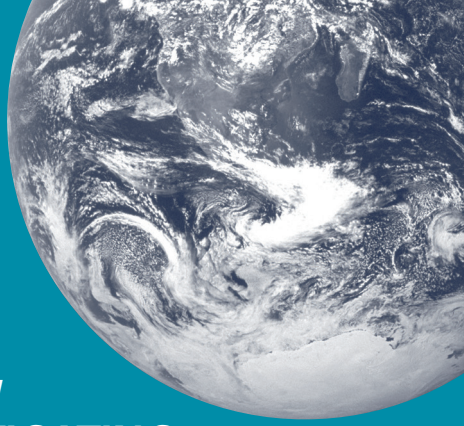
World Cancer Research Journal WCRJ 2017; 4 (1): e837



INVESTIGATING DIFFERENCES IN POLARIZED ELECTRICAL POTENTIAL OF CANCEROUS CELLS, SPECIFICALLY BREAST CANCER SPECIES: INVESTIGATING THE POTENTIAL OF A NOVEL THERAPEUTIC METHODOLOGY

S. GAMALENDIRA[1], A.W. ANSON[2]

[1]College of Engineering, Design and Physical Sciences, Brunel University, London, UK
[2]Experimental Techniques Centre, Brunel University, London, UK

Abstract – *Purpose:* *To demonstrate that human breast cancer cells can be attracted to an electrically polarized conductive surface with a matching voltage.*

Material and Methods: *Rotational experimental equipment setup with 18 centrifuge tubes (15 ml) each with an inserted titanium electrode plate fitted in each centrifuge tube. All 18 centrifuge tubes were loaded with breast cancer cells (MCF-7) suspended in Dulbecco's modified Eagle's medium with GlutaMAX™.*

Out of eighteen centrifuge tubes, eight centrifuge tubes/electrodes were connected anodically and the other eight tubes connected cathodically: the remaining two plates had no electrical connections and acted as an experimental control. The experimental setup was placed in an incubator at 37°C. Electrical power was turned on to supply the cathode's (59 mV ± 0.5 mV), and anode's (57 mV ± 0.2 mV). The rotating assembly, powered by small DC motor, completed two rotations per minute: this was intended to offset the effects of gravity (to the cells) and to impart agitation for the cells. The experiment was conducted for 3 hours. After that, electrical connections, remaining cells and buffer solution were removed and the anode and cathode carefully disassembled for both optical and scanning electron microscopy analysis.

Results: *Titanium electrodes with negative polarization had more adherent cells compared to the positively charged plate; breast cancer cells morphology also investigated while observing each electrode specimen. It was apparent that most of the cells migrated towards the negative polarity and they habituated on the plate with evidence of good adherency. Furthermore, most of the cells migrated as clusters rather a single cell unit.*

Conclusions: *The breast cancer cells have a higher capacity of depolarization and, when the MCF-7 cells are at a weakly metastatic stage, they exhibit migration towards the cathode.*

KEYWORDS: *Breast cancer, Electrical polarization, Cell migration, MCF-7.*

INTRODUCTION

Cancer is the most common cause of death in virtually all territories around the world. There were 7.6 million of people deaths because of cancer, it has been revealed that further 84 million people may die within next decade because of cancer[1].

Breast cancer is one of the most common cancers in the United Kingdom, and breast cancer incident rate is increasing from its current 10,000 deaths per year according to the data[2].

The genetic-based knowledge of cancer research is intensely growing over the past couple of decades. Cancer develops due to the series of genetic changes to cells[3]. Therefore; biochemical and physical properties of human tissues have been of interest for several reasons, especially in the development of cancer treatment[4].

This study investigated the electrical polarization properties of human breast cancer cells and experiments were conducted to examine the potential of applying electrotaxis techniques as a

