SSCF-Hyperthermia Study in MCF-7 Spheroids – In Silicio

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Abstract. Electromagnetic hyperthermia is an alternative treatment for cancer that has been carried out as an adjuvant to other cancer treatments such as chemotherapy and radiotherapy; this study shows the interaction between a cell culture of three dimensional MCF-7 spheroids (Michigan Cancer Foundation-7) inside a culture media and the electromagnetic radiation generated by a set of two applicator antennas. This study was performed by using an electromagnetic simulation model. The cell culture was modeled by using its conductivity and permittivity parameters and was placed in a 24-cell plate. The distribution of the electric field was analyzed in both, the culture media and the 3D spheroid culture MCF-7 when they were illuminated by the two applicators in different scenarios regarding the input power of the applicator and the distance between the cell culture and the applicator. Temperature behavior was analyzed from the electric field. Moreover, standardization of spheroids using microplates coated with Ultra-Low Attachment (ULA) surface for 3D cell culture (Corning® Costar®) through two methods, with and without the Geltrex™ LDEV matrixes is presented. The preliminary results indicate that the electric field is significantly more focused over the 3D spheroids in a scenario when the applicators are located 4 cm apart from the cell culture and are excited with a power of 5 W, in this scenario configuration the effect of hyperthermia will be successfully obtained for the 3D spheroids.

Keywords: 3D spheroid culture MCF-7 · Electromagnetic hyperthermia · Applicators

1 Introduction

Cancer is a disease characterized by the uncontrolled growth and spread of abnormal cells. About one in six deaths is caused by cancer [1]. In 2020, it was estimated 19 292 789 new cases of cancer. In these new cases 11.7% correspond to breast cancer. In both men and women, breast cancer causes 6.9% of deaths worldwide [2]. Immunotherapy, stem cell transplantation, radiotherapy, chemotherapy, targeted medicine therapies

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and surgery (lumpectomy, mastectomy, and axillary lymph node dissection) are standard treatments used to tackle breast cancer [3]. Along with the treatments mentioned above, electromagnetic hyperthermia is another treatment that is being implemented. Hyperthermia treatment involves direct electromagnetic radiation over the tumor area to increase its temperature to 39–45 °C, where tumor cell death by apoptosis is induced [4]. The main challenge in the hyperthermia treatment is to effectively increase the tumor temperature while the surrounding tissues temperature as little as possible [5]; the electromagnetic hyperthermia combined with other standard treatments is an alternative with a promising prognosis [6]. Currently, hyperthermia is under study in human patients and in human breast cancer cell lines [7].

To obtain a cell culture, first, the cells of a plant or an animal are withdrawn. Second, withdrawn cells are placed in a favorable artificial environment for their growth. Cells are withdrawn from the tissue directly, alternatively, they can be unbundled by mechanical or enzymatic means before culturing or they can stem from a cell line that has already been established [8]. In [9], it is presented a categorization of 84 breast cancer cell lines, this characterization has been carried out according to three receptors (ER, Estrogen Receptor, PR, Progesterone Receptor, HER2, Human Epithelial Receptor 2). Among the 84 cell lines presented in [9], it is of particular interest the human breast adenocarcinoma cell line MCF-7 (Michigan Cancer Foundation-7), this cell line has been used for more than 40 years as a standard model in cancer research in vitro [10] and it is one of the most used as a model for the investigation of processes that impact patient care [11].

On the other hand, in conventional monolayer cultures it has been observed that the cytotoxic effects obtained are not similar to those obtained in vivo [12].

The 3D growth of cell lines is considered a model closer to the tumor in vivo to perform drug selection or assess antitumor treatments [13] because cell-cell interactions are generated in this system [14], there is a production and extracellular matrix deposition [15]. 3D models of tumor spheroids are used in the experimental evaluation and screening of conventional chemotherapeutics. These chemotherapeutics are used in the treatment of lung cancer, breast cancer, head and neck cancer, and ovarian cancer [16]. Janati [17] has reported a thermally enhanced dose with radiofrequency combined with hyperthermia and external mega-voltage X-rays in spheroids in the DU145 prostate cancer cell line.

