Microwave Cavity with Controllable Temperature for *In Vitro* Hyperthermia Investigations

(Invited Paper)

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Abstract

Hyperthermia is a form of cancer treatment in which affected human tissue is exposed to >40℃ temperature. In this paper, our goal is to assess the efficacy of fullerene agents to reduce heating time for cancer treatment. Such agents can accelerate heating of cancer cells and improve hyperthermia treatment efficacy. Typically, *in vitro* testing involves cancer cell culturing, heating cell cultures in accordance to specifications, and recording cancer cell viability after hyperthermia. To heat cell cultures, we design and evaluate a 2.4-GHz microwave cavity with controllable temperature. The cavity is comprised of a polystyrene cell culture dish (diameter = 54 mm, height = 13.5 mm) and a printed monopole antenna placed within the cavity for microwave heating. The culture temperature can be controlled through the intensity and duration of the antenna's microwave radiation. Heating experiments were carried out to validate the cavity's performance for F-12K culture medium (Kaighn's F-12K medium, ATCC). Importantly, fullerene agents were shown to reduce heating time and improve hyperthermia treatment efficacy. The culture medium temperature increased, on average, from 24.0℃ to 50.9℃ (without fullerene) and from 24.0℃ to 56.8℃ (with 3 mg/mL fullerene) within 15 minutes.

Key Words: Cell Culture, Controllable Temperature, Fullerene, Hyperthermia, *In Vitro*, ISM Band, Microwave Cavity.

I. INTRODUCTION

Hyperthermia is a promising form of cancer treatment in which the affected human tissue is exposed to >40℃ temperature for treatment [1]. Such high temperatures are intended to damage proteins and structures within the cells and to kill cancer cells. It is important that heating is localized and, of course, it must cause minimal trauma to the surrounding healthy biological tissues. To raise the temperature of the affected region, different types of energy may be used, including microwave radiation [2, 3]. Typically, external or implanted microwave applicators are placed close to the body or organ to be treated. Subsequently, energy is focused on the affected tissue area to raise its temperature.

A key challenge prior to employing hyperthermia is to carry out *in vitro* assessment of the procedure's efficacy [4–7]. For our case, the goal is to assess fullerene agents to reduce heating time and improve hyperthermia efficacy. Typically, *in vitro* testing involves cancer cell culturing, heating of cell cultures to specifications, and recording cancer cell viability after hyperthermia. As an example, the potential of single-walled carbon nanotubes (SWCNTs) was recently assessed *in vitro* for microwave detection and treatment of breast cancer [4]. In [4], an *S*-band rectangular waveguide was employed for microwave heating. Specifically, tissue-mimicking mixtures were poured into glass capillary tubes

and placed at the center of the waveguide. By exciting the waveguide, temperatures up to 37℃ were achieved using 3 GHz illumination.

In this paper, we instead propose a cavity fixture to heat the culture with and without fullerene. Because of their resonant properties, cavities with metallic enclosures can achieve temperatures up to approximately 50℃ (viz. much higher than a direct waveguide illumination). Even more importantly, the temperature of the enclosed cell culture can be controlled based on the heating time and intensity. Herewith, we will adapt 2.4 GHz (Industrial Scientific and Medical [ISM] applications [8]) frequency for microwave radiation. The chosen cavity is comprised of a polystyrene cell culture dish shielded with copper, and a 2.4-GHz printed monopole antenna placed within the cavity for radiation [9]. Because of the surrounding metal shield, when the antenna is excited at 2.4 GHz, cavity resonance is achieved, giving constant rise in temperature as radiation continues. The temperature of the medium is controlled through the intensity and duration of microwave illumination. Therefore, as compared to previous works [4, 10, 11], the proposed set-up provides for consistent temperature increase even beyond 50℃ in a controllable manner.

This paper is organized as follows. Section II presents the design of the microwave cavity. Then, Section III gives the heating performance for different cell cultures.

II. Microwave Cavity Design

The proposed microwave cavity is shown in Fig. 1 [9]. It is comprised of a polystyrene (ε_r = 2.4–2.7) culture dish (diameter = 54 mm, height = 13.5 mm) covered with copper tape and a 2.4-GHz printed monopole antenna placed within the cavity for microwave heating. The latter is fed by a 50-Ω coaxial cable that penetrates into the cavity through a small hole drilled on its upper surface. The antenna is designed so that it resonates when approximately 2.75 mL of cell culture medium is placed within the cavity. Because of the surrounding metal shield, when the antenna is excited at this frequency, modes are formed to enhance heating and raise the temperature. We remark that the temperature of the culture can be controlled through the intensity and duration of the antenna's microwave radiation.

