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Evaluation of Non-Thermal Microwave Effects on Bovine Lens by Measuring S-Parameters Induced by Variations in Dielectric Coefficient

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ABSTRACT This study evaluated the non-thermal effects of microwave on bovine lens by measuring the S-parameters induced by the dielectric coefficient variation in bovine lens. A closed, integrated biological experiment system was designed to expose bovine lens to microwave radiation quantitatively, and S-parameters were measured. Morphological evaluations were also performed during microwave radiation to evaluate the reaction mechanism of the non-thermal effects of microwave on the bovine lens. The results showed that the bovine lens became gradually cloudy and its refractive index changed significantly with increasing doses of microwave radiation. A comparison of experimental and control bovine lenses stained with hematoxylin and eosin revealed that the change in bovine lens might be a result of the nuclear fragmentation of lens epithelial cells and disorderly arrangement of lens fiber cells, which were induced by the vibration and friction of fiber tissues upset by microwave radiation. Further, the fluctuating decrements of 3.8 dB in S_{21} showed that the non-thermal effects of microwave could lead to the variations in the bovine lens dielectric coefficient. These findings suggest that the dielectric coefficient may quantitatively describe the physiological state of bovine lens. This study might be the valuable reference for the establishment of new electromagnetic radiation safety standard.

INDEX TERMS Non-thermal microwave effects, bovine lenses, dielectric coefficient, microwave radiation safety standard.

I. INTRODUCTION

Popular use of wireless devices has induced growing public anxious whether the electromagnetic (EM) radiation from wireless devices could pose potential hazards to human health. Safety standards, such as ICNIRP Guidelines [1] and IEEE C95.1 [2], have been established to limit the EM exposure thermal effects which are defined by specific absorption rate (SAR) (>5 or 10 MHz) and absorbed power density (APD) (>6GHz). However, they neglect the possible health hazards caused by the non-thermal effects. To date, numerous studies have shown or indicated that the non-thermal effects of microwave could cause various types of molecular

transformations and alterations by disturbing the weak bonds which could cause special microscopic biological effects (protein synthesis [3]–[7], genotoxicity [8]–[13] oxidative stress [14]–[18], cell signaling [19]–[22], brain EEG [23], cell apoptosis [24], [25], sperm motility [26] and etc.). From the macro perspective, non-thermal effects of microwave might cause heat shock [3], infertility [7], [18], auditory system buzzing sound [27], visual loss [28] and etc. In particular, special biological structure with less blood flow and direct exposure to EM radiation make the ocular tissue become the research focus [14], [21], [28]–[31]. For example, Dovrat *et al.* reported that the low-power of EM exposure at 1 GHz for more than 36 hours could affect the optical function of cultured bovine lenses. They also found that the bovine lens damaged by EM radiation are quite different

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from that caused by conductive heat [21]. Balci *et al.* found that the EM radiation from mobile phone could result in oxidative stress in cornea and lens [28]. Research performed in [29] showed the milliwatt level EM radiation might cause thickening of the anterior corneal epithelium. EM radiation from mobile phone at 1.8GHz could block reactive oxygen species production and induce DNA damage of human lens epithelial cells [14]. The above researches indicated the non-thermal effects of microwave on ocular tissues lead to types of abnormal biological effects, and they are always too imperceptible to be quantitatively described. However, the safety standards [1], [2] usually use the operational thresholds to set restrictions which had been proved to be no health hazards to human. For purpose of determining thresholds for ocular tissues including all exposures, further researches about quantitative describing of non-thermal microwave effects on the ocular tissues are needed.

Many researches had proved that the dielectric coefficient, at microwave frequencies, could describe the physiological state of biological tissues. Dielectric coefficient measurement technology, acting as a crucial rapid and noninvasive method, have also been widely used for medical diagnosis. For example, dielectric coefficient of human breast cancer tissue showed remarkable different from that of health tissue [32]. This finding greatly drove the development of breast cancer detection [33], [34]. Mayrovitz *et al.* [35] found that the dielectric coefficient of foot dorsum with diabetes mellitus (DM) are larger than that without DM, which provided possible measurement method to screen for early variations in foot skin features of DM-related edema. The results in [36] proved the effective dielectric permittivity and conductivity of normal thyroid tissues were significantly lower than that of thyroid cancer tissues. Therefore, the effective dielectric permittivity and conductivity could be used to determine malignancy in thyroid cancer. In [37], the variations in dielectric coefficient of skin had been applied to the early diagnosis of skin cancer. Evaluations of dielectric property between normal and tumor human liver tissues proved the discrimination between healthy and pathological tissues was possible by measuring dielectric permittivity [38]. Dielectric properties of bones had obtained significant attentions to diagnose the osteoporosis due to the correlation between the dielectric property and bone mineral density [39], [40]. Further, dielectric coefficient measurement of single particle also induced plenty of interests on the differentiation of cancer cells [41], [42].