Hyperthermia as evidenced in different studies cause direct damage to cancer cells and sensitize them to other treatment modalities (such as chemotherapy and radiotherapy) [18], in these cases hyperthermia is an adjuvant treatment [4].

Verification of a positive response to cancer treatment is carried out through cell death. Cell death is the “irreversible degeneration” of vital cell functions (especially ATP (adenosine triphosphate) production and preservation of redox homeostasis) that culminates in loss of cell integrity (permanent permeabilization of the plasma membrane or cell fragmentation) [19].

Hyperthermia on cell lines is carried out by using electroporation ECM 830 (microsecond pulsed electric field), an incubator, an UHR-2000 microwave beam thermo device, A RF-capacitive system (Celsius TCS), Iron oxide magnetic nanoparticles, X-ray, laser, photothermal technique.

In this paper, the distribution of the electric field (simulated) in the cell culture (3D spheroid culture MCF-7 (Michigan Cancer Foundation-7) and culture media) was analyzed. The cell culture was in a 24-cell plate (Fig. 2a). Moreover, temperature behavior was analyzed from the electric field. The cell culture was illuminated with two applicators at 2.45 GHz. The applicators were located at 2 cm and 4 cm over cell culture and were excited with a power of 1W, 5W and 10 W. The applicators were developed in SIW (substrate integrated waveguide) technology. Furthermore, standardization of spheroids using microplates coated with Ultra-Low Attachment (ULA) surface for 3D cell culture (Corning® Costar®) through two methods, with and without the Geltrex™ LDEV matrixes is presented.

2 Methodology

The electromagnetic simulation was carried out using the finite element method (FEM) solver of ANSYS Electronics®. Cell culture was modeled by 3D spheroid culture MCF-7 and culture media. The simulation setup is presented in the Table 1.

Table 1. HFSS configuration setup

Properties	Values
Maximum number of passes	10
Maximum delta S	0.01
Maximum converged passes	3
Order of basis function	Mixed order

The maximum delta S values and maximum converged passes values were chosen to improve the mesh accuracy and the convergence. Mixed order basis function was chosen because 3D spheroid culture MCF-7 and culture media have different electrical properties (conductivity (σ) and permittivity (ε)). Mixed order assigns a lower order (base function elements) where the fields are weaker, and a higher order (base function elements) where more precision is required.

Table 2 display the electrical properties of 3D spheroid culture MCF-7 and culture media implemented in the electromagnetic simulation based on the properties presented in [20] for a frequency of 2.45 GHz.

Table 2. Electrical properties of 3D spheroid culture MCF-7 and culture medium

	Relative permittivity	Conductivity
3D spheroid culture MCF-7	23	1.2
Culture media	72.81	2.7

3 Applicator

The cell culture was illuminated with two equal applicators at 2.45 GHz developed in SIW technology, Fig. 1 shows the applicator.

This applicator is conformed of 3 parts. First, a cavity designed by using the analysis equations of waveguides and rectangular cavities [21]. TE_{101} mode is the cavity resonance mode. Second, a transition from microstrip to SIW cavity as described in [22] is used in the cavity's top wall. A 50-microstrip line is coupled to the coplanar waveguide (CPW) cavity access. Third, a meandered slot antenna printed on the cavity's bottom wall [23], meandered slot antenna is equivalent to a magnetic dipole antenna of $\lambda/2$. It is designed to operate at $f_0 = 2.45$ GHz.

The impedance of meandered slot antenna is obtained by using the expressions of Janaswamy and Schaubert (low permittivity ε_r) [24], the slot was designed according to [25].

This applicator is one of the applicator prototypes which are being developed for a hyperthermia system for the treatment of breast cancer. It has linear polarization and frequency selectivity due to the cavity.