The printed monopole antenna prototype is shown in Fig. 2. It occupies $33 \times 30 \times 1.524$ mm^3 and is fabricated on Rogers TMM4 (ε_r = 4.5, tanδ = 0.02) ceramic substrate. The design is inspired from the one presented in [12]. As designed, when this antenna is placed inside the proposed microwave cavity, filled with approximately 2.75 mL of cell culture medium, it resonates at 2.4 GHz. Measured versus simulated data of the antenna's reflection coefficient, $|S_{11}|$, are given in Fig. 3, indicating good agreement. Of course, any antenna may be used, as long as it has
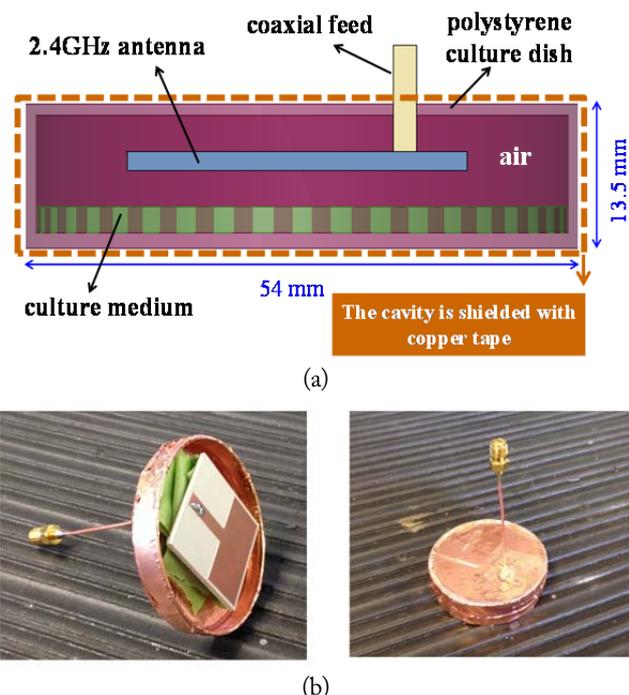

Fig. 1. Proposed microwave cavity for *in vitro* hyperthermia investigations: (a) cavity cross-section with radiating antenna and (b) photos of the fabricated prototype.
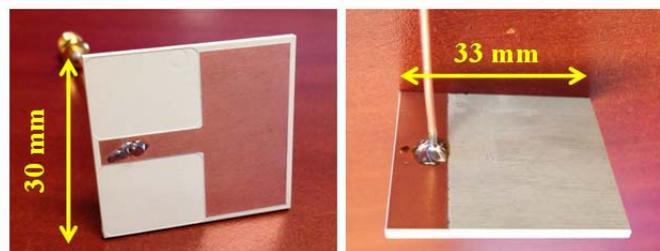

Fig. 2. Printed monopole antenna to be enclosed within the proposed microwave cavity.

been specifically designed to resonate at 2.4 GHz when employed within the configuration of Fig. 1.

