Microwave hyperthermia application with bioimplant single slot coaxial antenna design for mouse breast cancer treatment

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Abstract: In this study, a novel animal model for the breast cancer treatment which contains hyperthermia is proposed. For this main purpose a low cost, interstitial, bioimplant antenna by short ended single slot design is proposed to heat the cancerous tissues. Both the theoretical background of the proposed system and the simulation and measurement results of antenna design are presented. An artificial tissue phantom model has been created under laboratory conditions and then the utility of the proposed antenna has been tested on this model. Artificial tissues have been heated by 25W to (41-44 °C) in a short time like 25 s. Temperature measurement is performed in real-time and wireless by a digital temperature sensor using an embedded system platform. In addition, the effectuality of the proposed hyperthermia system has been tested by the study on live mice. For this purpose, breast cancer tissues created under laboratory conditions have been transferred to experimental group Balb/c mice to induce breast cancer. According to the results of hyperthermia treatment, the breast cancer has been inhibited by the proposed hyperthermia system. All studies have been animal care with ethics committee approval. All experiments have been repeated three times by at least two observers independently. The results of this study give hope that the breast cancer treatment method using this new antenna could be used in humans in the future. For this, the number of such types of ablation studies using this type of antenna should increase.

Key words: Microwave heating, hyperthermia, breast cancer treatment, coaxial antenna, in vivo, Balb/c mice

1. Introduction

Today, despite many advanced technologies developed, a fully effective method for cancer treatment has not been found yet. The most common method for the current treatment is drug-assisted chemotherapy; however, there is no guarantee of 100% success with this method. Although applying more chemotherapy increases the probability of success in cancer treatment, it has traumatic side effects that deeply affect the patient's life. Therefore, chemotherapy methods are not preferred unless they are compulsory. Besides, although microwave ablation is a promising alternative to chemotherapy, its technology for implementation has not been fully established yet and it has still been under development. In particular, it needs further nanotechnology supported R&D studies for its development as the microwave ablation lags behind the chemotherapy, considering its positive and negative effects. The main reason for this is its much more destructive effect on both healthy and cancerous

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tissues. Therefore, the microwave ablation is mostly considered as a last resort in hopeless patients who have not achieved successful results through a chemotherapy [1–3].

The microwave ablation is a special type of microwave heating, and it is defined as the heating of tissues from 60°C to 90°C [4]. These temperatures are very damaging to healthy tissues as well as they are destructive to a tumor or cancerous tissues. The hyperthermia, on the other hand, is described as the heating of tissues to 41°C-44°C [5, 6]. When compared to the microwave ablation, the harmful effects of the hyperthermia method on healthy tissues are much lower. In fact, the only reason for this is not to heat it to temperatures as low as 41°C to 44°C, rather than the ones as high as between 60°C and 90°C [7, 8]. Among these reasons, there are also structural differences between cancerous tissues and healthy tissues. As cancerous tissues contain much more water than healthy tissues, their conductivity values are much higher; thus, the incoming energy absorption rates, which are also known as the "Specific Absorption Rate" (SAR) values, are higher as well [9, 10]. The high SAR value of cancerous tissues causes these tissues to warm up much faster and much earlier than healthy tissues. While cancerous tissues are being heated up to 41°C to 44°C within the hyperthermia method, healthy tissues fall behind the tumorous tissues in terms of temperature [11]. For this reason, although the hyperthermia method is not considered as a curative method alone, it is accepted as a much more effective and much less harmful method than the microwave ablation method is, due to its use in conjunction with the chemotherapy method [1–3]. Studies have shown that the success rate of chemotherapy treatments which are not supported by hyperthermia is limited to 61% while the success rate of chemotherapy treatments supported with hyperthermia has increased to 76% [12].

There are many different hyperthermia techniques in the literature. Each provides different methods to heat the tissues to 41°C - 44°C. The most common hyperthermia methods can be listed as microwave-RF hyperthermia, laser hyperthermia, high focused ultrasound hyperthermia [13]. Among these methods, the microwave RF hyperthermia attracts more researchers than the other methods do. This is due to the microwave hyperthermia systems' potential superiority over the others, in terms of production and usage costs, energy efficiency, and compactness. In addition, since ultrasonic waves do not pass through formations such as bone air bubbles and gas, ultrasonic hyperthermia method is relatively unsuitable for cancer formation in organs such as the lungs and intestines [14, 15].

Breast cancer is the most virulent type of cancer, especially among women [16]. Although its lethality is high, the success rate in a treatment with an early diagnosis can be relatively higher than other types of cancer [17]. In addition, the distinction between healthy and cancerous breast tissues, in terms of their electrical properties such as conductivity and dielectricity, is more prominent than it is in the tissues of other cancer types. These encourage us to apply hyperthermia especially in the breast cancer [10]. On the other hand, breast cancer in women can occur either close to the surface or deep under the skin. Noninvasive hyperthermia might be utilized for the former one while a surgical intervention is often required if the latter is the case. However, it is possible to reach deep cancerous tissues by entering from a tiny area, which enables a minimal-invasive manner in hyperthermia [17, 18]. By means of this method, even deep cancerous tissues can be operated on without the need for a complex surgical operation [18, 19].

Although there are many publications on hyperthermia applications for cancer treatment in the literature, most publications have been published in multidisciplinary journals or in the medical field. However, electrical engineers and microwave and RF technology also play a key role in this area. In most of the studies such as [20–22], important design parameters, such as the electrical characterization of the radiating antenna,

reflection coefficient graphics, farfield radiation, SAR values on the tissue and other primary details that concern the engineers who will make the design, are not given sufficiently. In many studies showing details about the engineering aspects, publications are mostly limited to theoretical or simulation, antenna fabrication or measurements for application are not given [23–27] or the proposed designs are costly, far from being compact or production techniques of them are not suitable for mass production [25, 26, 28–30]. In this study, different from the others, all details concerning electrical and electronic engineering, such as theoretical background, simulation, fabrication, and measurements related to the microwave heating and hyperthermia are presented.