Applicators were developed in the substrate Rogers TMM 6 (tm), this substrate has a permittivity (ε) of 6.3 and an electrical strength (dielectric strength) of 14.6 MV/m.

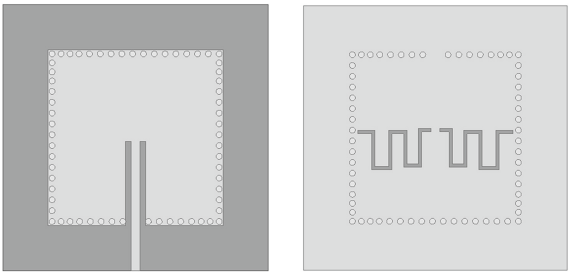


Fig. 1. SIW technology applicator at 2.45 GHz. (a) Top view. (b) Bottom view

4 Results

The cell culture was illuminated with two applicators at 2.45 GHz (Fig. 2a) for three different conditions, namely, with input power values of 1, 5 and 10 W. The applicators were located at 2 cm and 4 cm over the cell culture (Fig. 2a). 3D spheroid (0.5 cm diameter) culture is located in the center of the culture media, this location is due to cell culture feature (Fig. 2b), both 3D spheroids culture and culture media are located inside a 24-well plate (Fig. 2a).

The distribution of the electric field in both culture media and 3D spheroids culture was obtained on the 4 rows (Fig. 2a) of the 24-well plate.

The distribution of the electric field in both culture media and 3D spheroids culture located on row 1 (Fig. 2a) is presented in Fig. 3. The applicators were located at 2 cm