In designing the cavity and antenna, the dielectric properties (permittivity ε_r and conductivity σ) of the enclosed culture medium must be taken into account. The latter were measured for F-12K medium (ATCC, Manassas, VA, USA) using the Agilent 85070E Dielectric Probe Kit and the Agilent E8362B PNA Network Analyzer. Measured data are shown in Fig. 4 ('culture medium'). Specifically, the 85070E high-temperature probe (operation frequency range, 200 MHz–20 GHz) was employed, and measurements were taken by immersing the latter inside the cell culture medium. As depicted in Fig. 4, the dielectric properties of F-12K medium at 2.4 GHz were measured to be ε_r = 75.5 and σ = 3.04 S/m. For comparison, the measured dielectric properties of distilled water are also given in Fig. 4 ('distilled water').
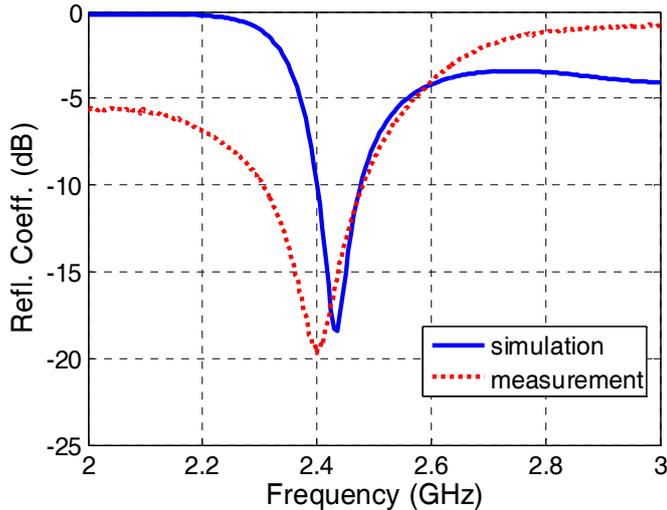
Fig. 3. Measured vs. simulated reflection coefficient, $|S_{11}|$, of the antenna. Antenna is placed within the cylindrical cavity filled with approximately 2.75 mL of cell culture medium.
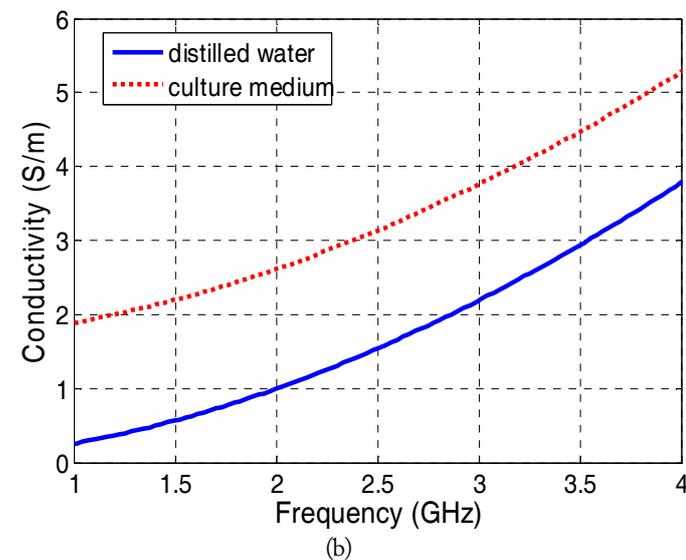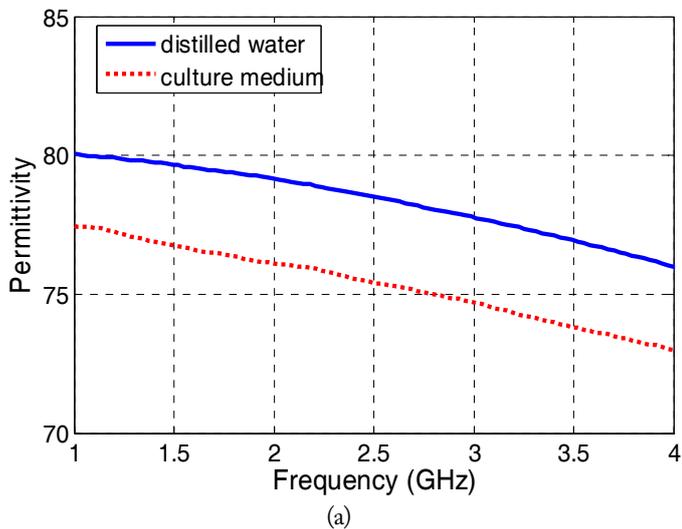

(a)

(b)

Fig. 4. Measured dielectric properties of the F-12K cell culture medium and distilled water: (a) permittivity ε_r and (b) conductivity σ.

III. THERMAL RESPONSE OF THE MICROWAVE CAVITY

1. Measurement Set-Up

The performance of the proposed microwave cavity is evaluated by carrying out heating experiments for the F-12K culture medium (ATCC). The measurement set-up is shown in Fig. 5. A 2.4-GHz signal generator (Anritsu 68369A/NV) with a power amplifier (Mini-Circuits ZHL-42) generate 31 dBm (1.26 W) of power into the cavity. The DC power to the power amplifier is provided by a 15-V DC power supply. Also, a 2.4-GHz sleeve balun is employed between the power amplifier output and the antenna input. In doing so, a balanced operation is achieved to minimize cable radiation. The cavity contains approximately 2.75 mL of the cell culture medium ($\varepsilon_r = 75.5$, $\sigma = 3.04$ S/m, see Fig. 4).