In the literature, it has been observed that there are only few studies in which the engineering details of the proposed systems are presented comprehensively and the biomedical functionality of these proposed systems is tested. For instance, a related study was only conducted on the rate of cooking the egg whites by volume, and the SAR values of the antenna immersed in a bowl filled with egg white in a simple but effective method [31]. As a more effective method than egg white, few publications work on these artificial fluids by creating tissue fluid using an artificial biological tissue (phantom model) [28, 29, 32, 33]. The study, which can be considered as a higher level one than the tissue fluid, is to work on organs such as kidneys, spleen, liver, or organ parts [34–36]. These studies, whether creating artificial biological tissue or working on nonliving organs, are called "in vitro". In order to fully test the scientific effectiveness of the proposed system, its results should be observed experimentally on living creatures such as mice and rats, which makes it an "in vivo" study. In this case, permissions must be obtained by the animal care and ethics committee and must be carried out in accordance with the Helsinki declaration. The proposed study differs from the rest of the literature since it is the first study to experimentally observe and test all these different methods and design stages and live animal experiments in real medical conditions with controlled experiments. The following sections present the designing steps and the results of an in vivo microwave hyperthermia which was performed on mice with breast cancer by the use of a designed minimal-invasive type bio-implant short ended single slot coaxial antenna.

1.1. Theoretical background of microwave heating

The microwave energy was transferred to target cancer tissues with coaxial cables for heating. For this purpose, the derivation of the SAR value and the heat equation, starting from time averaged power flow in the coaxial cable, transferred to the tissues is shown step by step through formula-1 to formula-6 [37]. After penetrating the cancerous tissue through a tiny hole in the skin, the coaxial antenna would be able to heat the tumors in the range of $41°C$ to $44°C$ to suit hyperthermia, under the guidance of these equations. Tissues exposed to these electromagnetic waves with the coaxial slot antenna absorbed these waves' power and distributed the power throughout the tissue, which means SAR. It can be calculated by the heat transfer equation. For this purpose, since coaxial antennas carrying electromagnetic waves are characterized by transverse electromagnetic field (TEM), the equations with the assumption of time harmonic fields containing phase information are presented below [37].

$$E = e_r \frac{C}{r} e^{j(wt-kz)} \tag{1}$$

$$H = e_\varphi \frac{C}{r} e^{j(wt-kz)} \tag{2}$$

$$P_{av} = \int_{r_{inner}}^{r_{outer}} Re(E \times H^*) 2\pi r dr = e_z \pi \frac{C^2}{Z} \ln\left(\frac{r_{outer}}{r_{inner}}\right) \tag{3}$$

Where C represents amplitude and ω denotes the angular frequency. Here, e_z shows the propagation direction of the electromagnetic wave. The variables of r, ϕ, and z are cylindrical coordinates centered on the coaxial cable axis. Z is the characteristic wave impedance in the dielectric, while r_{inner} and r_{outer} is the dielectric's inner and outer radius, respectively. The electric and magnetic fields flowing in the coaxial cable are defined in equations (1) and (2), respectively [37]. Equation (3) is the time-averaged power flow P_{av} in the cable. The antenna radiates into the tissue, and a damping wave propagates. The electrical properties required for determining the radiation characterization in the tissue are given in Table 1.

Table 1. Electrical properties of breast tissues for frequency of 2.45 GHz[38]

Electrical properties of materials	Dielectric constant ϵ'	Loss factor ϵ''	Loss tangent δ	Conductivity $\sigma[S/m]$
Skin	46.7	5.06	0.11	0.69
Fat	5.60	0.29	0.05	0.04
Breast phantom	4.44	0.95	0.21	0.13
Tumor	55.25	19.80	0.36	0.80

In order to evaluate the antenna's heating ability of the electrical properties in the tissues which are given on Table 1, the SAR is an important parameter. The SAR is indicated mathematically as follows,[37]

$$SAR = \frac{\sigma}{2\rho}|\overrightarrow{E}|^2 \tag{4}$$

In Equation (4), σ is tissue conductivity (S/m), ρ is the tissue density (kg/m^3) and SAR is specific absorption rate (W/kg). The bio-heat equation[37] at Equation (5) describes the stationary heat transfer problem as:

$$\rho C_b \frac{\partial T}{\partial t} + \nabla(-k\nabla T) = \rho_b C_b \omega_{b(T_b - T)} + Q_{met} + Q_{ext} \tag{5}$$

where k represents the liver thermal conductivity W/(m.K), p_b blood density (kg/m^3), C_b blood specific heat capacity J/(kg.K), ω_b blood perfusion rate (1/s), Q_{met} heat source from metabolism, and Q_{ext} external heat source, both measured in (W/m^3). This model neglects the heat source from metabolism[37]. Equation (6) presents the external heat source, which is the resistive heat generated by the electromagnetic field:

$$Q_{ext} = \frac{1}{2} Re\big((\sigma - j\omega\epsilon)EE^*\big) \tag{6}$$

2. Material and methods

2.1. Short ended single slot coaxial antenna design and simulation results

The proposed interstitial type antenna is based on a 47.5 mm long coaxial semirigid cable which is short ended with a single slot. The main radiation frequency is 2.45 GHz as it is compatible with Industrial Scientific Medical bands (ISM). The ISM bands are preferred on purpose because they can be broadcast over a certain output power limitation without a certificate or license for radio communication in many countries. Also, a compact design with the dimensions of 15 mm × 37 mm × 1.6 mm ($0.12\lambda_0$ × is suitable for interstitial microwave ablations or hyperthermia applications. In Figure 1, the produced antenna and its simulation results

are presented in detail. The simulation view and the dimensions of the antenna are given in Figure 1a. The fabricated antenna is shown in Figure 1b. An SMA connector feeds the antenna.