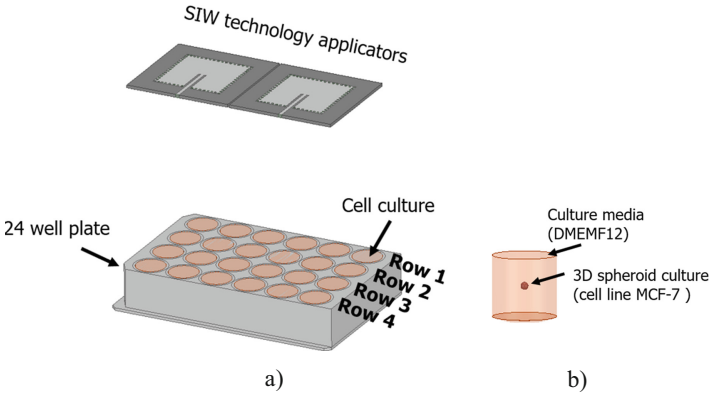


Fig. 2. 3D model. (a) Applicators located over cell culture. (b) Cell culture

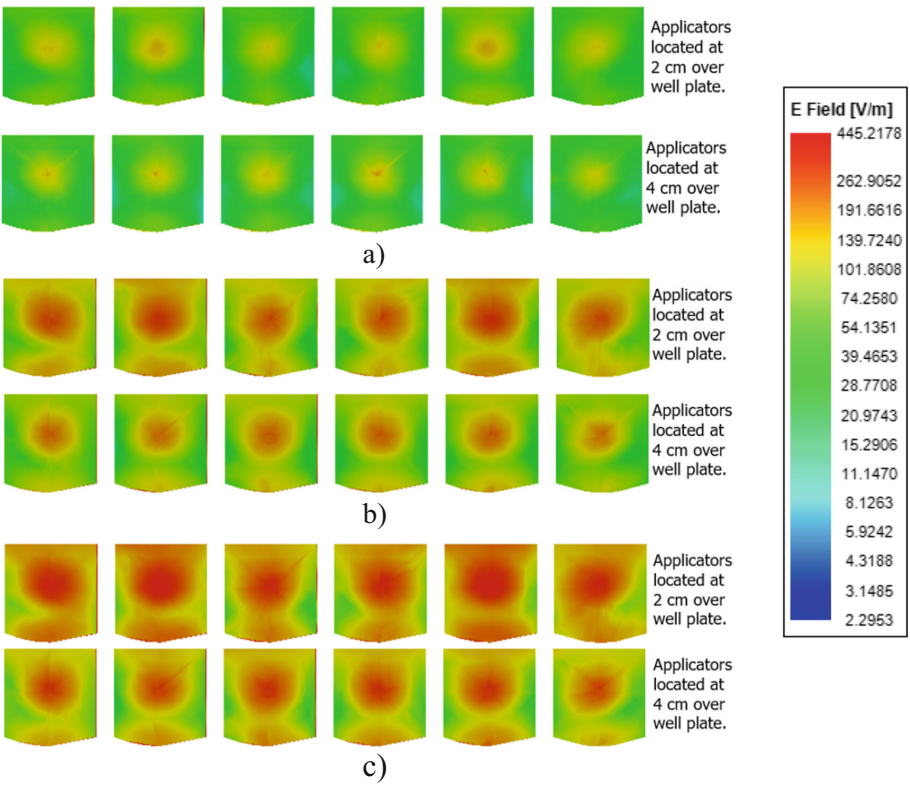


Fig. 3. Distribution of the electric field in both culture media and 3D spheroids culture located on row 1 (Fig. 2a), (a) the applicators were excited with a power of 1 W, (b) the applicators were excited with a power of 5 W, (c) the applicators were excited with a power of 10 W.

and 4 cm over the cell culture (Fig. 3a–c). The applicators were excited with a power of 1 W (Fig. 3a), 5 W (Fig. 3b) and 10 W (Fig. 3c).

Figure 3c shows that the electric field was absorbed by both, the culture media and the 3D spheroids culture when the applicators were located at 2 cm and 4 cm over the cell culture and were excited with a power of 10 W. This result is undesirable because the increase in temperature will be obtained in both culture media and 3D spheroids culture.

In Fig. 3a, it is observed that the electric field was poorly absorbed by the culture media and the 3D spheroids culture when the applicators were located at 4 cm over the cell culture and were excited with a power of 1 W.

In Fig. 3b, it is depicted how the electric field was significantly more absorbed by the 3D spheroids while the culture media interacted weakly with the electromagnetic radiation with the applicators located at 4 cm over the cell culture and excited with a power of 5 W, this result is important because in this scenario the temperature will be focused in the 3D spheroids, and therefore the effect of hyperthermia will be obtained.

