Original Article

Finite Element Analysis Of Electric Field For In-Vitro Electropermeabilization

In-Vitro Elektropermabilizasyon İçin Elektrik Alanın Sonlu Elemanlar Analizi

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Abstract

Background: The aim of this study was to investigate the electric field distribution inside the cell solution for different electric pulse amplitudes.

Materials and Methods: Breast cancer cells were loaded into a BTX 640 model cuvette with parallel aluminum plate electrodes and the cuvette were placed in the electroporation chamber which was connected to electroporator. Eight square pulses of duration 100µs (having repetition frequency of 1Hz) with 400V and 800V were applied to the electrodes. Since electroporation involves electrostatic properties, its analysis is performed using Ansys-Maxwell 3D Electrostatic transient module. In this direction, it is different from other studies in the literature. Firstly, the capacitance of the cuvette is calculated analytically by the parallel plate capacitor approach. Secondly; the capacitance value is calculated numerically by the software. The accuracy of the model is tested with analytical and numerical analysis. Thirdly, the electric field (E) distribution inside the cell solution was examined.

Results: The capacitance (C) was calculated as 0.5461125pF by using the Formula. C value in the numerical analysis was found to be 0.54387pF. The electric field distribution was found around 1998.7-2001,3V/cm. If E is too low, the potential value for electroporation can not be reached. Increasing E at the corners of the cell solution is an expected result. Because electrical charges accumulate at corner points. While the applied voltage is 400V, E value on the solution is around $5.66\times10^{4}-8.13\times10^{4}$ V/m. Thus, the membrane potential is calculated at about 0.56-0.81V. While the applied voltage is 800V, E is around $1.035\times10^{5}-1.82\times10^{5}$ V/m on the solution. Thus, the membrane potential is calculated at about 1.03 - 1.82 V.

Conclusion: The capacitance value error ratio between analytical and numerical analysis was 0.047%. It is expected that the actual model will be compatible with the model in the simulation. As the amount of the cell solution increased, a linear increase in the capacitance value was observed. For this reason, the charging time for electroporation of the cells is affected. In analyzes performed with solution, when 400V is applied, the permeability of the cells in the electric field values (1000 V/cm) is low. However, increasing the voltage value from 400V to 800V could significantly increase the permeability of the cells.

Key Words: Electropermeabilization, Electroporation, Electric Transient, In-Vitro, Finite Element Analysis.

Öz

Amaç: Farklı elektrik puls değerliklerinde hücre solüsyonundaki elektrik alan değişimlerinin incelenmesi amaçlanmıştır.

Materyal ve Metod: Meme kanseri hücreleri, paralel alüminyum plak elektrotlu 4 milimetre (mm) boşluklu elektroporasyon küvetlerine yerleştirildi. Küvetler, elektroporasyon cihazına bağlanan elektroporasyon odasına alındı. Hücrelere, 0-800 V aralığında genlik ile 8 karesel titreşim (tekrarlama frekansı 1 Hz'lik) uygulanmıştır. Her bir elektrik darbesinin süresi 100

µs idi. Elektroporasyon işlemi elektrostatik özellikler içerdiğinden dolayı Ansys Maxwell 3D Elektrostatik ve Elektrostatik transient modülü kullanılarak analiz işlemleri gerçekleştirilmiştir. Bu özelliğiyle literatürde yeralan diğer çalışmalardan farklıdır. İlk olarak, küvet içinde bulunan elektrotlar paralel plakalı kondansatör yaklaşımı ile kapasitans değeri analitik olarak hesaplanmıştır. İkinci olarak, yazılım ile kapasitans değeri nümerik olarak hesaplatılmıştır. Analitik ve nümerik analiz ile modelin doğruluğu test edilmiştir. Üçüncü olarak, hücre solüsyonundaki elektrik alan değişimi incelenmiştir.

Bulgular: Kapasitans (C) formül kullanılarak; 0.5461125 pF olarak hesaplanmıştır. Nümerik analizi neticesinde kapasitans değeri 0.54387 pF olarak bulunmuştur. Elektrik alan değişimi 1998.7-2001,3 V/cm aralığında görülmüştür. Eğer elektrik alan şiddeti çok düşükse, hücrelerde por için gerekli potansiyel değerine (0.7-1V) ulaşılamaz. Solüsyonun köşe kısımlarında elektrik alan değerinin artması beklenen bir sonuçtur. Çünkü yükler köşe noktlarında birikir. 400V uygulandığında, solüsyondaki E değeri $5.66\times10^{4}-8.13\times10^{4}$ V/m civarındadır. Böylece membran potansiyeli 0.56-0.81 V'tur. 800V uygulandığında ise, E değeri $1.035\times10^{5}-1.82\times10^{5}$ V/m civarındadır. Böylece membran potansiyeli 1.03-1.82 V'tur.