The proposed short ended single slot coaxial antenna is designed with CST Microwave Studio 3D electromagnetic simulation software. According to the simulation results in Figure 1c, 18.64 V/m max electrical field strength is obtained by the omnidirectional radiation with 2.15 dBi directivity. The reason why omnidirectional radiation is preferred is to achieve maximum microwave heating efficiency when the antenna is struck into the tumor center. The main strength is obtained at the edge of the antenna which aims cancerous tissues. The 2D SAR distributions are presented in Figure 1d while the return loss measurement and simulation results are given in Figure 1e. The heat distributions over the with multilayered tissues are shown in Figure 1f as well.
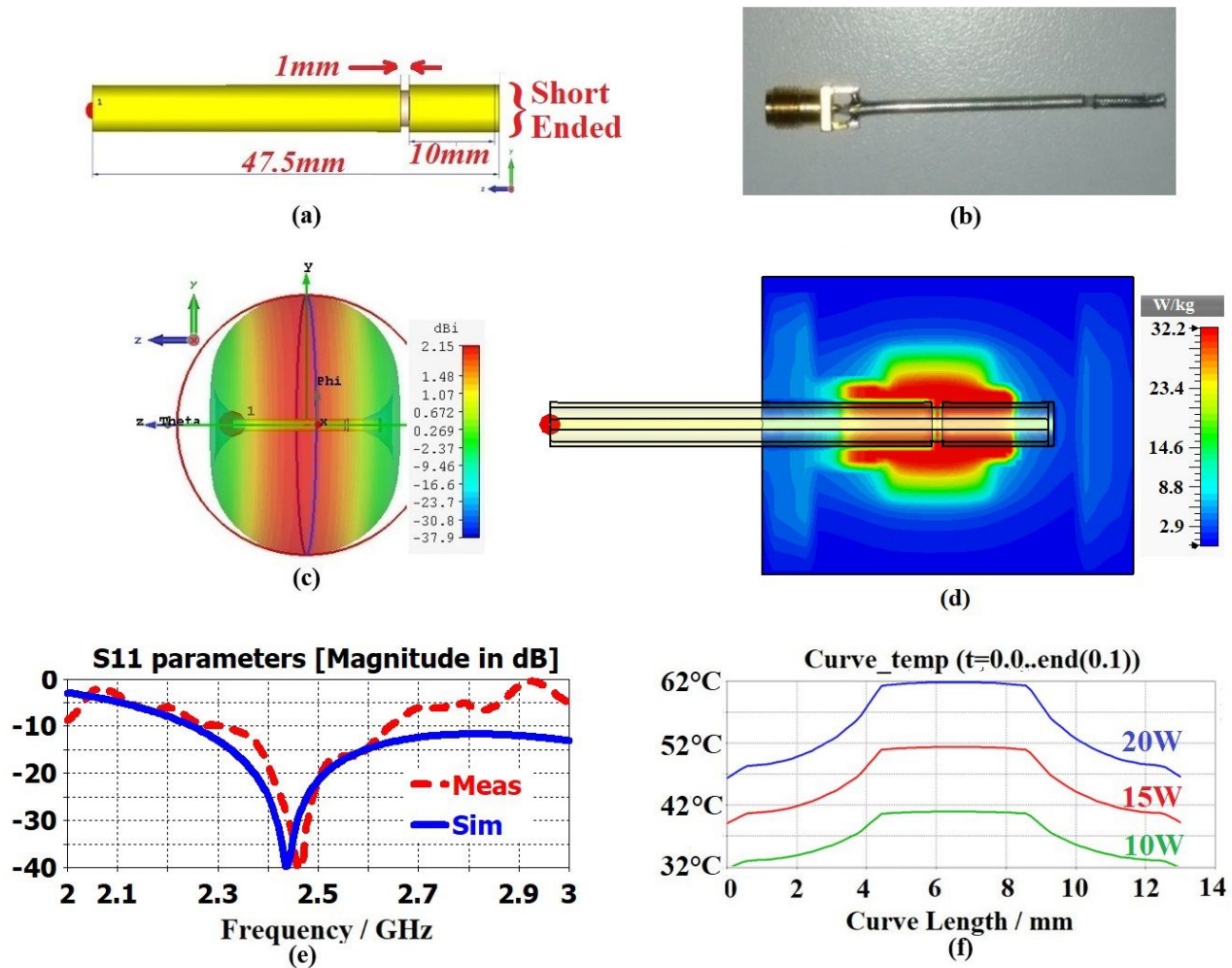

Figure 1. Measurement and simulation results of short ended single slot coaxial antenna design, a)CST 3D view, b)implemented design, c)3D directivity, d)2D SAR simulation (f = 2.45 GHz)(1gr), with tumor tissue, e)Return loss, f) Heat distributions over the with multilayered tissues

Since an application-oriented study is to be carried out, it is necessary to analyze the body or inside or within the tumor and the free space characteristics of the antenna as input impedance distortions or resonance frequency shifts can be observed when immersed in the body even if an antenna works perfectly in free space