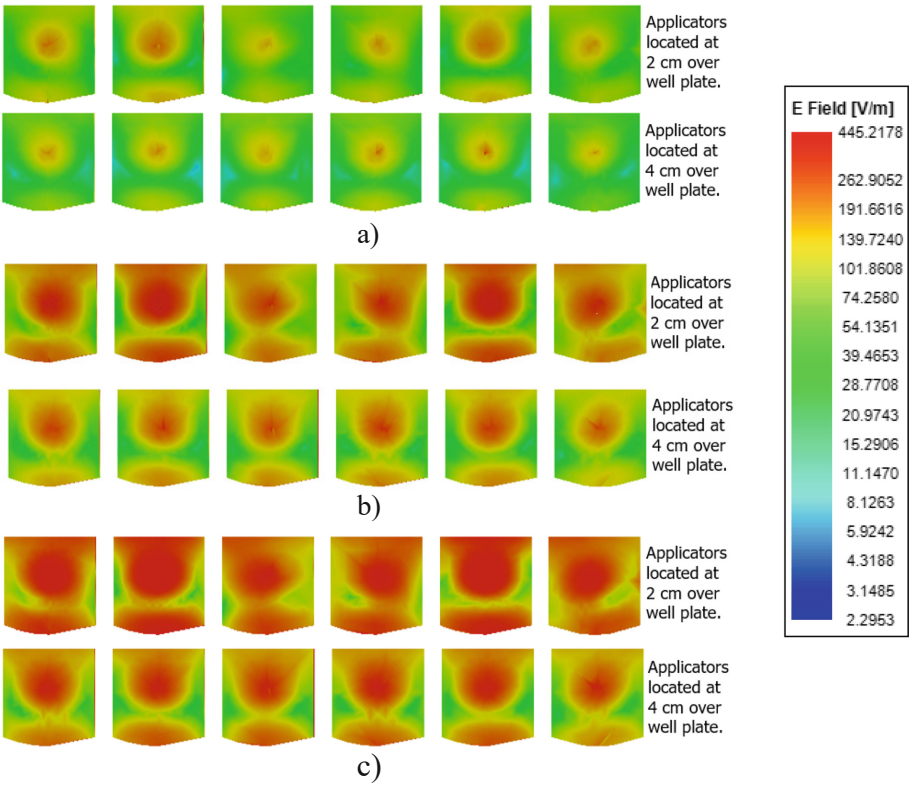


Fig. 4. Distribution of the electric field in both culture media located and 3D spheroids culture located on row 2 (Fig. 2a), (a) the applicators were excited with a power of 1 W, (b) the applicators were excited with a power of 5 W, (c) the applicators were excited with a power of 10 W.

The distribution of the electric field in both culture media and 3D spheroids located on row 2 (Fig. 2a) is presented in Fig. 4. The applicators were located at 2 cm and 4 cm over the cell culture (Fig. 4a–c). The applicators were excited with a power of 1 W (Fig. 4a), 5 W (Fig. 4b) and 10 W (Fig. 4c).

Figure 4a shows that the electric field was absorbed weakly by both, the culture media and 3D spheroids when the applicators were 2 cm and 4 cm apart from the cell culture and were excited with a power of 1 W, however, the magnitude of the electric field was significantly more focused in the 3D spheroids than in the culture media, this result is interesting because the temperature increment will be focused in the 3D spheroids, therefore achieving the desired hyperthermia effect.

In Fig. 4c, it is observed that the electric field was significantly absorbed by both culture media and 3D spheroids when the applicators were located at 2 cm and 4 cm over the cell culture and were excited with a power of 10 W. This is an undesirable result because the increase in temperature will be obtained in both media.

In Fig. 4b, it is depicted how the electric field was significantly more absorbed by the 3D spheroids while the magnitude of the electric field was weak in the culture media. In

