetramethylpiperidin-1-oxyl (TEMPOL), was purchased from Nacalai Tesque, Inc. and used as received. A 1.0-mM TEMPOL aqueous solution was mixed with a lotion (5:1 by weight) and topically applied to an area of a human finger and nail with a diameter of ~6 mm. The lotion was mixed to prevent the repulsion of the solution by the skin surface. Measurements were performed on the volunteer for 4–15 min.

EPR measurements

A commercially available JEOL RE-3X 9 GHz EPR spectrometer was used. All

continuous wave EPR spectra were obtained with a single scan. Typical EPR settings for surface-type detection are as follows: microwave power, 10 mW; time constant, 0.1 s; sweep time, 4 min; magnetic field modulation, 1.0 mT; and sweep width, 10 mT. All measurements were performed at ambient temperature.

Results and Discussion

Performance of the innovative 9-GHz EPR surface-type cavity compared with a JEOL commercial cavity

The surface-type cavity for measuring signals from a sample surface has been previously developed [4]. The present surface-type cavity is very different from a conventional cavity in its design of the field modulation coils and the sample aperture (Fig. 1). As shown in Figure 1, in the present surface-type method, a sample material is placed on the top of the cavity rather than being inserted into the cavity (a common usage). To observe the sample outside the cavity, a small aperture (8 mm in diameter) allowing microwave leakage was incorporated in the cavity wall. The aperture location and size were selected for measurement effectiveness, as shown in Figure 1. Therefore, it is possible to non-invasively measure biomedical samples such as skin surfaces.

The sensitivity of the surface-type TE_{111} cavity was compared with that of a JEOL commercial TE_{011} cavity. Figure 2 shows an EPR spectrum of 0.1 mM TEMPOL (10 μL) in two capillaries placed just above the cavity. A three-line pattern was obtained. The peak-to-peak linewidth (ΔH_{pp}) is 0.16 mT at the EPR center line. Figure 3 shows an EPR spectrum of the two capillaries with the same solution inserted into the cavity; ΔH_{pp} is

0.16 mT at the center line.

An exact comparison of two different detection types, i.e., surface-type and insertion detection, is not possible. A rough comparison of the two cavities can be made using the signal-to-noise (S/N) ratio. Insertion detection has ~14 times better S/N ratio than the surface-type detection. Surface-type detection has a lower sensitivity compared with ordinary EPR detection. The relatively low sensitivity of surface-type cavity detection compared with commercial cavity detection is because of the utilization of the microwave leakage from the 8-mm aperture (hole). Although surface detection has low sensitivity, one can use much high microwave power to measure the samples.

Application of surface-type detection to a human finger and nail

Figure 4 (A) shows the surface-type cavity used for the measurements. Figure 4 (B) shows an EPR spectrum of a human finger using surface-type detection. The spectrum shows an unequal three-line pattern, thereby suggesting that probe motion was the restricted motion in the finger.

Figure 5 shows an EPR spectrum of a 1.0-mM TEMPOL solution mixed with a lotion (approximately 5:1 ratio by weight) on a human finger obtained using surface-type detection. The EPR spectrum of the finger shows that the three lines were relatively broader than those of the TEMPOL solution (Fig. 2 or Fig. 3). Moreover, ΔH_{pp} is 0.18 mT at the center line. The slightly broad linewidth and unequal three lines suggest that the spin probe motion is relatively immobilized. The signal intensities do not decease as time elapses. TEMPOL signal intensities were stronger than those of the first spectrum.

TEMPOL solution on the finger may evaporate during the experimental period because of the temperature of the finger and the low humidity in the EPR room. After the finger was thoroughly washed using a soap, no EPR signal was observed. Therefore, the EPR results suggest that TEMPOL in an aqueous solution does not easily permeate the skin.