environment conditions. This is because the antenna's surface currents are affected by the reflected, refracted, or diffracted waves caused by ambient changes in the antenna's near field region. Since the human body is a lossy medium, the radiation properties of the bioimplant antennas inside the body vary in accordance with the free space radiation properties. This causes the efficiency and gain values of the antennas in the tissue to be low in simulation programs. However, the efficiency value obtained does not reflect the efficiency value of the antenna in the tissue; instead, the antenna and the tissue surrounding the antenna are taken as an integrated structure, and the Pout/Pin ratio scattering from this structure is presented as efficiency. Nevertheless, the fact that this value is low indicates that the surrounding tissues absorb the antenna's energy, and the energy scattering is very low. When the proposed antenna and surrounding tissues were simulated in our study, the efficiency value was 0.5 % in CST Microwave Studio 3D electromagnetic simulation software. This value does not indicate that the antenna is inefficient, but it can be determined that the healthy or unhealthy tissues surrounding the antenna absorb 99.5 % of the energy supplied to the antenna. As mentioned before, this amount of energy absorption does not cause a significant temperature increase in healthy tissues; on the contrary, it enhances the hyperthermia temperature (41°C - 44°C) on tumorous tissues. In addition, since the near field effect is much more complex, it is more accurate to examine the near field effect and SAR rather than the far-field effect of the lossy environment surrounding the antenna [39]. The antenna is designed to be 39.6 dB at 2.45GHz for the return loss when it is struck into the center of the tumor model and the body tissue. The simulation results show that the maximum SAR value from the tumor tissue is obtained when it is centered on the slot of the antenna. The red regions with the highest SAR value are the closest spots to the slot of the antenna, which is the radiation center. As the energy is transferred, it expands globally, decreasing in intensity. Therefore, the highest SAR value in the tumor tissues occurs around the radiating slot region of the antenna. In addition to these radiation aspects of the antenna's positioning, the electrical property(ies) of the tumor structures is also a significant factor for detecting a high SAR value. The highest SAR value obtained in CST simulations was 32.21 [mW/1g] obtained as the maximum SAR/1g value. Although the standards are not defined clearly in the field, the maximum SAR value we obtained is below the literature's limit values, which means the healthy tissues are not damaged [40]. The fact that cancerous tissues can have greater SAR values leads to a greater temperature increment in the tumor model than healthy body tissues. The highest temperature rise occurs at the points closest to the radiation zone of the antenna, where it is exposed to the maximum electric field, just as at the maximum SAR value. However, the high-temperature increase in tumor areas is due to the presence of a subject close to the radiation area and the electrical properties of the tumor. The highest temperature increases through microwave hyperthermia, the temperature distributions are observed for different power values such as 10Watt, 15Watt, and 20Watt. The highest temperature increase occurs in the region with tine tumor (approximately 4 to 8 mm). The temperature ranges were 47°C -62°C for 20W, 40°C -51°C for 15W, and 32°C -41°C for 10W while room temperature is considered at 20°C. As it can be seen in Figure 1d, the SAR distribution results in a relatively spherical or elliptical shape; nevertheless, obtaining a perfect sphericality in the ablation or around the heated zone is not among the primary priorities in hyperthermia applications, unlike ablation applications. The sphericity of the hotspot is more critical for targeting the tumor tissues with higher accuracy in especially microwave ablation applications, such as heating the temperature to very high temperatures such as 60°C to 90°C without distinguishing between healthy and unhealthy tissues. On the other hand, in a microwave hyperthermia application, the radiated energy from the antenna has low intensity; it is only sufficient to raise the temperature of the tumor tissues into the value of hyperthermia temperature (41°C - 44°C). The temperature of the healthy tissues is lower than the hyperthermia temperature due to

the different electrical properties between the tumorous and healthy tissues. This can be seen in the graph of heat distributions over the multilayered tissues in Figure 1f. To give an example in this graph, when an input power of 10W is applied to the antenna, the tumor tissues within the range of only 4 to 8 mm have reached hyperthermia temperature increase, on the other hand, the exhibition of an exponential decrease is far beyond the critical temperature limits that could cause damage to healthy tissues. All in all, although in most microwave ablation applications, the sphericity of the ablation zone is analyzed experimentally with "in vitro" studies, instead of this, as a hyperthermia application, the therapeutic effect of the proposed antenna is analyzed with a directly "in vivo" study with balb/c mice in the following parts.

2.2. Phantom model experiment in microwave hyperthermia system

The phantom model experiment investigates the effects of exposure time and power inputs on the muscle tissue-equivalent liquid before in vivo microwave hyperthermia experiment. The tissue simulations were performed by taking the target values of the dielectric properties into consideration, given as relative permittivity $\epsilon_r =$39.2, conductivity $\sigma = 1.80$ [S/m], at 2.45GHz as an operating frequency. The phantom liquid model includes water (49.75%), diacetin (49.75%) and bactericidal (0.5%) for the 2.45GHz [41]. The relationship between microwave hyperthermia application and power quantity, time, and temperature increase was investigated. The schematic diagram of the setup and the tissue-equivalent liquid is given in Figure 2.

The return loss and impedance measurements of the short ended single slot coaxial antenna and output impedance measurement of the power amplifier are measured with the HP8720A (130 MHz to 20 GHz) network analyzer. According to these measurements, the antenna performs a circular smith chart graph as expected. Overall, it can be characterized as a transmission line with short ended. However, when it is inside the phantom tissue liquid model, the reflection coefficient dramatically decreases. The impedance lines get closer to the origin in a broad range of frequencies, such as better than 10 dB return loss between 1 to 10 GHz. According to 2.46 GHz free space measurements, the return loss is 39.4 dB. The proposed antenna in 2.45 GHz tissue-equivalent liquid is measured as 14,734 dB as the return loss. The PA is pushed to work in AB-Class to get suitable parameters such as PAE and linearity. The load power is achieved as 25 W, and PAE is achieved as 60%. It is known that both the linearity and efficiency in RF PA (power amplifier) are in a trade-off.

In order to reach the ideal hyperthermia temperature 41°C - 44°C, two different input power sources are tested in the microwave ablation system. In both cases, Signal Hound VSGA25 is utilized as a signal generator which provides 2.45 GHz continuous sine wave with 1kHz modulation speed and 50 % depth. For the first case, output of the generator -5 dBm and for the second it is 0 dBm. In both cases, the signals are amplified with a CGHV40100F power amplifier which is fed via DC power supply. In the former setup, the amplifier circuit drew current 8.13A while it was 10.91A in the latter, which they lead the output power of the PA, 20.48W and 25.6W, respectively. These output powers applied to the short ended single slot coaxial antenna. As a result, in first case, the target hyperthermia temperature range of (41°C - 44°C) is reached within 42 s and for the second case, it is in 23 s which are presented in Figure 3. During the experiment, the temperature is monitored via Xbee & Arduino-based wireless data transfer through DSD18B20 sensors.