Corresponding Author: Gamalendira Shivapatham, MD; e-mail: sgamal07@gmail.com

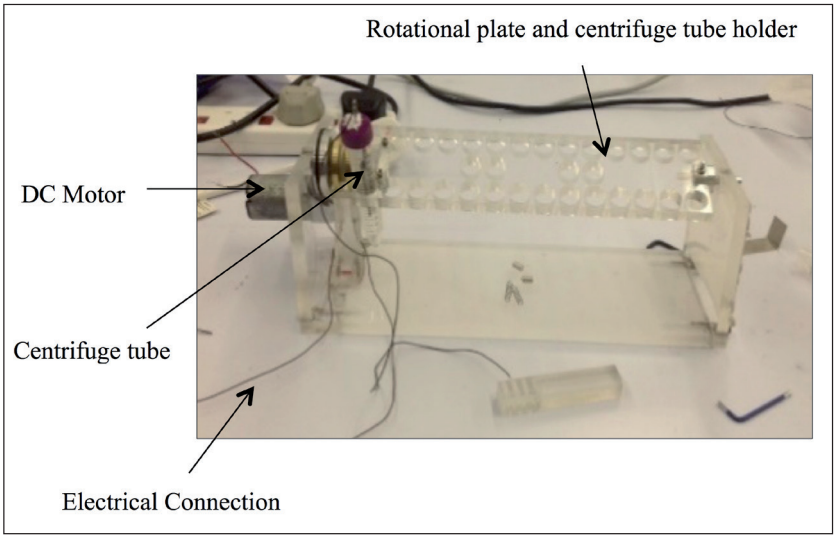


Fig. 1. Main experimental unit.

means to apply future diagnostic or therapeutic methods in the treatment of cancers. There are significant differences of electrical properties between the cancerous and non-cancerous cells because of the changes in cancerous cells, especially due to increased cellular water and salt content[5].

However, the action potential of each class of tissue is unique, for example, tissue such as liver, cardiac and kidney[6]. Liver cells have around have a potential difference across the cells lipid membrane of approximately 60 mV, while the tumour cells in the liver have lower than the normal liver cells, around 15 mV[7]. This specific electrical signature of each class could be used for minimally invasive diagnostic or therapies techniques for cancer.

METHODOLOGY

Rotational experimental equipment (Figure 1) was designed with 18 centrifuge tubes (15 ml). Each tube had a 98.9% purity titanium electrode inserted, attached and sealed through the centrifuge tube screw cap (Figure 2).

A small tail of the titanium metal was attached to the main electrode by micro resistance welding and the electrical integrity of each titanium assembly checked for consistency, using a micro-Ohmmeter. The same material was used for the tail to avoid any galvanic issues. Each tail was then connected by crimping, with wire for electrical supply. The choice of titanium for the electrodes was due to its low reactivity in the biological environment, evidenced in several publications[8].

The titanium plate inserted through the screw-top cap of the centrifuge tube, afterwards silicon elastomer applied for sealing between titanium plate and cap. A leakage test was conducted to make sure no fluid loss and to maintain sterility.

To confirm this interface space between lid and titanium plate, all the centrifuge tubes were filled with sterile water and placed horizontally (Figure 3) on paper tissue for four days. It was confirmed there was no leakage.

As this experiment is conducted with vertically rotating centrifuge tubes, it is necessary to ensure that electrical connection wires, from the supply to the rotating platform (Figure 4) do not

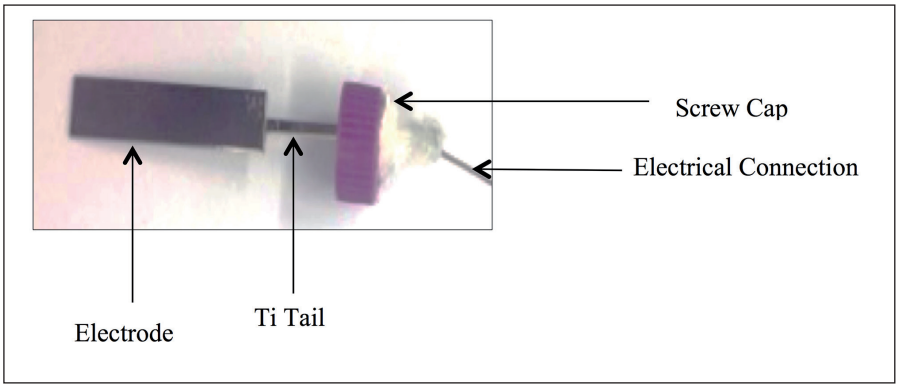


Fig. 2. The centrifuge's lid with inserted titanium plate.