Figure 6 shows an EPR spectrum of a 1.0-mM TEMPOL solution with a lotion (5:1 by weight) on a finger nail obtained using surface detection. The solution was placed on the nail before the measurements. The EPR spectrum shows that the three lines were relatively sharper than those of the finger. The EPR line-shape was an unusual EPR shape due to the phase change. Moreover, ΔH_{pp} is approximately 0.17 mT at the center line. The signal intensities of the nail were small and remain the same over time. The limited area of the curved nail surface attached to the aperture produces a small signal (Fig. 6). Note that the EPR intensities of the finger are stronger than those of the nail because the finger easily fits in the cavity aperture.

Moreover, surface detection is one of the most important aspects in biomedical research. EPR surface detection provides useful information regarding biomedical samples, including redox state, oxygenation, and membrane structures. Non-invasive method is a key element for human subjects and living animals. For the purpose of non-invasive measurements, various surface-type cavities (or resonators) have been developed by a few groups [6-8]. A bridged loop-gap resonator was developed and applied to measure free radicals in human skin. A perdeuterated ^{15}N spin probe with concentration of 0.5 mM or higher concentration of the 10-mM solution was used for the skin measurements. Although a live rat was measured with a surface-coil-type resonator, 1,1-diphenyl-2-picrylhydrazyl

(DPPH) powder was used.

Conclusions

We developed a 9 GHz surface-type non-invasive detection method that provides new possibilities to study paramagnetic species in fingers and nails. The present EPR results suggest that TEMPOL in an aqueous solution does not easily permeate into fingers and nails. Although the detectable radical concentration is relatively low, the surface-type method measures various sample sizes and shapes (e.g., human fingers, human nails, and parts of the arm and foot), unlike the conventional EPR method. Therefore, the EPR results suggest that EPR surface-type detection can be used to non-invasively study bulky samples such as thin materials, foods, and biomedical samples.

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Figure Legends

Figure 1. Schematic of the innovative surface-type cavity (TE_{111}). The field modulation coils are placed above the surface-type cavity. The aperture diameter is 8 mm.

Figure 2. EPR spectrum of 0.1-mM TEMPOL (10 µL) solutions in two capillaries. The spectrum was taken with the surface-type cavity.

Figure 3. EPR spectrum of 0.1-mM TEMPOL (10 µL) solutions in two capillaries. The spectrum was taken with a JEOL commercial cavity (TE_{011}).

Figure 4. (A) Picture of the surface-type cavity. The aperture diameter is 8 mm. (B) Demonstrative picture of non-invasive finger measurements.

Figure 5. EPR spectrum of a human finger obtained using the non-invasive surface-type cavity method. A ~1.0-mM TEMPOL solution mixed with a lotion was applied to the finger. The top spectrum shows the background. The stick spectrum in the bottom spectrum indicates an unequal three-line pattern for the relatively immobilized spin probe.

Figure 6. EPR spectrum of a human finger nail obtained using the non-invasive surface-type cavity method. A ~1.0-mM TEMPOL solution mixed with a lotion was applied to the nail.

Figure 7. Plot of EPR intensities of a finger and nail obtained as a function of time.

Figure 1.

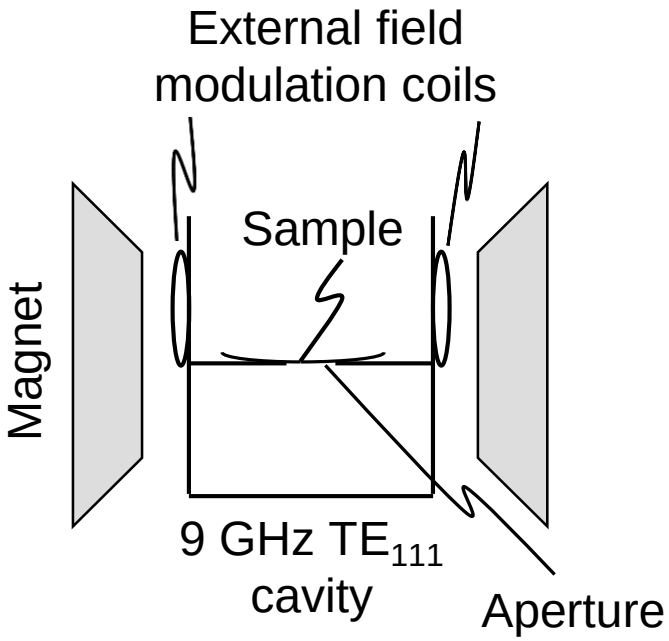
External field
modulation coils
Sample
Magnet
9 GHz TE_{111}
cavity
Aperture