2.3. In vivo study

All studies were animal care with ethics committee approval. Ethical approval was obtained from the Celal Bayar University Local Research Ethics Committee (Ethical approval number: 77.637.435-46-12/05/15). The study protocol was carried out in accordance with the Helsinki Declaration as revised in 1989. All experiments
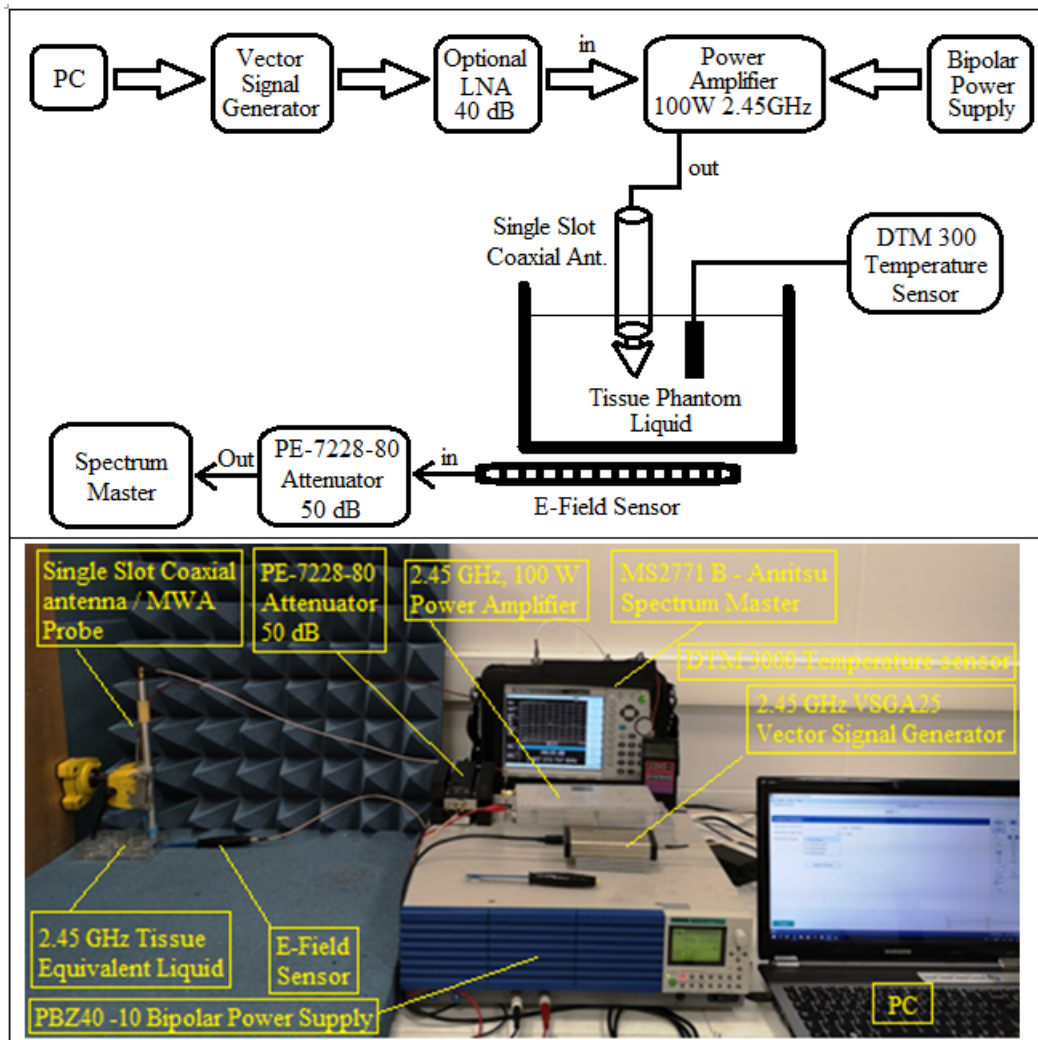
Figure 2. Realized setup for microwave hyperthermia application with tissue-equivalent liquid or in vivo microwave heating in mouse breast cancer.

were repeated three times by at least two observers independently. The Tukey - Kramer multiple comparisons test was used to express differences amongst the mean values (mean $\pm$ standard deviation). The values for <0.05 were considered statistically significant [41]. The schematic diagram of microwave hyperthermia setup with tissue-equivalent liquid and realized setup for microwave hyperthermia application with tissue-equivalent liquid or in vivo microwave hyperthermia in mouse breast cancer are given in Figure 4.

The in vivo studies started with the creation of breast cancer models and animal experiments. 6 - 8 weeks old Balb/c wild type female mice weighing 20 $\pm$ 5 gr were used in the study. The reason for selecting this genus is its genetic compatibility for cancer models [42]. Mice were housed for two weeks for checking any signs of health problems for adaptation. During the study, the animals were kept under stable conditions (22 °C temperature, 30-70 % humidity, light dark cycle 12/12 h). They were fed with standard mouse feed and tap water. The 4T1 mouse metastatic breast cancer cell line was used to construct the experimental breast cancer model. The proliferating cells were then collected and counted under the microscope. The
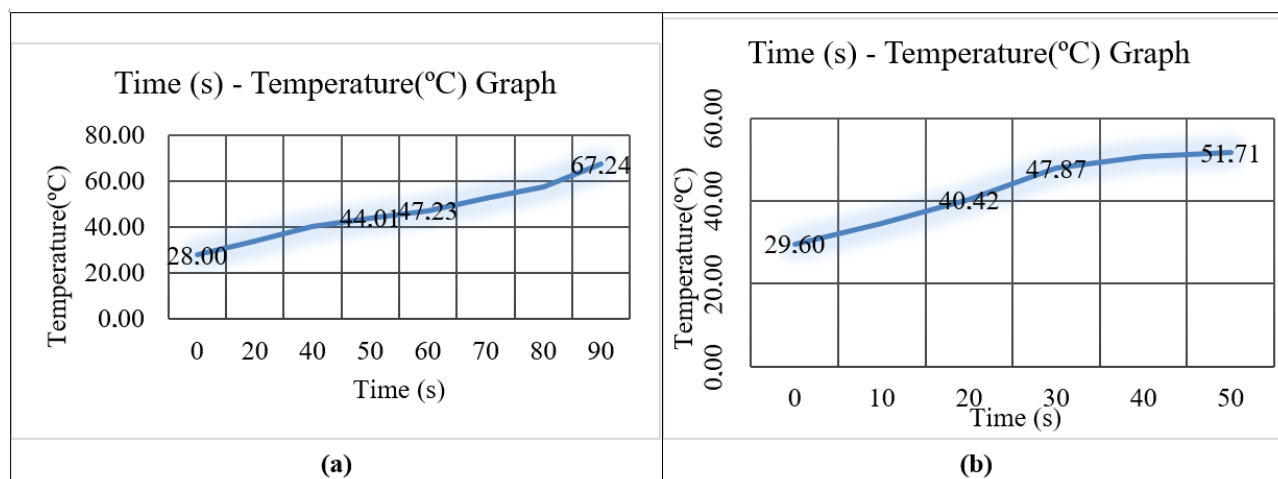
Figure 3. a) Time (s) - temperature($^\circ$C) graph of tissue simulation application in phantom experiment for 20.48 W PA output power b) time (s) - temperature($^\circ$C) graph of tissue simulation application in phantom experiment for 25.6 W PA output power.