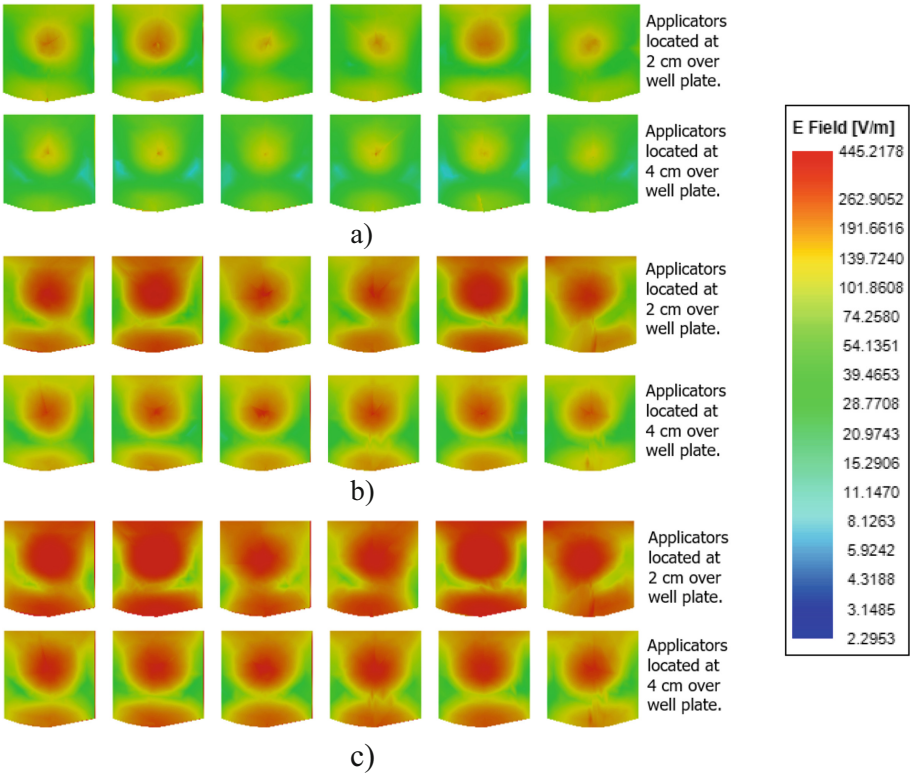


Fig. 5. Distribution of the electric field in both culture media and 3D spheroids culture located on row 3 (Fig. 2a), (a) the applicators were excited with a power of 1 W, (b) the applicators were excited with a power of 5 W, (c) the applicators were excited with a power of 10 W.

this scenario the applicators were 4 cm apart from the cell culture and were excited with a power of 5 W, this is an interesting scenario that results in a temperature increment of the 3D spheroids only.

Generally, the magnitude of the electric field is more intense in both, the culture media and the 3D spheroids located on row 2 compared to row 1 because row 2 is located below the center of the applicators ("main radiation zone" of the applicator).

The distribution of the electric field in both culture media and 3D spheroids located on row 3 (Fig. 2a) is presented in Fig. 5. The applicators were located at 2 cm and 4 cm over the cell culture (Fig. 5a–c). The applicators were excited with a power of 1 W (Fig. 5a), 5 W (Fig. 5b) and 10 W (Fig. 5c).

In Fig. 5, it is displayed that the distribution of the electric field on row 3 in both culture media and 3D spheroids is similar to that observed in row 2 (Fig. 4). This behavior is due to the fact that row 2 and row 3 are localized below the center of the applicators ("main radiation zone" of the applicator).

The distribution of the electric field in both culture media and 3D spheroids located on row 4 (Fig. 2a) is presented in Fig. 6. The applicators were located at 2 cm and 4 cm

