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***Abstract*—While electromagnetic fields are now quasi-ubiquitous on earth and are constantly growing in use, RF radiation impact on living beings still continues to question. Within this context, a near-field RF applicator compatible with routine *in vitro* microplates for cellular investigations is proposed. This paper specifically focuses on its dosimetry performances, which are evaluated through simulation and measurements at 225 MHz, and its facility in biological post-analysis enabled by the use of standard bio-microplates.**

***Keywords*—Dosimetry, RF applicator, RF effects, SAR, biological cells.**

I. Introduction

Evaluating the effect of electromagnetic fields on living beings remains a challenging topic due to controversial scientific results, as mentioned for instance in [1] with the cancer occurrences in laboratory rats. Such an investigation is however requested, as new equipment and standards such as 5G are developed, and the presence of ElectroMagnetic (EM) waves in our environment is continuously spreading out.

To evaluate the effect of RadioFrequency (RF) fields on living models, studies are conducted at different scales: *in vitro* using cultured cells, *in vivo* with animals and through epidemiological investigations at the human level. These different types of studies currently help to evaluate if there may be or not any beneficial or harmful effect of electromagnetic fields on living systems, depending on the RF exposure conditions [2-3]. As far as experimental studies are concerned, they require developing appropriate RF exposure systems and test setups in conjunction with post-analysis performed by biologists and physicians. Actually, a large variety of RF exposure systems exist, with various electromagnetic approaches (far-field, near-field, antennas, rectangular waveguides) [4-6]. For *in vitro* evaluation, most of these solutions employ traditional Petri dishes, which present limitations in terms of compactness, dosimetry, and handling.

This paper therefore introduces an original near-field RF applicator specifically designed to radiate electromagnetic fields to cells, which are located into a standard biological 96-well plate. The structure permits not only to illuminate biological cells with controlled and calibrated electromagnetic conditions, but it drastically facilitates the biological post-analysis of the cells due to the use of a standard biological accessory employed in routine analysis by biologists.

After describing the microwell plate-based near-field RF applicator in a first section, its electromagnetic characterization performed at 225 MHz is presented. The results obtained with electromagnetic simulation are given in section III, whereas the section IV is dedicated to the experimental dosimetry of the applicator. The next section provides then the application of the RF applicator with biological cells and its association with a biological post-analysis.

II. Description of the MultiWell-Plate-Based Near-Field RF Applicator

Figure 1 presents a photography of the RF applicator. It includes two radiating elements, RF1 and RF2, located on the top of a Printed Circuit Board (PCB). The biological microplate may then be placed on top of the PCB, whereas the back side of the board is used to connect the applicator to the RF generation chain. Metallic vias through the PCB are also employed to interconnect the two sides of the PCB.

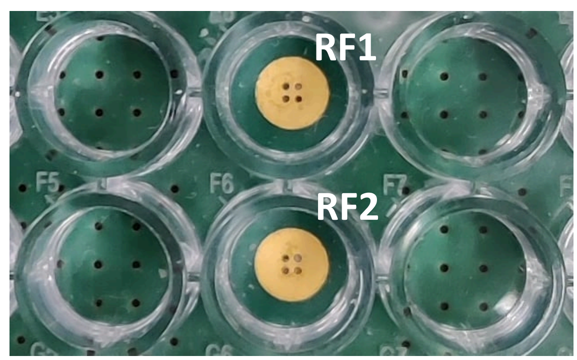

Fig. 1. Photography of the RF applicator developed to radiate cells with a biological 96 well plate placed on top and composed of two radiating elements, RF1 and RF2.

The configuration of illuminating two well plates simultaneously with the same electromagnetic field may appears astonishing at a first sight. Nevertheless, it is defined on purpose. Biological studies request to systematically replicate the experiments due to the intrinsic inhomogeneity of living beings. The integration of two radiating elements powered by one cable consequently permits to apply the same electromagnetic field to two wells without needing to replicate RF cables on the back side. This configuration is presented in Figure 2 a and b, which indicates the biological microplate placed on top of a PCB, a soldered SMA connector and an RF cable.

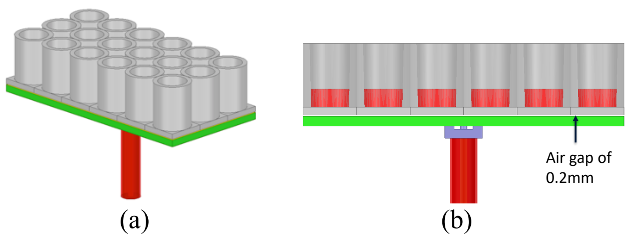

Fig. 2. Schematics of the RF applicator board (green) with the microwell plate placed above (grey): (a) Tilted version and (b) Cross section. Back side RF cable is in red, whereas the RF connector in purple.