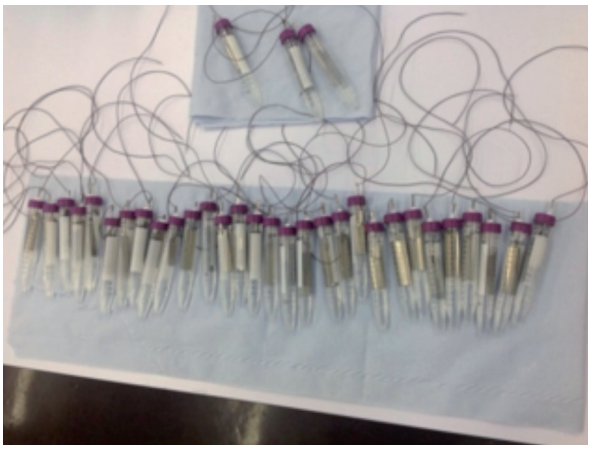


Fig. 3. Titanium plate inserted centrifuge tubes filled with water.

become entangled. This was addressed by incorporating a two-channel slip-ring assembly into the rotating assembly: electrical resistance and supply continuity was verified in ambient conditions and at 37°C.

It was considered that having a slowly rotating experiment tube fitted with electrodes would prevent cells from settling due to gravity while at the same time allowing continuous movement of the cells to facilitate occasional close passes near to the electrode. The attraction force between cell and electrode is weak and the cells have to be in close proximity to show the attraction between the polarized elements (cell-electrode).

For thawing of cells, several steps were taken to remove protective agents to facilitate the best recovery method for the cell cultures. The agent removed as much as quickly and gently as possible. Cells recovered normally within 7 hours after removing the protective agent. Then the contents were transferred into a container with Modified Eagle's medium, on an orbital shaker and agitated

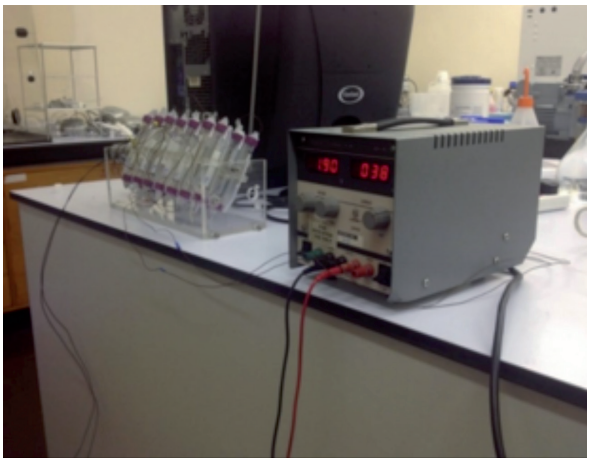


Fig. 4. Main experimental unit connected with DC power supply.

Fig. 5. Main experimental unit inside the incubator.

for 16 hours and incubated normally at 37 degree Celsius which allowed them to warm up.

Before starting the experiment, the cells were observed through an optical microscope and their morphology checked. Afterwards, all 18 centrifuge tubes were loaded with 14 ml media/breast cancer cells (MCF-7) suspended in Dulbecco's modified Eagle's medium with GlutaMAX™. These eighteen centrifuge tubes were placed in the dedicated rotational experiment system (see Figure 1).

From these eighteen centrifuge tubes, there were eight centrifuge tubes connected anodically, other eight tubes connected cathodically. The remaining two plates had no any connections. The centrifuge tubes without electrical connections were considered for control of this study.

The whole experimental setup was placed in a biologically conducive environment (incubator) at 37ºC (Figure 5). Electrical power was turned on to supply the cathode (59 mV ± 0.5 mV), anode (57 mV ± 0.2 mV) and the motor (1.9 V) to rotate the experimental set up during the investigation.

The experiment was run for 180 minutes and monitored closely to ensure all parameters were stable, for example, anode/cathode voltage, temperature, rotational speed. At the end of three hours, all electrical connections were removed carefully, and the system transferred into a biological safety cabinet.

Then each centrifuge tube's cap with fitted titanium electrode was gently unscrewed and media removed by means of a 20 ml syringe fitted with a 5 mm diameter silicone elastomer tube. The collected cells and media were deposited in a container for appropriate disposal.

The titanium electrodes underwent the following steps to preservers and analyse cells attached to each titanium electrode. The process involved series of steps; fixation, dehydration, and drying.

Solution			
	Volume	Concentration	Time
3 ml ethanol	7 ml Distilled water	30% ethanol	10 Minutes
5 ml ethanol	5 ml Distilled water	50% ethanol	10 Minutes
7 ml ethanol	3 ml Distilled water	70% ethanol	10 Minutes
9 ml ethanol	1 ml Distilled water	90% ethanol	10 Minutes
10 ml ethanol	/	100% ethanol	10 Minutes
10 ml ethanol	/	100% ethanol	10 Minutes

TABLE 1. Prepared chart for dehydration process.