Sonuç: Analitik ve nümerik analiz arasındaki kapasitans değeri hata oranı %0.047 olarak görülmüştür. Benzetim çalışmasındaki model ile uygulamadaki gerçek model uyuşması beklenmektedir. Solüsyon miktarı arttıça kapasitans doğrusal olarak değişmektedir. 400V uygulandığında yada 1000 V/cm elektrik alan değerlerinde solüsyondaki hücrelerin geçirgenliği azdır. Ancak gerilim 800V değerine yükseltildiğinde hücrelerin geçirgenliğinde önemli bir artış beklenmektedir.

Anahtar Kelimeler: Elektropermabilizasyon, Elektroporasyon, Elektrik Geçici Hal Analizi, In Vitro, Sonlu Elemanlar Analizi

INTRODUCTION

'Electropermeabilization' or 'Electroporation'; a phenomenon that is defined by an additional voltage component along the cell membrane through exposure of the cell to an external electric field (1). Electroporation in terms of electrical properties; applied in the form of short pulses (5KHz or 1Hz) of high voltage value (2). The permeability of the cell membrane is significantly increased and nano-sized pores are formed.These electropores enable the transport of deoxyribonucleic acid (DNA), ribonucleic acid (RNA), proteins, fluorescent markers, peptides, polar and charged molecules, plasmids and cancer drugs into the interior of the cell. Electroporation may be reversible or irreversible depending on pulse parameters such as; amplitude, duration time, number of pulses, repitation frequency, polarity and etc. The most important electroporation parameters to be to be optimized are amplitude of electrical pulses. For instance, at low amplitude voltage values, the cell permeability level is not affected, but DNA transfer is facilitated (2). This method has been used in the last decade for in vitro application of genetic material to the cell (3). This approach was first applied in vitro. But it retained its validity in vivo (4). Theoretical and practical studies; the level of cellular granular permeability has been shown to depend on the electric field, cell size, shape and interaction with the surrounding cells. Some factors related to cell permeability are, for example, cellular tiny particles, ambient conductance, cell position, critical transmembrane

voltage (TMP) affect effective tissue conductivity. In addition, the local electric field intensity at a site is the key parameter for permeability (2). The electroporation process is carried out by means of an electropulsor or a capacitor discharge. The electrodes, which consist of two rectangular, flat and stainless steel materials, are arranged in parallel to form a regular electric field. Electric field parameters can be adjusted by controlling the square wave (pulse) from the electropuls source (4). Castiello et al. (5) have performed tests of different in vitro models. They argued that the new device had faster and homogenous voltage pulses than standard electropuls sources. In addition, the Electropuls system can be used to treat tumors by taking cancer drugs into cells. This system is known as electrochemotherapy which the effect of anticancer drugs can be increased and the side effects of drugs can be reduced (6). For example, Low dose Cisplatin can be used in the treatment of neuroblastoma with electroporation, but when used in combination with tamoxifen electroporation, it is effective in breast cancer treatment (7,8).

Tumor and healthy tissue can be determined by solving the electric field changes the electrostatic-based programs. The solution of the solution methodology included in some commercially available programs (finite element method, finite difference method, etc.) does not change the basic field rules used for the solution. However, the electrical conditions required for the application require the same characteristics of the simulated conditions. In short, the electroporation system should be considered as a transient electrostatic problem because of the electrostatic problems in the field of electrical parameters and pulse source. Sreeet al. (9) has shown that the electric field distribution of ductal carcinoma is 11.5% higher than that of normal touch in Maxwell 3D simulation program. Different geometries have been created for a number of new large needle array configurations required for electroporation of larger areas for alternative cancer treatments (1,10-11). Miklavčič et al. (12) have studied the change of electric field with the needle diameters for in vivo application of electroporation.