It is necessary to include a temperature sensor, pierced through the cavity side, to sense the temperature of the enclosed medium. The temperature sensor is a custom-made ungrounded probe (diameter = 0.062", length = 2") with a type-K thermocouple at its tip (W. H. Cooke & Co. Inc., Hanover, PA, USA). Network analyzer measurements verified that the presence of this temperature sensor does not perturb the cavity's resonance. The temperature sensor data are displayed using a digital thermometer with a resolution of $0.1\,°C$ and an accuracy of $\pm0.3\%$ (Extech TM100). We remark that for thermal insulation purposes, the microwave cavity is enclosed inside styrofoam.

2. Heating Response Results

Heating experiments were carried out using the measurement set-up of Fig. 5. The cavity was filled with cell culture medium (F-12K, ATCC), that was measured and then dispensed with an adjustable pipette. The initial temperature of the culture medium was $24.0\,°C$ prior to hyperthermia. To induce hyperthermia, we turned 'ON' the 2.4 GHz signal generator and power amplifier to deliver 31 dBm of power at the antenna terminals. The RF generator remained 'ON' for 15 minutes, and the heating response was recorded as a function of time (heating cycle). The microwave source was then turned 'OFF' to allow the medium to cool. The exhibited cooling response was recorded for another 15 minutes (cooling cycle). We note that during the cooling cycle, the cavity was still encased with Styrofoam. All measurements were conducted at room temperature of approximately $24\,°C$.

To assess the repeatability of the experiments, three sets of measurements were performed, each for different F-12K samples. The heating and cooling cycle data were then recorded and compared in Fig. 6(a). Fig. 6(b) shows the average thermal (heating/cooling) response along with error bars as a measure of the associated standard deviation (SD). We note that the SD is seen to be always less than $1.9\,°C$, confirming the repeatability of
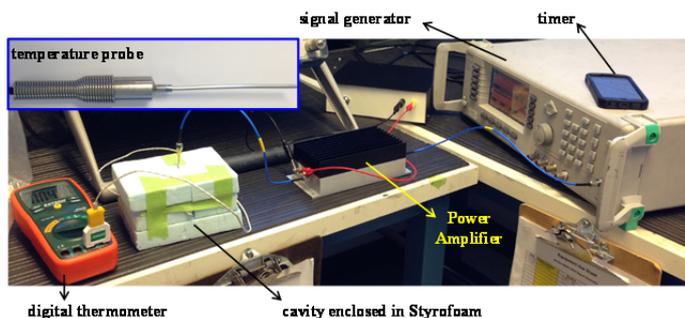
Fig. 5. Experimental set-up for measuring the thermal response of cell culture samples using the proposed microwave cavity.
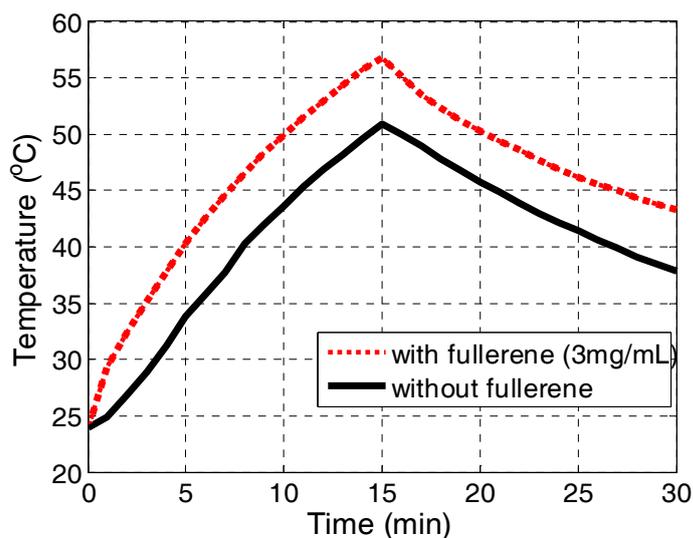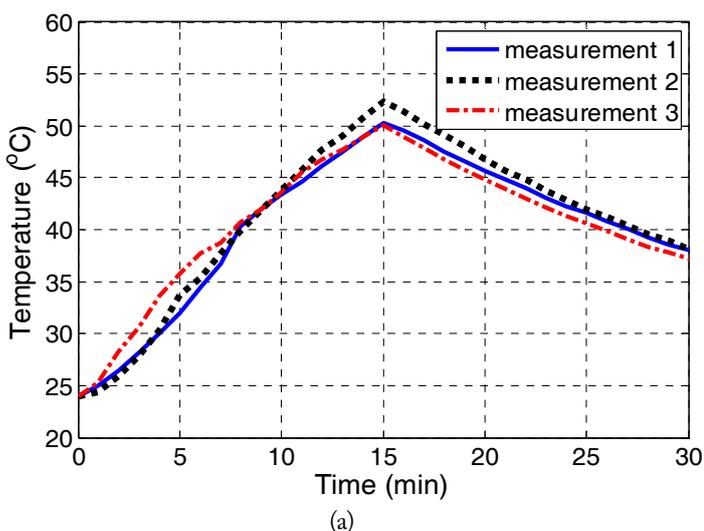