A typical laboratory procedure involved preparation of the fixative solution, in this procedure Glutaraldehyde is used. Initially prepared a Phosphate-buffered saline (PBS) at pH 7.2 and placed at room temperature. 25% of 500 ml Glutaraldehyde prepared as 2% (10 ml) of concentration and then mixed with 250 ml of phosphate-buffered saline (PBS).

The titanium plates again were suspended with 2.0% of Glutaraldehyde in buffer for two hours at room temperature on the rotating instrument, each tube filled with 14.5 ml of glutaraldehyde solution.

The dehydration process facilitated removal of the water, and followed a standard laboratory practice of sequential dilution through different, increasing concentrations of ethanol, each for 10 minutes interval as given in Table 1.

Immediately after the dehydration process was completed the tail of the titanium electrode was removed and each titanium plate carefully transferred into new centrifuge tubes, corresponding identification number written on centrifuge tube's wall. Then all the centrifuge tubes were placed inside a freeze drying system for 15 hours to remove residual water.

After the freeze drying process centrifuge tubes were divided into three categories control, negative, polarization and positive polarization. Subsequently, all the categorized titanium electrodes underwent staining to enhance the visualizations of cells. Therefore, the first group of nine, including control titanium electrodes were treated with a Coomassie blue staining solution, applied on both sides of the titanium electrodes then and washed with a buffer solution to remove residual staining solutions from the electrodes and allowed to dry. Afterwards, from each category, the tubes were randomly divided into two groups for analysis; one group for optical microscope and one group for Scanning Electron Microscopic analysis

An Olympus optical microscope (Olympus, Tokyo, Japan) was used to approximate the stained cells resident on the titanium electrodes. A ZEISS Supra 35 VP scanning electron microscope was used in this investigation and four samples examined. Secondary Electron (SE) image technique was used to analyze the specimens, those scanning parameters selection has been considered carefully to protect cells turgidity.

The scanning started with the lowest set of parameters to avoid damage to cells; those parameters are given in Table 2.

Titanium plates were carefully stuck on the specimen holder using conductive carbon tape and the cell's morphology and cell adherence was observed and noted: the scan initiated with 10 kV, 17 Pa vacuum with 22 mm specimen working distance, it was noted those parameters were not enough to have a clear visualization; therefore, all the parameters were changed as given in Table 2.

RESULTS

Optical microscope analysis did not give significant results due to the limitations of the external lighting system and magnification factor.

There were four titanium plates' analyzed under scanning electron microscope (SEM), untreated, control, positive polarization and negative polarization. Observation images were captured mainly divided into three areas from each titanium plate respectively, top of the plate (which is proximal to the lid of the centrifuge tube), middle of the plate and bottom of the plate.

The experimental electrodes were, in addition, investigated to observe topographical features, the surface appearances showed cracking; this is assumed to be naturally occurring surface oxides rather than the underlying titanium metal. It is interesting to note that many of the scanning electron microscope (SEM) images show cells

Voltage	Pressure	Working Distance	Magnification
10 kV	20 Pa	11 mm	X10000

TABLE 2. Scanning electron microscope's parameters.

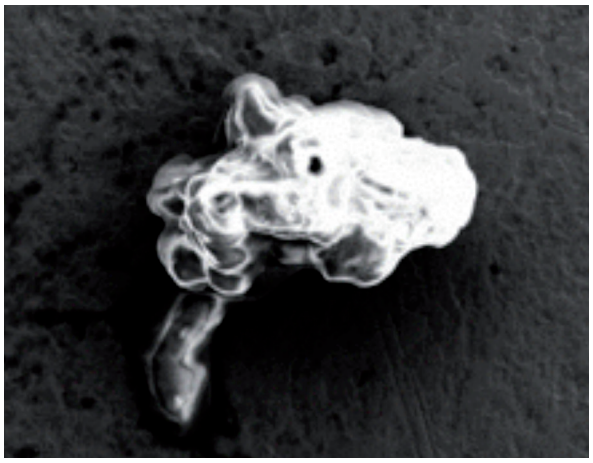


Fig. 6. Control titanium plate under SEM.

clustering in the vicinity of these crack/fissures: the oxide layer is non-conductive and this may give some credence to the suggestion that cells are preferentially attracted to an electrically conductive, oppositely polarized zone. Equally, as shown in Figure 6, cancer cells were observed on a control electrode, which was not connected with electrical supply but may have residual electrical fields surrounding the titanium.