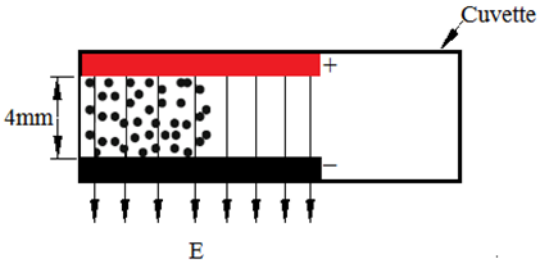

Figure 1. Two-dimensional appearance of DMEM solution with Cuvette

In this study, the electric field change of the BTX 640 model 4 mm cavity cuvette, which is currently produced for in vitro application, was investigated by the finite element method. A train of 8 square pulses (having repetition frequency of 1 Hz) with amplitude ranging from 0 to 800 V were applied to 100 µL DMEM (Dulbecco's Modified Eagle's

Medium) cells placed in the cuvette. The duration of each electric pulse was taken as 100 µs. Since electroporation involves electrostatic properties, its analysis is performed using Ansys-Maxwell 3D Electric transient module. In this direction, it is different from other studies in the literature. Firstly, the capacitance of the cuvette is calculated analytically by the parallel plate capacitor approach. Secondly; the capacitance value is calculated numerically by the software. The accuracy of the model is tested with analytical and numerical analysis. Thirdly, the electric field (E) distribution inside the cell solution was examined.

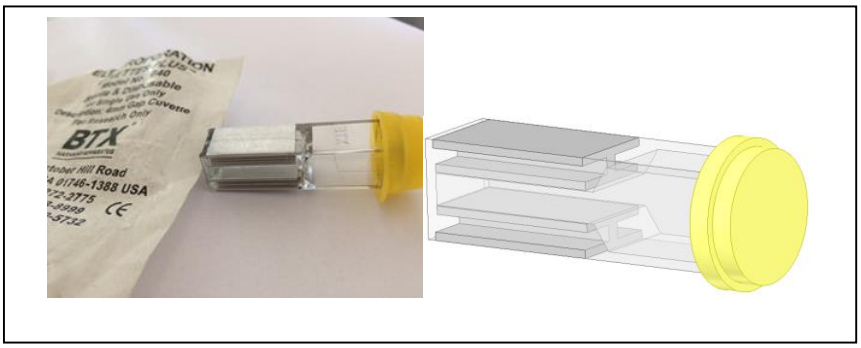

Figure 2. a) Harvard BTX 640 model cuvette, b) Simulation Model

MATERIALS AND METHODS

The size of the field distribution of an electrode system depends on the magnitude of the voltage, the electrode gap. Numerical calculation methods are usually used to find the maximum electric field in mixed electrode systems that require long calculation processes. In Figure 1 a two-dimensional view of the cuvette system is given.

The solution contained in the cuvette is defined as 1.5 S/m and the dielectric constant 80. Due to the conductivity of the solution, the entire surface of the plates is coated with insulating material and is produced hollow.The realization of the solution cuvette and the simulation model are given in Figure 2:

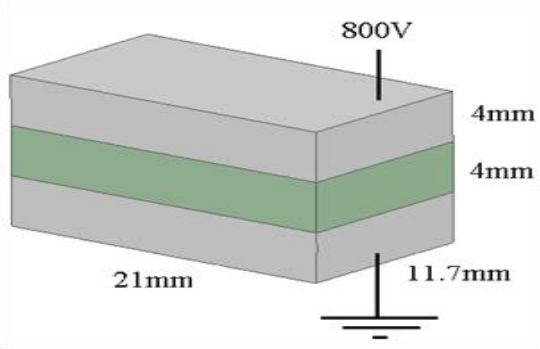

Figure 3. Simulation with parallel plate of Figure 2b

The error rate between analytical and numerical calculation significantly changes the application results. For this reason, calculations like the model parallel plate condenser in Figure 2b have been performed. For this reason, the analysis of the model given in Fig 3.