(a)

(b)

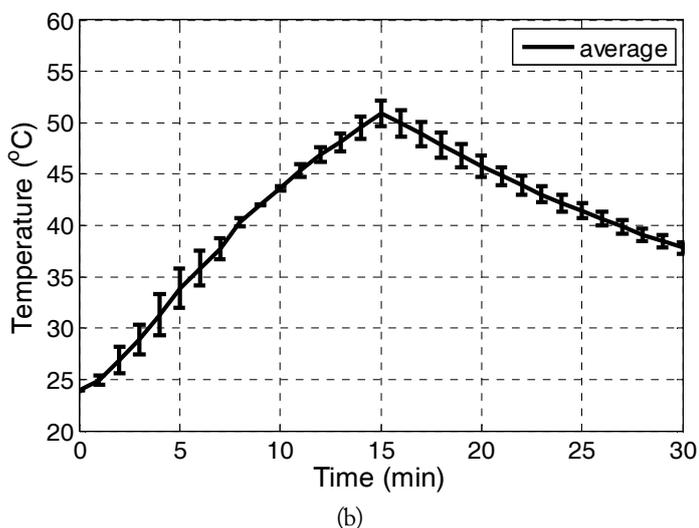Fig. 6. Measured heating/cooling response of three F-12K samples using the proposed microwave cavity: (a) individual measurements and (b) average thermal response with SD error bars.

the proposed microwave cavity set-up. On average, hyperthermia temperatures (>40℃) are reached in less than 8 minutes. Also, temperature increase from 24.0℃ to 50.9℃ is achieved within a 15 minutes heating cycle. Further, it was found that the temperature drops by about 13.1℃ (from 50.9℃ to 37.8℃) after the 15 minutes cooling cycle. Of importance

Fig. 7. Measured heating/cooling response of F-12K samples with (3 mg/mL) and without fullerene.

is that the temperature of the enclosed cell culture medium can be controlled. To do so, the intensity (radiated power) and heating (exposure time) should be varied.

An example temperature profile of the F-12K culture medium with (3 mg/mL) and without fullerene is given in Fig. 7. Average temperature increase by 32.8℃ and 26.9℃ is achieved within the 15 minutes heating cycle, respectively. As such, fullerene agents can accelerate heating of cancer cells and improve hyperthermia treatment efficacy.

IV. CONCLUSION

Hyperthermia is a form of cancer therapy in which the affected tissue is exposed to >40℃ temperature for treatment. Typically, the effectiveness of various hyperthermia methods can be assessed by cancer cell culturing, heating cell cultures in accordance to specifications, and recording cancer cell viability after hyperthermia. For *in vitro* hyperthermia assessment, we designed and validated a resonant cavity to achieve a temperature rise via microwave heating. The cavity is comprised of a polystyrene cell culture dish shielded with copper and a 2.4-GHz printed monopole antenna placed within the cavity for microwave heating. Of interest was temperature control by adjusting the microwave power and exposure time.