However, there are many challenges and limitations *in vitro* and *in vivo* experiment with respect to the dielectric coefficient measurement of biological tissue. Take the bovine lens as an example, it is a soft irregular ellipsoid composed of multilayer biological membranes which makes it difficult to be completely filled in a microwave sensor with a fixed structure. In addition, the irregular ellipsoid led to difficulties in building an accurate numerical model which resulted in large errors in inversion calculation. Therefore, it is difficult to directly measure the dielectric coefficient of

bovine lens. Moreover, biological experiment is very special because there is usually no compromise at all in some aspects during the entire experiment processes. For example, the requirement of absolutely sterile would limit the usage of measurement devices. With regard to the *vivo* experiment, the variations in body temperature could affect dielectric coefficient which would result in unpredictable measurement errors. In addition, the dielectric coefficient measurement is limited to evaluate the whole characteristic of organ under test rather than the individual tissue. Thirdly, the non-invasive dielectric coefficient sensor is difficult to be installed in the living body (such as bovine head) which also bring difficulties to the real time implementation.

Due to these above reasons, this study will evaluate the non-thermal effects of microwave on bovine lens by measuring the S-parameters induced by the dielectric coefficient variations in bovine lens. A closed integrated biological experiment system will be designed in this study. Not only does it expose the bovine lens to microwave radiation quantitatively, it also could measure the S-parameters which are sensitive to the bovine lens dielectric coefficient variations. To evaluate the reaction mechanism of the non-thermal effects of microwave on the bovine lens, this study, as well, compared the transparency, refraction index and microscopic structure between the bovine lenses in experimental and control group.

II. EXPERIMENT AND MATERIAL

A. HIGH SENSITIVE MICROWAVE MEASUREMENT SYSTEM

The proposed microwave biological experiment system is shown in Fig. 1. Input signal from port 1 is divided and transmitted into two co-planar waveguide (CPW) branches by a 3 dB power divider (model number, MNK_MPD-0.5/6-2S), where magnitude and phase are same. Then two signals, respectively, pass through the testing sensor port (TSP) and the reference sensor port (RSP) which are both located on the middle of the CPW branch. Fig. 2 shows the structure of sensor port, which is a custom-made quartz tube with a bottom. Two channels (quartz tubes with 5mm diameter) locate on the tube wall

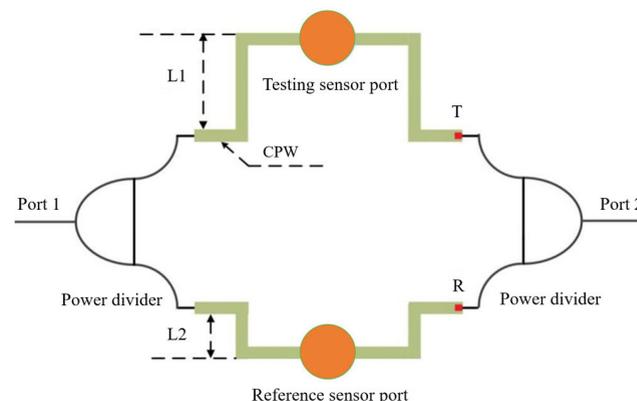


FIGURE 1. Schematic of the microwave device.