Figure 2.

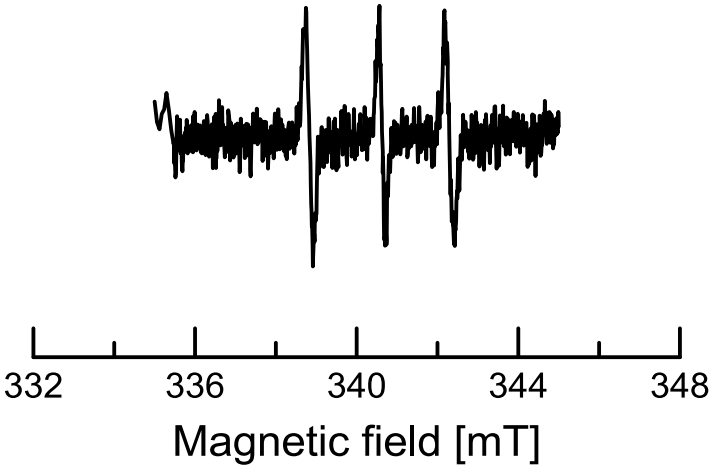

Figure 3.

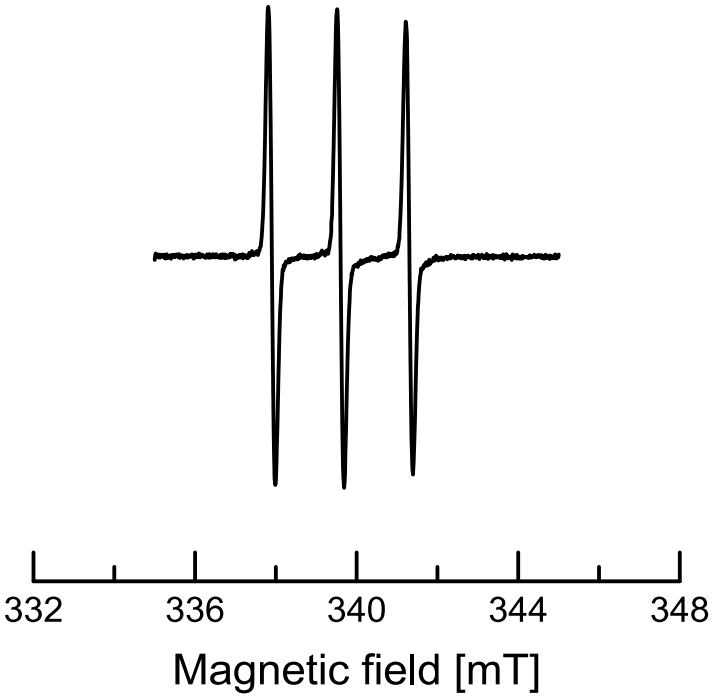

Figure 4.