Heating efficacy was validated with experiments using F-12K medium (ATCC). Three sets of measurements were carried out for three separate, yet identical, samples. These measurements indicated an average temperature increase of 26.9℃ within a 15-minute heating cycle and an average temperature decrease of 13.1℃ within a follow-up 15-minute cooling cycle. Fullerene agents were shown to accelerate heating of cancer cells and improve hyperthermia treatment efficacy. Average temperature increase by 32.8℃ was achieved for F-12K medium with 3

mg/mL of fullerene. As compared to previous works [4, 10, 11], the proposed set-up provides for consistent temperature increase beyond 50℃. SD errors of less than 1.9℃ were recorded, indicating the repeatability of the set-up.

Future work will assess the effects of fullerene on cancer cell viability as a function of heating time and/or transmitted power.

REFERENCES

[1] P. Wust, B. Hildebrandt, G. Sreenivasa, B. Rau, J. Gelermann, H. Riess, R. Felix, and P. M. Schlag, "Hyperthermia in combined treatment of cancer," *Lancet Oncology*, vol. 3, no. 8, pp. 487–497, Aug. 2002.

[2] X. He, S. McGee, J. E. Coad, F. Schmidlin, P. A. Iaizzo, D. J. Swanlund, S. Kluge, E. Rudie, and J. C. Bischof, "Investigation of the thermal and tissue injury behavior in microwave thermal therapy using a porcine kidney model," *International Journal of Hyperthermia*, vol. 20, no. 6, pp. 567–593, Sep. 2004.

[3] M. G. Skinner, M. N. Iizuka, M. C. Kolios, and M. D. Sherar, "A theoretical comparison of energy sources-microwave, ultrasound and laser-for interstitial thermal therapy," *Physics in Medicine and Biology*, vol. 43, no. 12, pp. 3535–3547, Dec. 1998.

[4] A. Mashal, B. Sitharaman, X. Li, P. K. Avti, A. V. Sahakian, J. H. Booske, and S. C. Hagness, "Toward carbon-nanotube-based theranostic agents for microwave detection and treatment of breast cancer: enhanced dielectric and heating response of tissue-mimicking materials," *IEEE Transactions on Biomedical Engineering*, vol. 57, no. 8, pp. 1831–1834, Aug. 2010.

[5] W. Rao, W. Zhang, I. Poventud-Fuentes, Y. Wang, Y. Lei, P. Agarwal, B. Weekes, C. Li, X. Lu, J. Yu, and X. He, "Thermally responsive nanoparticle-encapsulated curcumin and its combination with mild hyperthermia for enhanced cancer cell destruction," *Acta Biomaterialia*, vol. 10, no. 2, pp. 831–842, Feb. 2014.

[6] H. H. Kampinga, "Cell biological effects of hyperthermia alone or combined with radiation or drugs: a short introduction to newcomers in the field," *International Journal of Hyperthermia*, vol. 22, no. 3, pp. 191–196, May 2006.

[7] M. Kakehi, K. Ueda, T. Mukojima, M. Hiraoka, O. Seto, A. Akanuma, and S. Nakatsugawa, "Multi-institutional clinical studies on hyperthermia combined with radiotherapy or chemotherapy in advanced cancer of deep-seated organs," *International Journal of Hyperthermia*, vol. 6, no. 4, pp. 719–740, 1990.

[8] International Telecommunications Union, "ITU-R radio regulations section 5.138 and 5.150," http://itu.int/home.

[9] A. Kiourti, S. Luther, and J. L. Volakis, "Microwave cavity with controllable temperature for hyperthermia treatment investigations," presented at the *IEEE International Symposium on Antennas and Propagation*, Memphis, TN, Jul. 2014.

[10] M. Kim and K. Kim, "Development of a compact cylindrical reaction cavity for a microwave dielectric heating system," *Review of Scientific Instruments*, vol. 83, no. 3, article no. 034703, Mar. 2012.

[11] F. Irin, B. Shrestha, J. E. Canas, M. A. Saed, and M. J. Green, "Detection of carbon nanotubes in biological samples through microwave-induced heating," *Carbon*, vol. 50, no. 12, pp. 4441–4449, Oct. 2012.

[12] S. M. Abbas, B. Aftab, E. Qamar, F. Muzahir, S. Shahid, and H. Zahra, "High gain broadband monopole antenna for wireless communications," in *Proceedings of the IEEE International Conference on Emerging Technologies*, Islamabad, Pakistan, pp. 1–6, 2012.

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