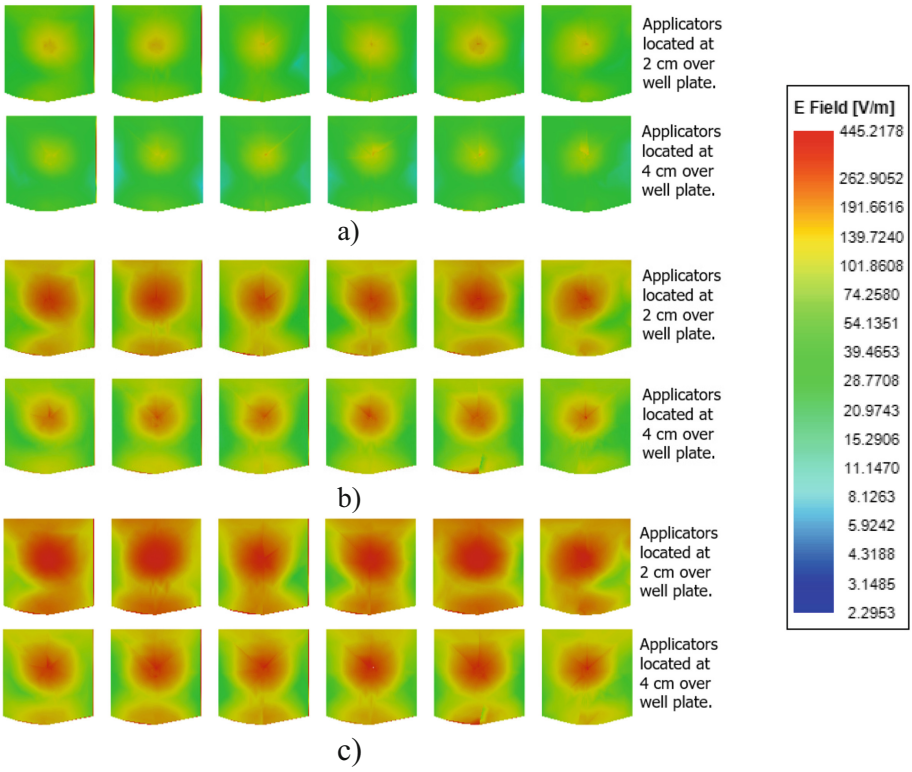


Fig. 6. Distribution of the electric field in both culture media and 3D spheroids culture located on row 4 (Fig. 2a), (a) the applicators were excited with a power of 1 W, (b) the applicators were excited with a power of 5 W, (c) the applicators were excited with a power of 10 W.

over the cell culture (Fig. 6a–c). The applicators were excited with a power of 1 W (Fig. 6a), 5 W (Fig. 6b) and 10 W (Fig. 6c).

In Fig. 6, it is depicted how the distribution of the electric field on row 3 in both culture medium and 3D spheroids is similar to that observed in row 1 (Fig. 3). This behavior is due to the fact that row 2 and row 3 are localized below the center of the applicators ("main radiation zone" of the applicator).

5 Cell Culture Conditions

The results presented in Sect. 4 were obtained through simulation of the interaction between the cell culture and the electromagnetic waves radiated by the antenna applicator, all the simulations were performed for 3D spheroids centered inside a culture media on a 24-well plate. This section describes the culture conditions in which the 3D spheroids of the MCF-7 line are being currently developed.

5.1 Culture of MCF-7 Cell Line

Currently, cell culture standardization is being carried out, that is, human breast epithelial adenocarcinoma cells MCF-7 (ATCC) were seeded as monolayer culture, cells were maintained in Dulbecco's modified Eagle's medium F12 (DMEM F12) supplemented with 10% fetal bovine serum (FBS-Gibco), and 1% antibiotics penicillin/streptomycin/amphotericin b (Lonza) at 37 °C in a humidified atmosphere with 5% CO_2.

5.2 MCF-7 Cell Line-Derived Spheroids

Spheroids were obtained using Microplates coated with Ultra-Low Attachment (ULA) surface for 3D cell culture (Corning® Costar®) through two methods, with (Fig. 7a) and without (Fig. 7b) the Geltrex™ LDEV matrix. MCF-7 cells were seeded at a density of 1.000, 2.000 5.000, and 10.000 cells/well in ULA 96-well plates. Spheroids were cultured in DMEM F12 (Dulbecco's Modified Eagle's Medium/Nutrient Mixture F-12) supplemented with 10% fetal bovine serum (FBS-Gibco) and 1% antibiotics penicillin/streptomycin/amphotericin b (Lonza). Spheroids were incubated in a humidified atmosphere and 5% CO_2 at 37 °C. After 4 day spheroid formation was determined under an inverted microscope.

5.3 Morphological Parameter Estimation

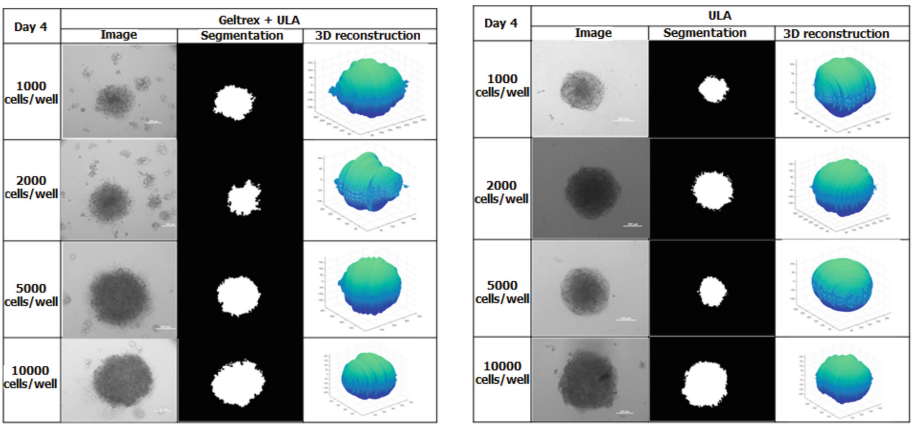
Spheroid formation was assessed through a light microscope Zeiss Imager.M2BX connected to a digital camera CCD monochromatic AxioCam HRm. Morphology parameters (diameter, solidity, convexity, sphericity, and volume) were assessed with AnaSP image analysis software. Tridimensional reconstruction with ReVisp software was made. Spheroids with more sphericity and larger diameter will be chosen to be used in this study.