The plate which is connected with positive polarization was investigated under scanning electron microscope (SEM), it shows overlapping crypts and three-dimensional cell sponge appearances. Further, bunch of tails like filaments (Figure 6) and considerable length of membrane appearance (Figure 7) were also observed in few areas. Overall, there were more cells clusters observed, compared to the control.

Finally, the negative polarization plate was investigated. There were so many cell fragments (Figure 8) and varieties of elongated and polygonal shaped cells were observed during the investigation, and histological appearances were poorly

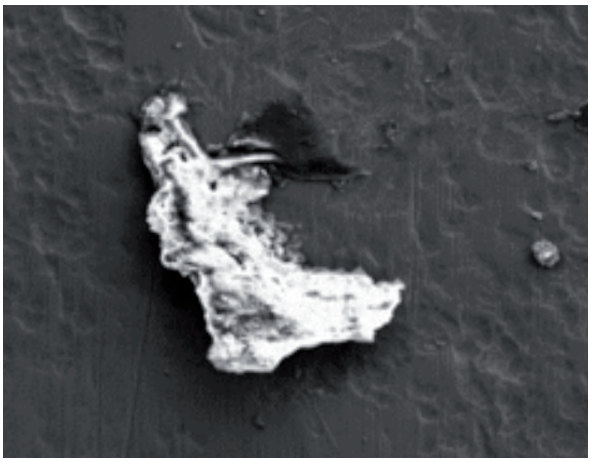


Fig. 7. Bottom of the titanium plate, positive connection.

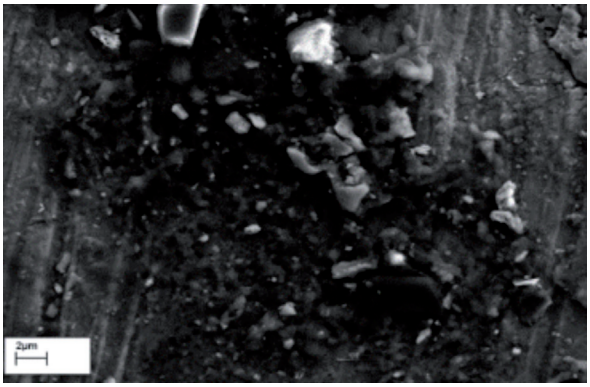


Fig. 8. Top of the titanium plate, negative connection.

differentiated. Further, an apical surface of filaments noticed and tortuous and direct lumen-like structures were also observed (Figure 9).

Overall, it seems most of the cells migrated towards the negative polarity, compared to the positive or control and they happily habituated on the plate with evidence of filament spread. Almost all of the cells adhered in a cluster form and more spreading in an elongated way.

DISCUSSION

As titanium electrodes are hydrophobic which would restrict cell migration through the titanium plate and adherence on their surface, the consequence, is insignificant numbers of cells were to be seen resident on the surface of the control samples in this study[9]. The titanium plates were not smooth, and that also can be a reason for cell adhesions ratio to be high in control[10].

Human breast cancer cells have a higher ability to migrate than the other types of cancer cells, especially those MDA-MB-231 and MCF-7 demonstrated as highest[11].

The result appeared as small groups of two or many cells crowded on the titanium plate. Normally, MCF-7 displays these kinds of the pattern

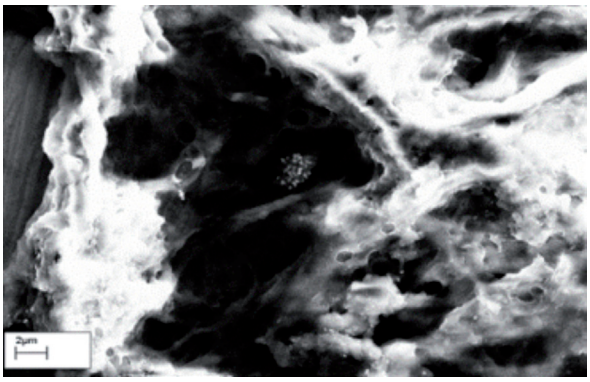


Fig. 9. Middle of the titanium plate, negative connection.

during their migration[12]. Most of the published data show almost all the cancer cells exhibit negative polarizations and its trend to moves towards the anode[13].

This result was explained by Nuccitelli in 2003[14]. When cancer cells exposed to a small electric field there could be three chances; some of them move towards the cathode, some of them move towards the anode and others show no effects.