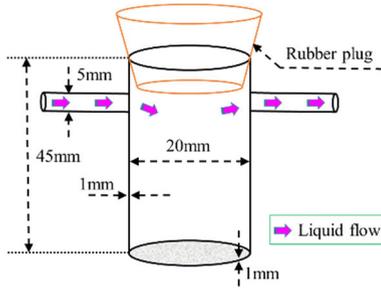


FIGURE 2. Cross section of microwave sensor.

which allow the liquid to flow into and out of the sensor port. The thickness of sensor port (both the bottom and tube wall) is just 1mm which helps to improve the measurement sensitivity. Finally, the two signals enter the two branches of the other 3dB power divider (model number, MNK_MPD-0.5/6-2S). The phase differences between T and R (Fig. 1) could be calculated by:

$$p = \frac{2*L_1 - 2*L_2}{\lambda_1} + \left(\frac{D}{\lambda_2} - \frac{D}{\lambda_3} \right) \quad (1)$$

where λ_1 is the microwave wavelength in CPW at the given frequency (900MHz). λ_2 and λ_3 represent the microwave wavelength in CPW of TSP and RSP sensing-zone. D is the external diameter of sensor port. λ_1 is decided by the waveguide structure and substrate material. There is no need to calculate the absolute values of λ_2 and λ_3 because this experiment is based on the balanced measurements. The phase differences P is altered to $\pi/2$ by adjusting the length of L1 when the TSP and RSP are filled with the same medium. S_{21} , in this situation, equals to 0. When the materials in the TSP and RSP are different, S_{21} does not equal to 0. This design (cancellation circuit) could effectually measure the minor differences of dielectric coefficient in the TSP and RSP by canceling the background signals [41], [42]. Compared with the conventional transmission lines method, the measurement sensitivity is improved by more than 20 dB [42].

B. HARVEST AND CULTURE BOVINE LENS

The bovine lenses used in this experiment were all excised from 1-year-old healthy calves in Chengdu Hongye slaughterhouse. This experiment, firstly, harvested the intact eyeball by removing the muscle around the eyeballs and rinsed it with the 0.9% normal saline. Then the eyeball was put into a sealed wild-mouth bottle full of 0.9% normal saline. To keep the bovine lens active, the whole harvest process must be finished within 30 minutes after the death of calves. Secondly, the wild-mouth bottle including the bovine lens was kept in an incubator which has the temperature of 8° C and transported to sterile laboratory of Chengdu University of Traditional Chinese Medicine. The transportation process takes about one and a half hours. Third is to harvest the bovine lens under the sterile environment. The eyeballs were put into a wild-mouth bottle full of Chloramphenicol Eye drops

for 20 minutes. Then the eyeball is cleaned by the 0.9% normal saline for 5 times.

This experiment uses a 6 wells culture plate to culture four bovine lenses which are used in experimental (microwave exposed) and control group, respectively. All the bovine lenses are completely immersed in culture medium which consists of M199 with Earl’s balanced salt solution, 20% fetal calf serum and 10% antibiotics solution (Penicillin-Streptomycin Solution) [21]. The experiment begins after the 6 wells culture plate with bovine lenses had been kept in the incubator (SANYO MCO-15AC) for three hours at the stable temperature of 35° C. The choice of the temperature is according to the previous literature [20] which has the ability to culture the bovine lens *in vitro* state for more than 15 days. The results in the literature [20] also have proved the existence of microwaves non-thermal effects in the bovine lens under 35° C.

C. EXPERIMENT MEASUREMENT METHOD

As is shown in Fig. 3, both the TSP and RSP are filled up with culture medium, while the experimental bovine lens is immersed only in the TSP. This study used the diaphragm pump to circulate the culture medium in the closed system which is composed of EP pipe, TSP and RSP. EP pipe is located in the thermostatic water bath which could keep the temperature of the bovine lens and culture medium stable at 35° C. This design could avoid the influence of temperature variations on the dielectric properties of bovine lens and culture medium. The closed systems also could reduce the possibility of external contaminate avoiding the dielectric properties change caused by the biomass denaturation. The vector network analyzer (R&S-ZVL) acts as the microwave source. Meanwhile, it could measure S_{21} parameter which are sensitive to dielectric coefficient variations in bovine lens. Such a design would help to exclude the unnecessary microwave exposure to the experimental bovine lens.

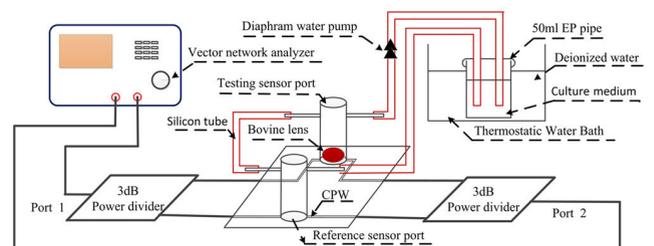


FIGURE 3. Experiment system.