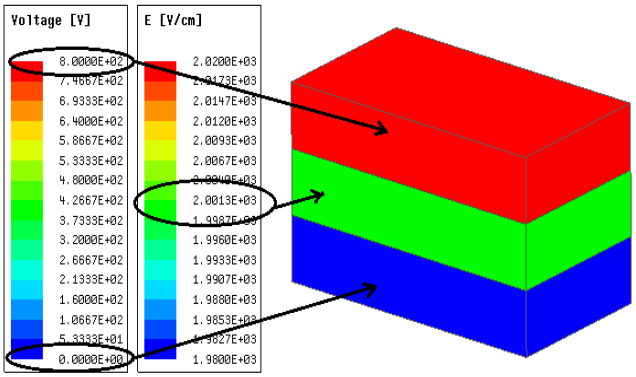

Figure 4. Plate potential and electric field change

It is difficult to investigate on electrical activity of cells, tissues and organs due to measurement and ethical reasons. For this reason, software that uses the finite element method is preferred in literature to investigate the electrical effects. In general, the electrical properties of a biophysical problem can be electromagnetic, electrostatic, or two-domain problems. As the electroporation process contained electrostatic properties, analyzes were performed using the Ansys-Maxwell 3D Electrostatic and Electric transient module.

The electrostatic field simulator computes static electric fields arising from potential differences and charge distributions. The electrostatic solver calculates the value of the electric potential at each tetrahedron node. To produce the optimal mesh, used an adaptive analysis, in which the mesh is automatically refined in critical regions (13). As the Electric transient solver does not provide adaptive meshing, so we will first solve using Electrostatic solver and import its adaptively refined mesh for use in Electric transient solution. However, the two model geometries must be identical. Electrostatic field computes the static electric field that exists in a structure given a distribution of DC voltages and static charges. A capacitance matrix, force may also be computed from the electric field (14). For electrostatic solver (14):

- It is assumed that all objects are stationary,
- There is no time variation of any of the electromagnetic quantities,
- There is no current flow in conductors,
- All conductors are considered to be perfect,

Electric field (E) is automatically calculated from the scalar potential (φ). $\varphi$is represented by the Poisson equation,

$$\nabla^2\varphi = -\rho/\varepsilon_r\varepsilon_o \qquad (1)$$

Electrostatic solver solves for the electric field using the general formulation of fields,

$$E = -\mathrm{V}\varphi \qquad (2)$$

Following the calculation of E, it creates the solution files and performs an error analysis on the solution. Using the adaptive analysis, refining the regions of the mesh in which the largest error exists. Refining the mesh makes it denser in the areas of highest error, continues solving until the stopping criterion is meet, and resulting in a more accurate field solution.

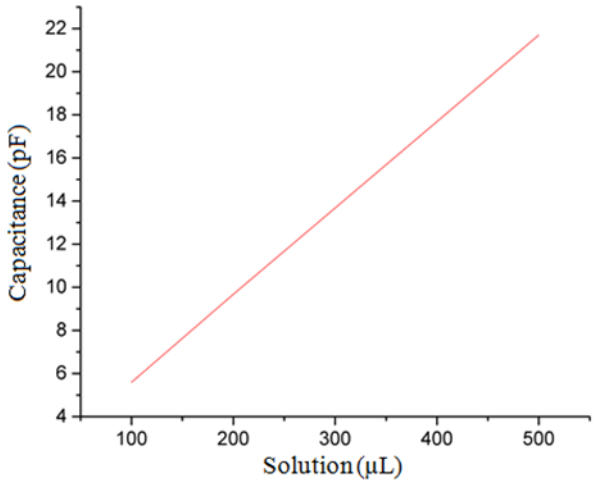

Figure 5. Capacitance change with solution

The Electric transient module can be used to study the field distribution in objects subjected to lightning-induced overvoltage, produced by power transmission line and other applications. The solver uses time-varying applied potentials, total charge and volume charge density, time-varying current excitations. The quantity for which the electric transient field simulator solves is the electric potential; the electric field, the current density and the electric flux density are automatically calculated from the potential. Derived solution parameters such as the electric energy, ohmic loss, surface charge density; maximum and minimum electric field can be obtained for each solid (14).

$$-\nabla \cdot \left(\varepsilon \nabla \frac{\partial \varphi}{\partial t}\right) - \nabla \cdot (\sigma \nabla \varphi) = 0 \qquad (3)$$

The electrostatic model is described by defining the geometry, material properties, voltages, external conditions in the Cartesian coordinate system. Solution process is repeated until energy errorvalue decreases.Unlike an electrostatic solution, an electric transient is drawn to the design data sets of interest voltage graphs. The corresponding voltage value is assigned as pwl (amplitude, time).