animals were given general anesthesia subcutaneously at the right first breast level for cancer formation [43, 44]. After the experiment, the histopathological evaluation and staining process are initiated. The tumor lesions were removed under general anesthesia and fixed in 10 % buffered formalin solution for 24 – 72 h. After the microwave hyperthermia applications, cell proliferation and vascularization parameters were evaluated by immunohistochemistry by PCNA and VEGF. Also, the TUNEL assay was applied to detect apoptotic cells. The differences between the groups were evaluated by light microscopy. The immunohistochemical staining levels were determined as no staining (0), weak (+), moderate (++), and severe (+++) in the five different fields, and the H-score formula used the evaluation of immunohistochemical results: σ Pi (intensity of staining + 1). Pi means the percentage of stained cells for intensity, varying from 0% to 100% [45]. To determine the apoptotic cell death, Terminal Transferase dUTP Nick End Labeling (TUNEL, S7101) staining method was used. TUNEL positive cells were detected by the blind method, and the apoptotic index was calculated.

After forming the breast cancer model, the subjects were divided into two groups; control (n:6) and microwave hyperthermia group (n:6). In the mouse model breast cancer performed with the 4T1 cell line, it was observed that the cancerous cells produced tightly interlocked tumor tissue and the tumor growth started on the 10th day and reached approximately 1 cm in twenty days and the animals died showing signs of weakness and indulgence which are terminal clinical signs of cancer. It was observed that the cells contained typical carcinoma features in their morphological images and regressed with microwave hyperthermia applications. Microwave hyperthermia was performed by using a short ended single slot coaxial antenna in vivo experiments with 4T1 mouse metastatic breast cancer model. In Figure 5, the microwave hyperthermia device and intra-tissue applications are illustrated.

3. Results and discussion

In Figure 5, the coagulation diameters achieved in microwave hyperthermia parameter groups to reach microwave hyperthermia temperature (25 W for 3-5 min) were represented. It is used histologic as HE and immunocytochemical parameters; such as PCNA, VEGF, and TUNEL, for evaluating the application on tumor tissue.
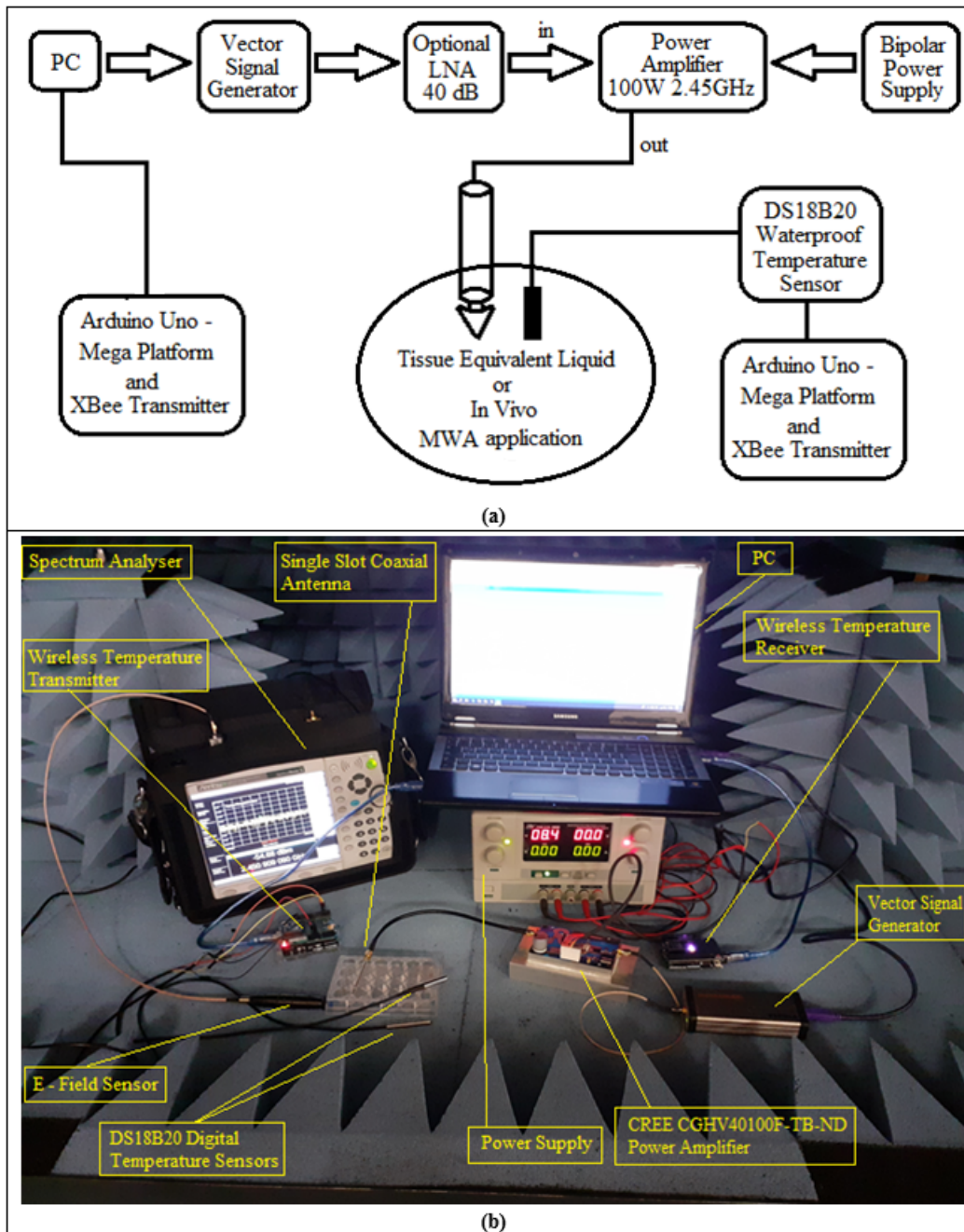
Figure 4. Realized setup for microwave hyperthermia application with tissue-equivalent liquid or in vivo microwave heating in mouse breast cancer.