Figure 3 presents the dimensions of the applicator with both top and bottom views. The radiating elements exhibit a coplanar configuration. The metallization is indicated in yellow, whereas the capacitive slots are presented in green respectively. Vias are present in the middle of the signal patch to interconnect both sides of the PCB. The purple strip on the back side is used to convey the electromagnetic field to the two radiating elements. The ground is specifically designed to allow the soldering of an SMA connector.

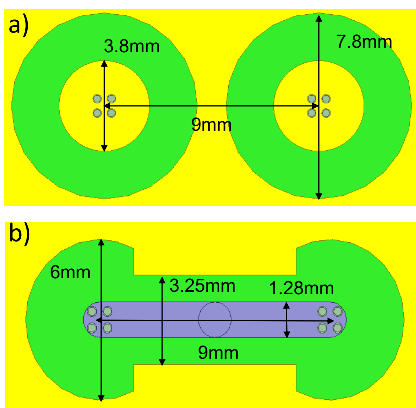

Fig. 3. Schematics and dimensions of the RF applicator, which radiates electromagnetic waves into 2 wells: (a). Top view (b). Bottom view.

The dimensions are defined to illuminate the well, which presents an internal diameter of 6.35 mm, with a center to center distance of 9 mm.

The two next sections are dedicated to the dosimetry study of the applicator with electromagnetic simulation and experiments respectively.

III. Electromagnetic Simulation of the Applicator at 225 MHz

An electromagnetic simulation is conducted with the finite element software named HFSS® to evaluate the dosimetry characteristics of the applicator. The corresponding results are indicated in Figure 4, which presents the Specific Absorption Rate (SAR) distribution within the two illuminated wells, as well as in surrounding ones. The illuminated wells are easily recognizable with the red color in their center, whereas the non-illuminated ones exhibit a blue color. One may notice the symmetry versus both x and y axis of the applicator and wells configuration. The electromagnetic study may consequently be simplified by evaluating SAR values in specific locations, mentioned as a, b and c in Fig. 4.

A SAR value of 15 W/kg is obtained in the center of the two radiating elements (location a in Fig. 4), whilst an incident power of 1W is applied. At a distance of 2 mm from this center (location b in Fig. 4), the SAR presents a lower simulated value (9.7 W/kg). This is however not an issue as cells beyond this limit will not be considered in biological experiments during the RF effects investigations.

One may also notice that the surrounding wells, which are not directly exposed to an electromagnetic field, present nevertheless a light blue color of SAR in the vicinity of the RF illuminated wells. This is due to parasitic electromagnetic fields, with SAR values a decade lower than the maximal one in the radiating elements (see location c in Fig. 4). All these SAR values have been defined while wells are filled with a culture medium (Dulbecco's Modified Eagles Medium – DMEM in our case) and at a height of 10 µm from the bottom of the plastic well. This height corresponds to the average value for the size of the cells used later for RF impact evaluation.

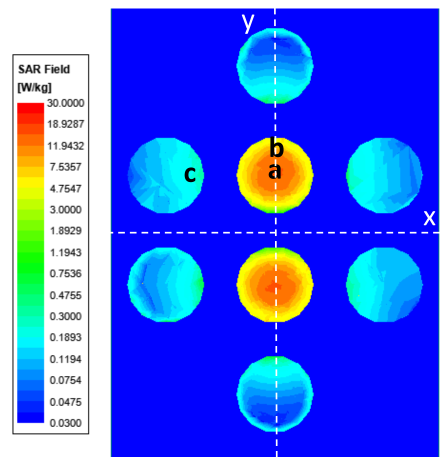

Fig. 4. Simulated SAR distribution in the wells placed above the RF applicator and their nearest neighbors.

To complete this study, the experimental dosimetry is also conducted and presented in the next section.

IV. Experimental Dosimetry of the RF Applicator

A. Method applied to extract the SAR value and the thermal increment

The experimental protocol applied to evaluate the SAR value and the thermal increment within the device at 225 MHz is based on temperature measurements, as mentioned in [7] (cf. pp. 67, eq. (3)). Wells are filled with culture medium (DMEM), whereas a strong incident power is applied on the RF applicator to obtain a measurable thermal increment. A fiber optic temperature sensor permits to get the temperature versus time at the bottom of the well. Based on the corresponding curve, the SAR value may be directly extracted from the slope, which starts once RF power is ON. Further details on the experimental test setup, temperature measurements and SAR extraction are similar to those in [8]. For the experiments, the RF applicator and the temperature probe are both placed in an incubator at 37°C.

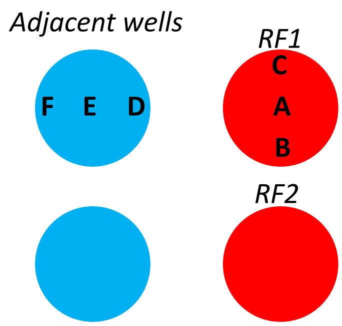

Fig. 5. Location of temperature kinetic measurements for SAR evaluation.