For the model in Figure 2b, the fringing of the electric field at the edges in the solution-free cuvette, the electric field is smooth and stable. Equal to $E = \frac{V}{d}$. Where V is the applied DC voltage (Volt), d is the distance between parallel plates (m). The electrostatic energy density is

$$W_e = \frac{1}{2}\int_V \varepsilon E^2 dv \qquad (4)$$

Capacitance expression C (F); Equation 6 is obtained when the plate surface area is S (m^2) and the electric field in Equation 4 is written.

$$W_e = \frac{1}{2}(\varepsilon \frac{S}{d})V^2 \qquad (6)$$

The expression given in parentheses here is expressed as the capacitance of the parallel plate model. It should also be noted that the ε expression is the dielectric product of the material placed between the gap and the plates. In this case, the relationship between the energy stored in a capacitor model and the capacitance is given in Equation 7.

$$W_e = \frac{1}{2}CV^2 \qquad (7)$$

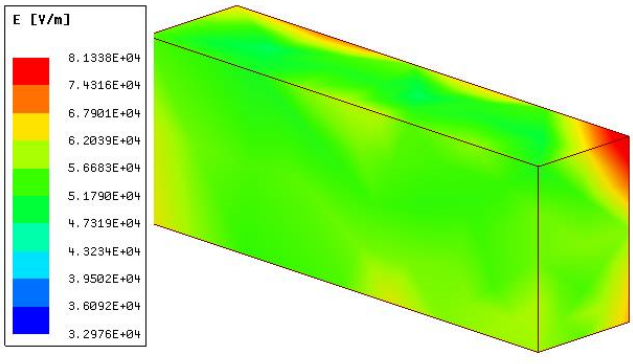

Figure 6. Electric Field Variation in Solution (400V)

$$C = \varepsilon \frac{S}{d} \qquad (5)$$

RESULTS

The change in electric field of the model in Fig. 2b without solute was investigated by three-dimensional analysis. In addition, parallel plate capacitor calculations were carried out and the capacitance was calculated analytically and compared with the three-dimensional model. The surface area of a plates is equal to 0.2457 m^2 .Distance between two plates are 0.004 m and the permittivity of free space is equal to approximately 8.85×10^{-12} (F/m). The capacitance (C) was calculated as 0.5461125 pF by using the Equation 5. The capacitance value in the numerical analysis was found to be 0.54387 pF. The electric field and the potential change are given in the Figure 4.

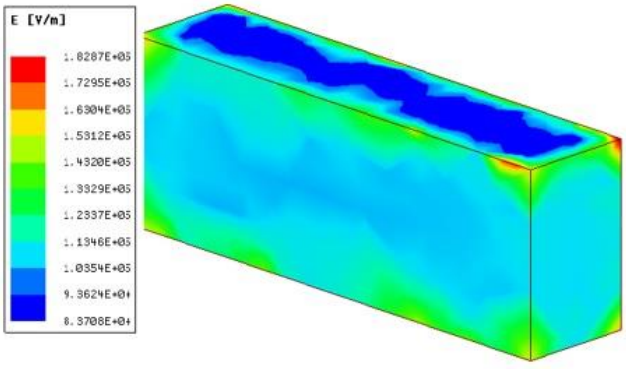

Figure7. Electric Field Variation in Solution (800 V)

The electric field distribution was found around 1998.7-2001.3 V/cm. If the pulse length is too short, the membrane capacitance cannot be charged to the potential value required for the cell (15). For this reason, the capacitance variation with solution is given in Figure 5: If the magnitude of the electric field is too low, the potential value for electroporation can not be reached (15). This potential value is larger for large cells with constant electric field value ($V_{cell} = 1.5rE\cos\theta$) (15). WhereE electric field, r cell radius (10-100 μm), θ is given as the angle between the cell surface and the electric field.When 400V and 800V are applied, the distribution of the electric field in the solution between the plates is given in Figures 6 and 7.

Increasing the value of the electric field at the corners of the cell solution is an expected result. Because electrical charges accumulate at corner points.While the applied voltage is 400 V, E value on the solution is around $5.66\times10^4 - 8.13\times10^4$ V/m. Thus, the membrane potential is calculated at about 0.56-0.81 V. In green areas, the permeability of the cells is very low.

While the applied voltage is 800 V, E is around $1.035\times10^5 - 1.82\times10^5$ V/m on the solution. Thus, the membrane potential is calculated at about 1.03 -1.82 V. The permeability of the cells at green and solution corner points is very low. However, the permeability of the cells in the interior of the solution in general and in the areas near the plate surfaces will be very high. Increasing the voltage value from 400V to 800V has been shown to significantly increase the permeability of the cells.