Further, an interesting result was found in Djamgoz et al's investigation in 2001[15]. The investigation explained the migrations towards both sides; anode and cathode. Migration ability evaluated for the human breast cancer cells; MDA-MB-231 and MCF-7. MDA-MB-231 showed strong and rapid migration towards the anode whereas weakly metastatic MCF-7 cells moved slowly and unexpectedly towards the cathode. Therefore most acceptable concept behind our investigation is the cells that we used were at the weak stage of metastatic MCF-7 as we found more cells migrated towards cathode in our study. However, it should also be considered that cells depolarize (or equally, re-polarize) depending upon their activity and metabolic status, this will determine the migration towards either the anode or the cathode.

CONCLUSIONS

The mechanisms by which cancer cells migrate towards electrodes are complex because there are many factors that influence membrane potential voltage, which plays an important role in cancer development. Gaining a better understanding of electrical parameters, particularly polarization, may give insight for new therapies in oncology for low invasivity for cancer treatment. This could be facilitated by a catheter-based treatment having an array of electrically charged electrodes, able to differentiate cells by carefully selected voltage matching (cell specific voltage) with suitable polarization.

The experimental result has shown that cancer cells can migrate towards the anode or cathode and some of them had no response under applied electric field. Further, it was found that significant numbers of cells attached on to the cathode, in this investigation.

Therefore, from the experiment result it can be concluded that the breast cancer cells have a higher capacity for depolarization and when the MCF-7 cells are at the stage of weakly metastatic they exhibit migration towards the cathode.

Acknowledgements

This study was supported by Brunel University London, United Kingdom.

Conflict of Interests

There is no conflict of interests

REFERENCES

1. World Health Organization. Cancer control: knowledge into action: WHO guide for effective programs. World Health Organization; 2007.
2. Osborn G. Management of breast cancer: basic principles. Surgery (Oxford) 2013; 31: 27-31.
3. Nass SJ, Henderson IC, Lashof JC, editors. Mammography and beyond: developing technologies for the early detection of breast cancer. National Academies Press; 2001 Aug 23.
4. Fricke H, Morse S. The electric capacity of tumors of the breast. J Cancer Res 1926; 10: 340-376.
5. Zou Y, Guo Z. A review of electrical impedance techniques for breast cancer detection. Med Engin Phys 2003; 31; 25: 79-90.
6. Northover, B. J. Electrical Properties of Mammalian Tissues. 1st ed. London: Chapman & Hall, 1992.
7. Persinger MA, Lafrenie RM. The cancer cell plasma membrane potentials as energetic equivalents to astrophysical properties. International Letters of Chemistry, Physics and Astronomy 2014; 17: 67.
8. Tengvall P, Lundström I. Physico-chemical considerations of titanium as a biomaterial. Clin Mater 1992; 9: 115-134.
9. Ko TJ, Kim E, Nagashima S, Oh KH, Lee KR, Kim S, Moon MW. Adhesion behavior of mouse liver cancer cells on nanostructured superhydrophobic and superhydrophilic surfaces. Soft Matter 2013; 9: 8705-8711.
10. Chen W, Weng S, Zhang F, Allen S, Li X, Bao L, Lam RH, Macoska JA, Merajver SD, Fu J. Nanoroughened surfaces for efficient capture of circulating tumor cells without using capture antibodies. ACS Nano 2012; 7: 566-575.
11. Stylli SS, Kaye AH, Lock P. Invadopodia: at the cutting edge of tumour invasion. J Clin Neurosci 2008; 31; 15: 725-737.
12. Bozzuto G, Condello M, Molinari A. Migratory behaviour of tumour cells: a scanning electron microscopy study. Annali dell'Istituto Superiore di Sanità 2015; 51: 139-147.
13. Fraser SP, Diss JK, Chioni AM, Mycielska ME, Pan H, Yamaci RF, Pani F, Siwy Z, Krasowska M, Grzywna Z, Brackenbury WJ, Theodorou D, Koyutürk M, Kaya H, Battaloglu E, De Bella MT, Slade MJ, Tolhurst R, Palmieri C, Jiang J, Latchman DS, Coombes RC, Djamgoz MB. Voltage-gated sodium channel expression and potentiation of human breast cancer metastasis. Clin Cancer Res 2005; 11: 5381-5389.
14. Nuccitelli R. A role for endogenous electric fields in wound healing. Curr Top Dev Biol 2003; 58: 1-26.
15. Djamgoz MB, Mycielska M, Madeja Z, Fraser SP, Korohoda W. Directional movement of rat prostate cancer cells in direct-current electric field. J Cell Sci 2001; 114: 2697-26705.