Estimation of the exposure dose in experimental bovine lens is crucial in this experiment. However, it is impossible to assess the induced electric field in non-invasive manner. Therefore, numerical approaches for electromagnetic dosimetry assessment had been adopted. This study calculated the local specific absorption rate (SAR) in the bovine lens by the Comsol Multiphysics. The relative dielectric coefficient and conductivity of bovine lens are 35.8 and 0.49 S/m,

respectively [43]. The real and imaginary part of effective culture medium permittivity were measured by DAK (Dielectric Assessment Kit, Schmid & Partner Engineering AG) system shown in Fig. 4(a). In order to decrease the effects of temperature nonuniformity about the culture medium on the measurement results, we used the water bath to heat the culture medium in the baker for 20 minutes before it is measured. We take the average values (68.9 and 39.6 for real and imaginary part, respectively) of 5 times measurement results (Fig. 4(b)). The output power of the vector network analyzer was 10dBm (at 900MHz) which was based on the previous literature [21]. The magnitude of electric field strengths within the RSP and TSP are shown in Fig. 5 which show relative uniform distribution in the bovine lens. The peak value of the local SAR is 0.179W/kg. The peak value of average SAR for 10g is therefore smaller than the basic restriction (2W/kg over 10g) of ICNIRP guidelines because local SAR is larger

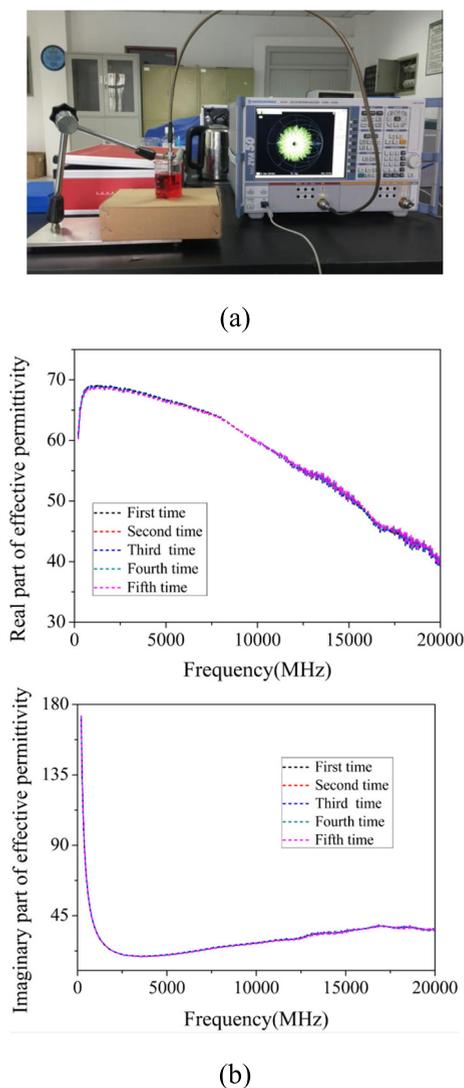


FIGURE 4. Measurement of culture medium permittivity by DAK system, (a) DAK system, (b) real and imaginary part of effective culture medium permittivity.

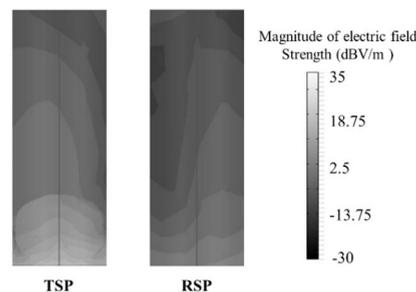


FIGURE 5. Magnitude of electric field strength distribution within the RSP and TSP.

than the average SAR over 10g. Under such exposure scenario, the non-thermal effects of microwaves would happen in the bovine lens [21] where the experiment time is shorter than the largest *in-vitro* saving time.