Morphology parameters (diameter: It is calculated with the diameter of the circle that has the same area as the cross-sectional area of the analyzed spheroid; solidity; It is related to the cohesion of the cells that make up the spheroid about its density; convexity: the index allows the integrity of the spheroid to be determined before treatment; and volume: calculated as the number of voxels) will be obtained by image analysis. The software converts images gray levels to make the segmentation of the spheroids and finally achieve a binary mask that allows extracting the morphological parameters through equations. It is important to highlight that these parameters will be evaluated only in in vitro conditions. Because they are treatment effect predictors, any decrease of sphericity may be due to loss of spheroid integrity. It can be related to losing cell-cell or cell-matrix adhesion. Additionally, all parameters correlate with other assays.

The magnitude of the diameter of the spheroid has been used to carry out the electromagnetic simulation, the diameter of 0.5 cm has been used because with it the best cohesion of the cells was obtained. Likewise, the value of the permittivity and the conductivity of the spheroid has been considered to carry out the electromagnetic simulation.

5.4 Evaluation of Cell Culture Formation

Spheroid formation was evaluated with a light microscope (10×). The results showed that spheroids with morphological characteristics more spherical were obtained using the method without the Geltrex™ LDEV matrix (Fig. 7b). Three-dimensional cell culture models like spheroids; allow accurately mimic treatment responses in vivo. Because of this, we proposed to generate and standardize a breast cancer 3D crop to know how hyperthermia affects cell viability, morphological and structural organization of the tumor cells. We found that a better method to MCF-7 spheroids is using ULA only, without any matrix.



a) b)

Fig. 7. Spheroids of MCF-7 cultured (a) with Geltrex and ULA, segmentation, and their three-dimensional reconstruction. (b) Cultured with ULA only, segmentation and their three-dimensional reconstruction.

Figure 7(a) shows that the most representative images of the spheroids seeded at three cells numbers (1000–2000–5000 and 10000). Scale bar 200 μM. Note that density color schematically shows the location of the voxels within the three-dimensional figure.

Figure 7(b) shows that the most representative images of the spheroids seeded at three cells numbers (1000–2000–5000 and 10000). Scale bar 200 μM. Note that density color schematically shows the location of the voxels within the three-dimensional figure.

6 Conclusions

This paper presented the simulated distribution of the electric field in both 3D sphe-roid culture MCF-7 and culture media when they were illuminated by a set of two applicators with input power values of1 W, 5 W and 10 W. Located at a distance of 2 cm and 4 cm from the cell culture. The results indicate that the magnitude of the electric field was greatly focused in the 3D spheroids MCF-7 when the applicators were located at 4 cm over the cell culture and were excited with a power of 5 W, in this case, the best effect of electromagnetic hyperthermia in the 3D spheroids MCF-7 was obtained.

Spheroids with the best spherical morphological characteristics were obtained using Ultra-Low Attachment (ULA) only. The three-dimensional reconstruction shows spheroids as more homogeneous and circular.

Currently, a study of the distribution of the electric field (simulated) in the cell culture when the applicators are located at other distances (different at 2 cm and 4 cm) is being carried out. Furthermore, different applicators are being used.

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