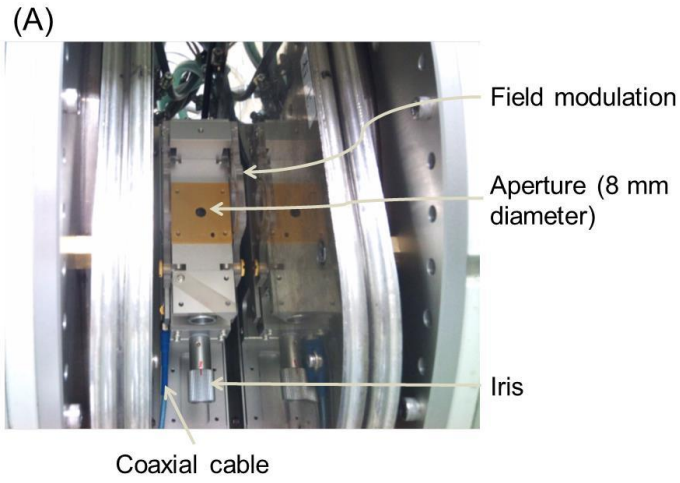
(A)
Field modulation
Aperture (8 mm diameter)
Iris
Coaxial cable
(B)

Figure 5. (finger)

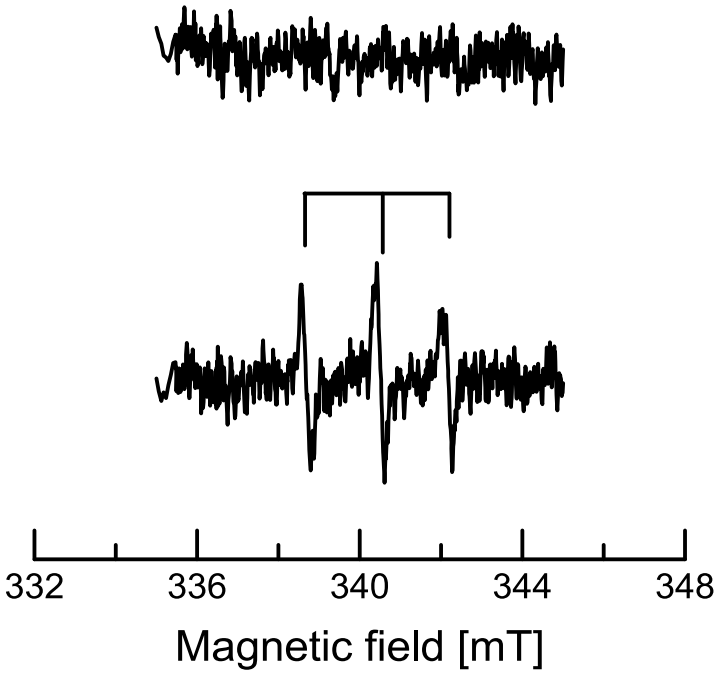

Figure 6. (Finger nail)

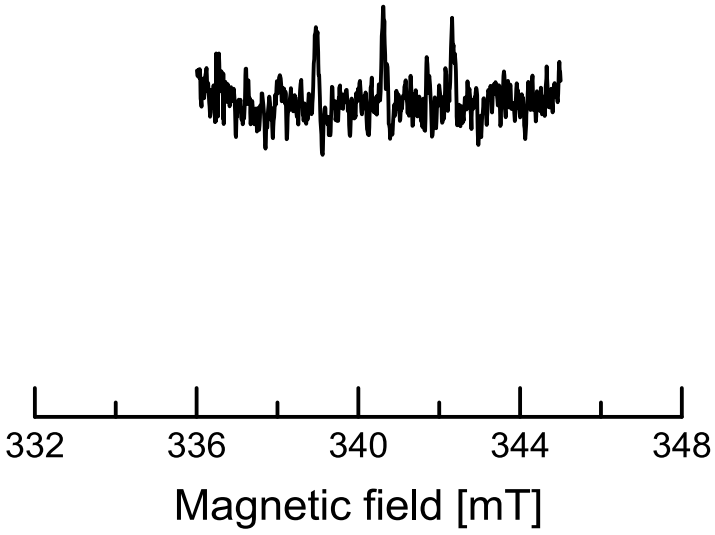

Figure 7.