D. MORPHOLOGICAL STUDY OF BOVINE LENS

To evaluate the reaction mechanism of the non-thermal effects of microwaves on the bovine lens, hematoxylin and eosin (HE) staining assay were performed on the experimental and control bovine lenses. To this end, the control bovine lens (day zero) and the experimental bovine lens (day 5) were fixed with 10% formaldehyde and embedded in paraffin. The wax block was placed in a -20°C freezer for 30 minutes and then cut into 4-μm sections. The sections were baked at 70° for 4 hours, dewaxed, hydrated in distilled water, stained with hematoxylin (1min), differentiated in hydrochloric acid alcohol, blued in ammonia water, counterstained with eosin (7 seconds), dehydrated with ethanol at different concentrations (75%, 90%, and anhydrous ethanol), transparentized with xylene I and xylene II, and mounted in neutral gum. Five fields of the bovine lens sections were randomly selected at ×1000 magnification and photographed to count the number of cells.

III. RESULTS AND DISCUSSIONS

To reduce the possibility of external pollution, the entire experiment was carried out under the laminar flow (Fig. 6). In this study, the bovine lens was exposed to microwave radiation for 107 cycles. Each cycle consisted of 50 minutes of microwave radiation and 10 minutes of break [21]. All the experiment parameters are shown in Table 1.

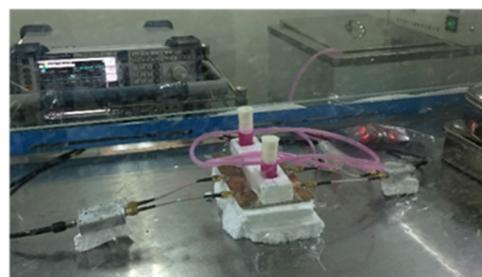


FIGURE 6. Experimental setup under laminar flow.

Fig. 7 showed the transparency variations in the experimental bovine lens from the first day to the fifth day

TABLE 1. Experiment parameters.

Experiment parameters	Value
Measurement temperature	35°C
Output power of vector network analyzer	10dBm
Radiation frequency	900MHz
Peak value of the local SAR in bovine lens	0.179W/kg
Exposure cycle	1 hour (50 minutes microwave radiation and 10 minutes break)
Total exposure time	107 cycle

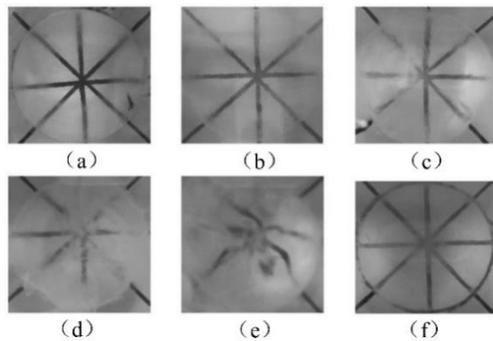


FIGURE 7. Transparency variations in bovine lenses during the experiment. Figure 7(a)-(e) represent the experimental bovine lens from day 1 to day 5. Figure 7(f) represents the control bovine lens at day 5.

of microwave exposure. We found that the experimental bovine lens became gradually cloudy with increasing doses of microwave radiation. By comparison, the control bovine lens remained clear (Fig. 7(f)). The marker line under the experiment bovine lens appeared obviously distorted starting on the third day (Fig. 7). These results illustrate that microwave radiation could result in a change in refraction index and transparency of the bovine lens. To evaluate the reaction mechanism for the observed changes, HE staining assay were performed on the experimental and control bovine lenses. The results revealed that the cytoplasm and nucleus of the control lens was uniformly stained and normal (Fig. 8). By comparison, nuclear condensation began to appear and the staining became deeper in the experimental lens. The cell density decreased significantly, and cell debris was visible. Furthermore, the fibrous structure of the experimental bovine lens was not as orderly as that of the control group. We speculate that the variations of transparency and refraction index are the results of the nuclear fragmentation of lens epithelial cells and disorderly arrangement of lens fiber cells, which might be induced by the vibration and friction of fiber tissues upset by the non-thermal effects of microwaves [18].