In order to perform the dosimetry study of the structure, the characterization method mentioned above is performed at different locations above the RF applicator and in some surrounding wells. These positions are summarized in Fig. 5. The two red circles symbolize the RF applicator, whereas the two blue circles are surrounding wells. Three different locations on RF1 are identified: A at the center, B and C at a distance of 2 mm from A, with B toward the radiating element RF2 and C toward the opposite direction respectively. As far as the adjacent and non-illuminated wells are concerned, three positions are tested, similarly as with RF1, and referenced as E in the middle, D and F for the two others. Due to the symmetries of the structure, only the points indicated above are measured. Each point is measured with three independent experiments.

B. *Experimental results*

Table 1 gives a summary of the measured SAR values and thermal increments with an incident power of 1W in continuous wave mode, at 225 MHz. The initial temperature is 37°C.

Table 1. Measured dosimetry characteristics of the RF applicator for an incident power of 1W at 225 MHz.

Location of measurements	SAR Value (W/kg)	ΔT (°C)
Point A above RF1 element	16.9 ± 0.9	0.7 ± 0
Point B above RF1 element	16.9 ± 1.03 (100% / point A)	0.7 ± 0
Point C above RF1 element	15.9 ± 1.3 (94% /point A)	0.67 ± 0.06
Point D in the adjacent well	3.3 ± 0.3	0.32 ± 0.03
Point E in the adjacent well	2.4 ± 0.3	0.3 ± 0.02
Point F in the adjacent well	1.95 ± 0.2	0.28 ± 0.03

Above the center of the RF1 element, an average SAR value of 16.9 W/kg is measured with a standard deviation of 0.9 W/kg, whereas the temperature increment is of 0.7°C. These values are also reached in point B at 2 mm from A on RF1. On the opposite side of RF1, in point C, the SAR value is decreased by 1 W/kg, leading to a global SAR homogeneity of 94% in a 4 mm disk diameter in the RF1 well.

V. Application of the RF Applicator to Human Living Cells

To validate the developed applicator regarding its interest for EM effect evaluation on living cells, this one is applied to biological human cells and associated to a biological post-analysis, which evaluates the homeostasis of cells [9]. This technique, called LUCS (for Light Up Cell System) is relevant of any perturbation of the cellular equilibrium and is based on a fluorescence detection realized directly with cells placed in standard 96-well microplates. The detection is only performed within a 4 mm diameter disk at the bottom of the wells, due to the high SAR homogeneity in this location. Three independent experiments have been performed on the human liver HepG2 cell line culture in DMEM medium. Fig. 6 presents the results of one representative experiment for the two conditions: with or without (negative control) RF application. Due to the parasitic fields in the nearest neighbor wells as indicated in section IV.B, negative controlled wells are chosen several columns further on the microplate.

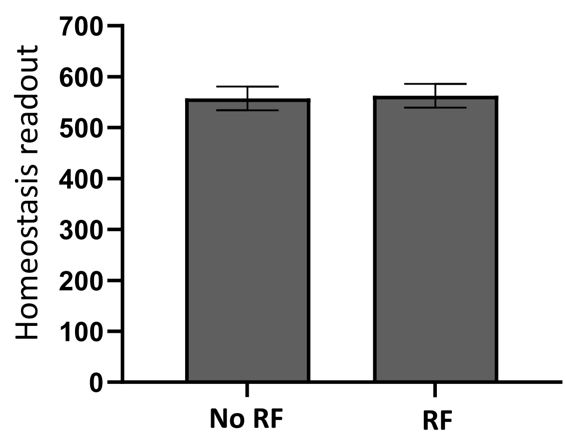

Fig. 6. LUCS homeostasis assay readout obtained for HepG2 cells submitted or not to an electromagnetic illumination at 225 MHz, with a SAR of 16.9 W/kg, for 24 hours.

No significative impact for this specific RF scenario applied to HepG2 cells is obtained for all the experiments.

VI. Conclusions

A near-field RF applicator specifically designed to radiate a controlled and calibrated electromagnetic field on cultured cells, which are located in standard biological microplates, is introduced. Its dosimetry study is performed at 225 MHz, leading to a homogeneous SAR value on a large surface at the bottom of the microplates' wells. This applicator has then been applied to illuminate biological cells with a SAR value of 16.9 W/kg. No significant effect on cells was detected. But the key issue of this experiment was to demonstrate the power of allying specific RF applicators with standard biological consumables, i.e microplates. The bio-post-analysis are extremely facilitated due to the use of standard bio-microplates, which are compatible with all the traditional and routine bio-equipment.

Acknowledgment

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