Cancer cells proliferate and produce vessels for getting bigger and migrate to metastasis. One of the basic aims of chemotherapy and radiotherapy is to decrease their proliferation and vessel formation. Unfortunately, these therapies have important side effects, and their cost is very high. Moreover, they are not successful enough in getting over cancer cells completely. Nevertheless, our system decreased proliferation as shown by PCNA staining and vessel formation by VEGF staining. The best effect was that our application increased the apoptosis
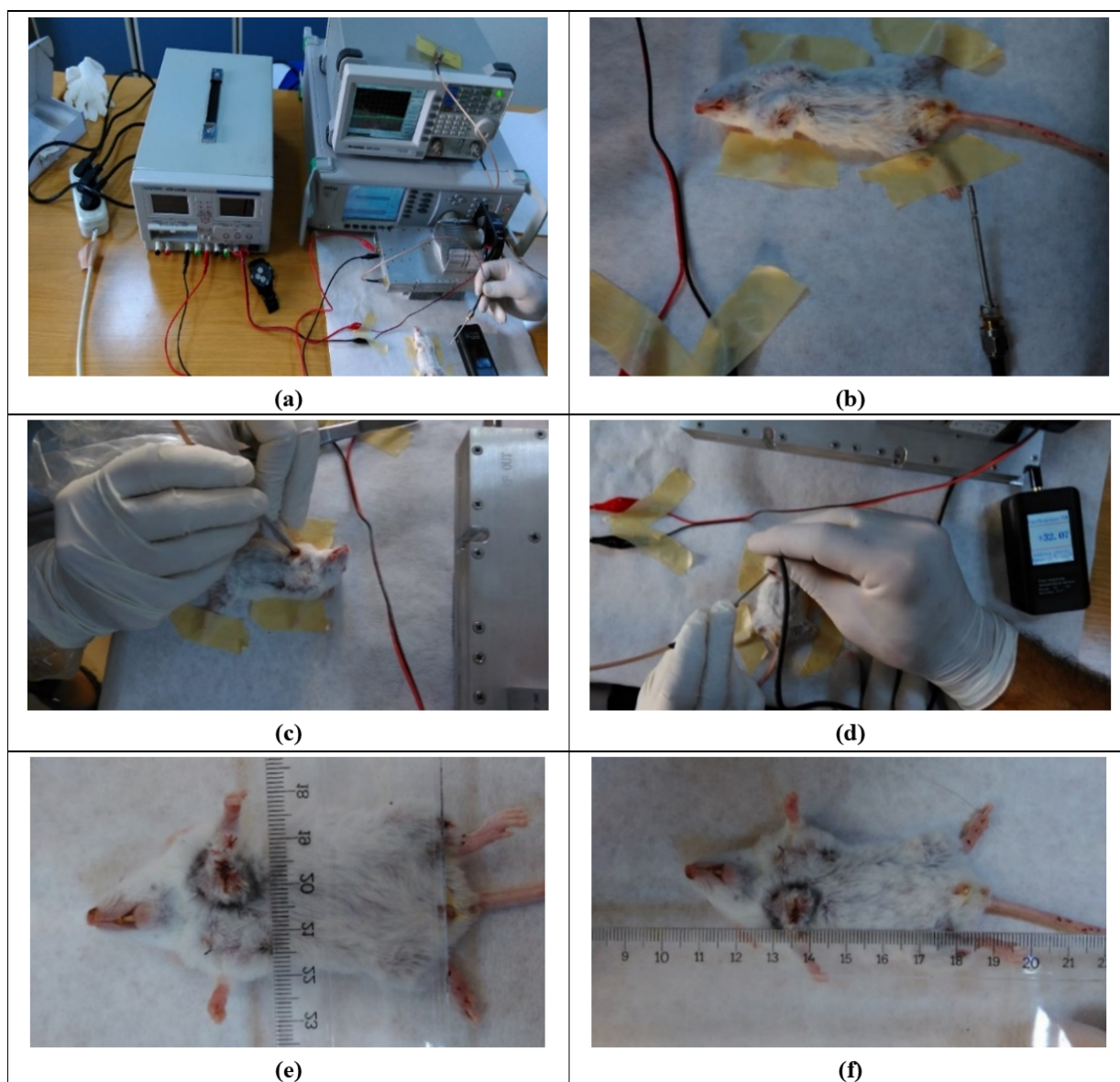
Figure 5. Microwave hyperthermia on mouse breast cancer (in-tissue application).

which can kill them all. It would be much better if these experiments are done with adding chemotherapy less amount than normal use. Then, we may see a much better treatment without side effects and high cost.