Fig. 9 shows the S-parameters of microwave radiation system during the 107 microwave radiation cycles. The magnitudes of S_{11} and S_{21} were less than -16dB and -23dB , which confirmed that the microwave radiation system used in this study met the design requirements and showed good sensitivity [41], [42]. S_{21} fluctuated with microwave exposure time and exhibited a declining trend of 3.8 dB overall. We also found that the magnitudes of S_{21} decreased sharply

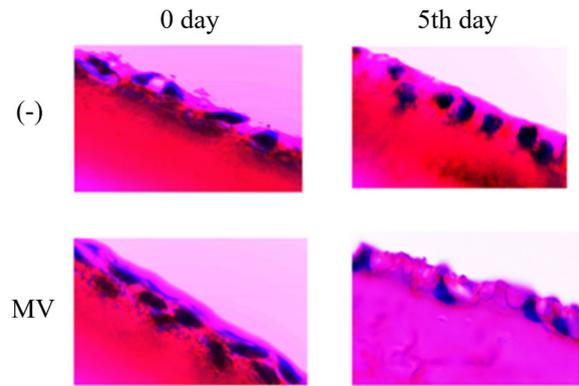


FIGURE 8. Comparison of morphological changes in the HE-stained lens epithelial cells of the control and experimental groups, (-), control group, (MV), experimental group.

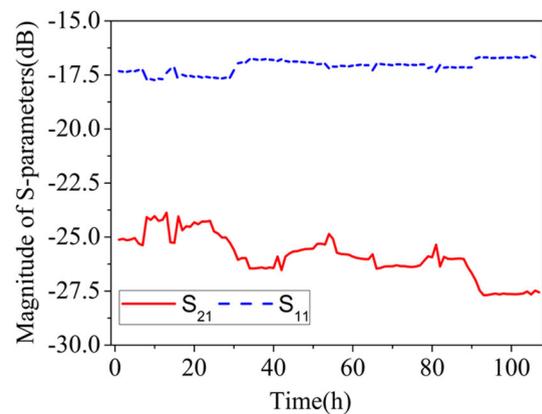


FIGURE 9. S-parameter during microwave exposure of 107 hours.

after 85 radiation cycles. This might be due to the protein denaturation of the experimental bovine lens. However, the magnitudes of S_{21} remained stable after 93 hours which may be the results of inactivation of the experimental bovine lens. The magnitudes of S_{11} , by contrast, showed fewer variations and increased by only 1dB . For the control group, we measured the magnitudes of S-parameters at the 0 and 93 hours, respectively. The variations in S_{21} and S_{11} were -0.2dB and 0.1dB , respectively, which indicates that the *in vitro* lens without exposure to microwave radiation had minor effects on the S_{21} parameters. These findings suggest that microwave radiation leads to dielectric coefficient variations in bovine lens. In summary, these results reveal that the dielectric coefficient, to some extent, could quantitatively describe the physiological state of bovine lens.

Moreover, S_{21} showed irregular behavior. This was owing to the biological denaturation (nuclear condensation and epithelial cell apoptosis (Fig. 8)) in experimental bovine lens which occurred in a relative short time rather than the linear process and resulted in significant variations in bovine lens dielectric coefficient [32], [35]. The fewer variations in S_{11} indicated that the input power of the measurement system are relative stable and the variations in S_{21} were primarily induced by the bovine lens dielectric coefficient. Obvious deformation in bovine lens (Fig. 7(e)) as a result of

breakage epithelial cell could change the living condition of fiber cell and resulted in apoptosis. Hence the magnitudes of S_{21} remained stable after 93 hours.

IV. CONCLUSION

This study evaluated the non-thermal effects of microwave on the bovine lens by measuring the S-parameters induced by the variations in bovine lens dielectric coefficient. Morphological evaluations were also performed to evaluate the reaction mechanism of non-thermal microwave effects on the bovine lens. The results showed the experimental bovine lens became gradually cloudy and their refractive index changed significantly with increasing doses of microwave radiation. The variations in bovine lens might be a result of the nuclear fragmentation of lens epithelial cells and disorderly arrangement of lens fiber cells, which were induced by the vibrations and friction of fiber tissues upset by microwave radiation. Further, the fluctuating decrements of 3.8 dB in S_{21} show that the non-thermal effects of microwave could lead to variations in dielectric coefficient. These findings suggest the dielectric coefficient may quantitatively describe the physiological state of bovine lens. This study could provide valuable references for the potential applications in the future that the physiological state of lens could be described by the dielectric coefficient (S-parameters). However, due to the special requirements (non-invasion, sterility, safety, size, structure and etc.) of biological experiment to the microwave measurement devices, this experiment is just limited to in-vitro scenario. More important, the reaction mechanism of non-thermal effects of microwave on bovine lens are not clear. Therefore, more in-vivo and interdisciplinary theoretical and experimental evaluations are needed to decipher the reaction mechanism in the future.

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