DISCUSSION

In this study, the electric field distribution of the BTX 640 (4mm cavity cuvette) was investigated by the finite element method. Without adding solution into the cuvette is likened to a parallel plate capacitor model. The capacitance value error ratio between analytical and numerical analysis was 0.047% in the Capacitance. It is expected that the actual model will be compatible with the model in the simulation. As the amount of the cell solution increased, a linear increase in the capacitance value was observed. For this reason, the charging time for electroporation of the cells is affected. In analyzes performed with solution, when 400V is applied, the permeability of the cells in the electric field values (1000 V/cm) is low. However, increasing the voltage value from 400V to 800V could significantly increase the permeability of the cells. In the experimental study given in reference (7,8), it has been shown that cell permeability and electric field value change in a similar manner. Besides, the design is because of

solving step by step in the electric transient module, there is an increase in the analysis time. Importing the mesh geometry from the electrostatic module is crucial in terms of giving correct results.

Declaration of conflicting interests

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References

1.Ongaro A, Campana LG, De Mattei M, Dughiero F, Forzan M, Pellati A, Rossi CR, Sieni E. Evaluation of the Electroporation Efficiency of a Grid Electrode for Electrochemotherapy From Numerical Model to In Vitro Tests. Technology in cancer research &treatment 2015;15(2):296-307.
2.Cukjati D, Batiuskaite D, Slivnik T, Mir LM, Miklavčič D. Sequential finite element model of tissue electropermeabilization. IEEE Transactions on Biomedical Engineering 2005;52(5):816-827.
3.Dev SB, Rabussay DP, Widera G, Hofmann GA. Medical applications of electroporation. IEEE Transactions on Plasma Science 2000;28(1):206-23.
4.Rols MP, Golzio M, Gabriel B, Teissié J. Factors controlling electropermeabilisation of cell membranes. Technology in cancer research & treatment 2002;1(5):319-27.
5.Castiello M, Dughiero F, Scandola F, Sieni E, Campana LG, Rossi CR, De Mattei M, Pellati A, Ongaro A. A new grid electrode for electrochemotherapy treatment of large skin tumors.IEEE Transactions on Dielectrics and Electrical Insulation 2014;21(3):1424-32.
6.Hong Z, Hao Z, Wei H, Zishu W, Qin G, Hong L.An exploration for optimal parameters of electromagnetic impulse on electrochemotherapy (ECT) of tumor.CEEM Proceedings2003;118-121
7.Esmekaya, M. A., Kayhan, H., Coskun, A., &Canseven, A. G.Effects of Cisplatin Electrochemotherapy on Human Neuroblastoma Cells. The Journal of membrane biology 2016;249(5):601-610.
8.Esmekaya, M. A., Kayhan, H., Yagci, M., Coskun, A., &Canseven, A. G. Effects of Electroporation on Tamoxifen Delivery in Estrogen Receptor Positive (ER+) Human Breast Carcinoma Cells. Cell biochemistry and biophysics 2017;75(1):103-109
9.Sree G, Velvizhi VK, Sundararajan R. Electric field distribution of malignant breast tissue under needle electrode configuration. InElectrical Insulation and Dielectric Phenomena (CEIDP) 2012:267-270
10.Campana, L. G., Dughiero, F., Forzan, M., Rossi, C. R., &Sieni, E. A prototype of a flexible grid electrode to treat widespread superficial tumors by meansof Electrochemotherapy.
Radiology and oncology 2016; 50(1): 49-57.
11.Bommakanti S, Agoramurthy P, Campana L, Sundararajan R. A simulation analysis of large multi-electrode needle arrays for efficient electrochemotherapy of cancer tissues.InElectrical Insulation and Dielectric Phenomena (CEIDP) 2011: 187-190
12.Miklavčič, D., Šemrov, D., Mekid, H., & Mir, L. M. A validated model of in vivo electric field distribution in tissues for electrochemotherapy and for DNA electrotransfer for gene therapy. BiochimicaetBiophysicaActa(BBA)-General Subjects 2000; 1523(1): 73-83.
13.Ansys Maxwell v16 Training Manual Lectures 4,5,6.
14.Ansys Maxwell v16 Help File.
15.Jaroszeski J. M., Heller R., Gilbert R. Electrochemptherapy, Electrogenetherapy and Transdermal Drug Delivery, Methods in Molecular Medicine, Humana Press, 2000:1-488