It is clearly seen in Figure 5, a single-slot coaxial antenna of microwave energy with 25 W for about 3-5 min yielded a mean coagulation size of $(0.3 \text{ cm} \pm 0.1 \text{cm}) \times (0.7 \text{ cm} \pm 0.1 \text{ cm})$ (Short Axis $\times$ Long Axis). After the microwave hyperthermia, there was a significant decrease in the distribution of PCNA and VEGF in the tumor tissues ($P < 0.001$). Also, the number of apoptotic cells was increased with the application of microwave hyperthermia ($P < 0.001$) in Figure 6. The heat has been checked in all applications into tumors. Also, peripheral tissues of the breast, such as skin and lungs, were examined as a precaution for any harmful effect. As a result, no morphological or histological damage was observed in such tissues. In Figure 5, no

indication of skin damage was observed during the controlled heating process at the center of the tumor. The black circle of tumor edge in Figure 5 indicates tumor area.
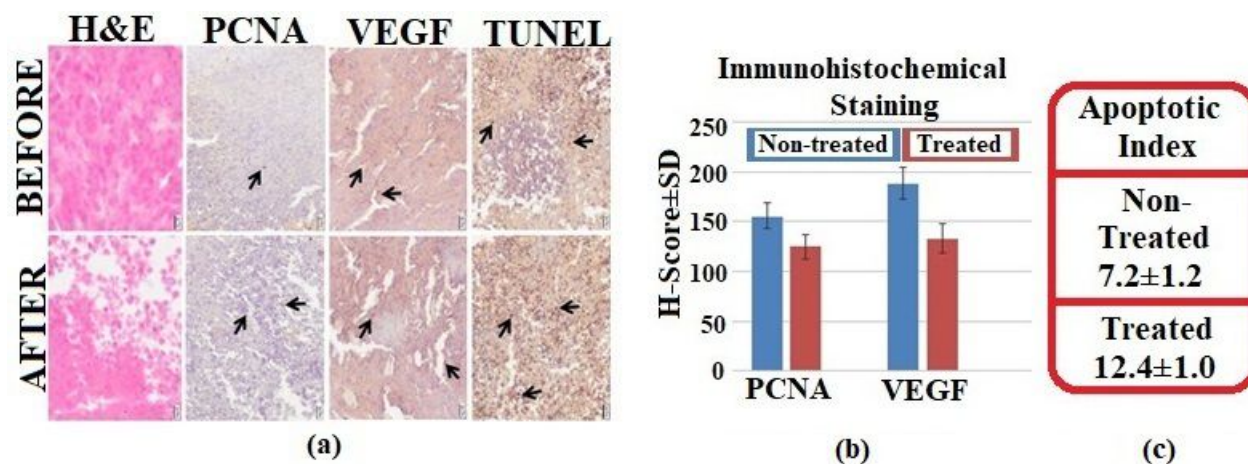

Figure 6. a) The histochemical and immunohistochemical stainings of PCNA and VEGF, TUNEL for apoptosis in the tumor tissues treated and nontreated with microwave hyperthermia. Arrows: Positive staining cells, Scale bars: 50 m. b) H-score analysis of the immunostainings of PCNA and VEGF, and (c) apoptotic index.

A comparative literature analysis is presented in Table 2. According to this table, the proposed research combines different interdisciplinary fields of study under a single publication and explains all the details from design to cancer treatment with in vivo. A patient-specific disposable model was chosen as a priority in the design criteria, which was low-cost, fast, and suitable for mass production. In the treatment of cancer with hyperthermia, although electrical and electronics engineers play an essential role in the design phase, the vast majority of articles published on the subject present the results in medicine. From electrical engineers' point of view, the electrical characterization of the radiating antenna, reflection coefficient graphics, farfield radiation, SAR values have been studied in detail. Also, most publications perform microwave ablation instead of microwave hyperthermia or aim at high-cost complex designs. Moreover, in these publications, in vivo studies are negligible, and a limited number of in vitro studies can be found.

4. Conclusion

In this study, after a significant literature review, covering all stages in hyperthermia applications for cancer treatment, microwave hyperthermia application with short ended single slot coaxial antenna design for mouse breast cancer model is realized. According to experimental results, the phantom liquid model can reach the hyperthermia temperature range of ($41°$C - $44°$C) in 42 s, and for the second case, it is 23 s. In this study, at the in vivo stage, breast cancer tissues are transferred to both groups under laboratory conditions. Therefore, cancer has been grown in breast tissues. Except for control group, treatment group has been treated by specific antenna in the stage of hyperthermia. Control group has been kept in the same environmental conditions. The antenna with microwave energy of 25 W for about 3-5 min yielded a mean operation area size of (0.3 cm $\pm$ 0.1 cm) $\times$ (0.7 cm $\pm$ 0.1 cm) (Short Axis $\times$ Long Axis). Morphological images of the cells are analysed to contain typical carcinoma features and regressed by microwave hyperthermia application. It has a significant effect on cell proliferation performed on the microscopic appearance of adenocarcinoma cells. Tumor volume was compared with sensitized dendritic cells by immuno-flap application in the tumor model, and a significant

Table 2. Planar metal plate antenna performance comparison from literature

Studies	Ablation=0 Hyperthermia=1	Electrical engineering details are given? (Yes=1,No=0)	High cost =0 Low cost =1	Complicated=0 Compact=1	Only simulation=0 With measurement=1	Tissue equivalent liquid model is used? (Yes=1,No=0)	in vitro=0 in vivo=1	Applied to on body=0, in body=1	Evaluation of the application on tumour tissue is done? (Yes=1, No=0)
[20]	0	0	1	1	1	1	0	-	0
[21]	0	0	1	1	1	1	1	1	1
[22]	0	0	0	1	1	0	1	1	0
[23]	1	1	1	1	0	0	-	-	0
[24]	0	1	0	0	0	0	-	-	0
[25]	1	1	0	0	0	0	-	-	0
[26]	1	1	0	0	0	0	-	-	0
[27]	1	1	0	0	0	0	-	0	0
[28]	1	1	0	0	1	1	0	0	0
[29]	0	1	0	0	1	1	-	0	0
[30]	0	1	0	0	1	0	-	-	0
[31]	0	1	1	1	1	0	0	-	0
[32]	1	1	0	1	1	1	0	-	0
[33]	1	1	0	0	1	1	0	0	0
[34]	0	1	1	1	1	1	0	-	0
[35]	0	1	1	1	1	0	0	1	0
[36]	0	1	1	1	1	0	0	1	0
Proposed	1	1	1	1	1	1	1	1	1

reduction in tumor volume was observed. Consequently, the results of hyperthermia treatment, applied and not applied cancer mice, are examined, and breast cancer has been inhibited with the proposed hyperthermia system. Another impressive concluded point is cost effective treatment in some cancer types will be provided by our antenna in the near future. Some other in vivo/in vitro studies should be performed.

5. ACKNOWLEDGMENT

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