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Feasibility Study for Focusing Electric Fields to Mediate *In vitro* Drug and Gene Delivery

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***Abstract*— Electric field mediated drug and gene delivery is a novel method that uses pulsed electric fields to improve permeability of cell membranes and therefore desired agent uptake by tissues. In this paper, we describe the modeling and experimental proof of concept of a method to direct electric fields to subsequently focus drug or gene uptake at a desired site. The *in vitro* experimental results presented are consistent with simulation models and could be scaled into different *in vivo* applications that can concentrate the effects of electroporation and overcome several problems related to localized effects near the electrodes.**

I. Introduction

The application of electric fields mediates the transport of a desired agent into cells in a target tissue. This physical method, electroporation (EP), is used to deliver therapeutic molecules including genetic material to tissues. The pulsed electric fields act on tissues by enhancing cell permeability and allowing flow of molecules into the intracellular space [1]. EP is a transient phenomenon that enhances membrane permeability either in cell culture (*in vitro*) or in tissues (*in vivo*). EP has been extensively used in drug delivery *in vivo* as a means for improving cell uptake of chemotherapeutic agents in the treatment of solid tumors; its efficacy has also been proven for delivering plasmid DNA *in vivo* [2, 3].

The distribution of the electric fields generated for EP purposes is dependant upon many factors including electrode geometries, tissue electrical characteristics, and electric pulse parameters. A typical electric field distribution used in EP has higher intensity closer to the electrodes; this creates a distribution of electric fields that will result in differential cell permeability[4]. Improved control of this field distribution will increase delivery efficiency.

The impact the electric field has on cells depends on the magnitude of the applied potential between the electrodes. The application of pulses might result in areas with higher electric fields where some cells die due to irreversible permeability, increased Joule heating, or local electrochemical effects, areas with appropriate electric fields where cells have reversible permeability resulting in successful EP, and areas with no EP due to lower electric field. Thus, only electrical parameters within a certain range will generate the desired results for a given electrode geometry and materials.

The ability to focus electric fields should provide a means for delivering the appropriate electric fields to the target site while reducing the localized adverse effects at the electrodes due to higher electric fields at these sites. In this study, we use modeling and simulation to design and then an *in vitro* experiment protocol to test a means of focusing electric fields. Results from this study will be used later for the design of *in vivo* electroporation devices.

II. Materials and Methods

A. In-vitro Electric Field Simulations

Electric field simulations were carried out using a commercially available package from Ansoft Corporation (Maxwell 2-D V.11). This package allowed electrostatic simulations and electric field mapping based on different geometries and applied potentials. The aim of these initial simulations was to find a geometry and material combination in an *in-vitro* environment that could focus the electric field away from the electrodes used to apply the potential.

After examining several models with varied geometries and materials, the setting in Fig. 1 was finally selected for further exploration; this geometry had four concentric

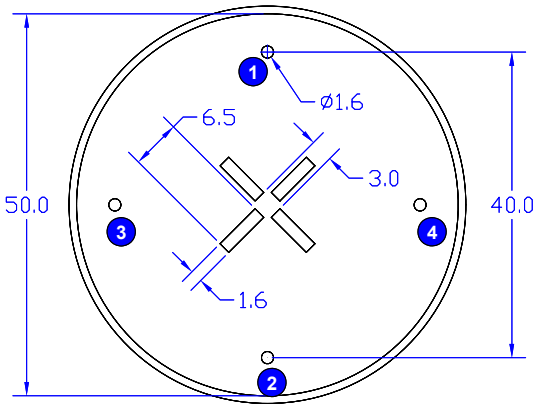

Fig. 1. Electrode configuration; the four electrodes work in pairs (1 with 2, and 3 with 4). The rectangular shapes in the center of the dish are stainless steel plates that are not grounded. All units in millimeters.

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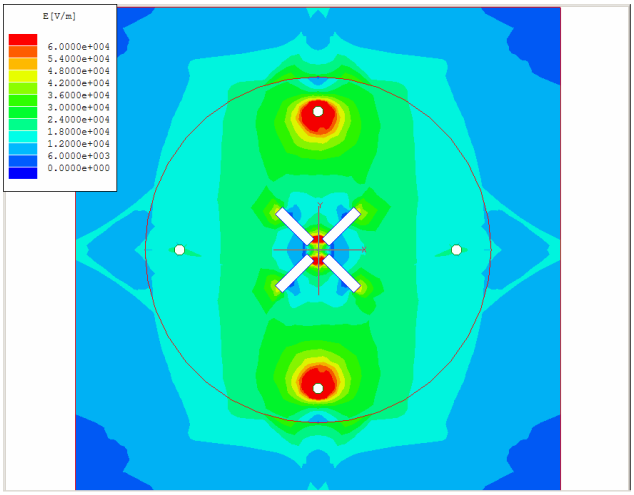

Fig. 2.A.

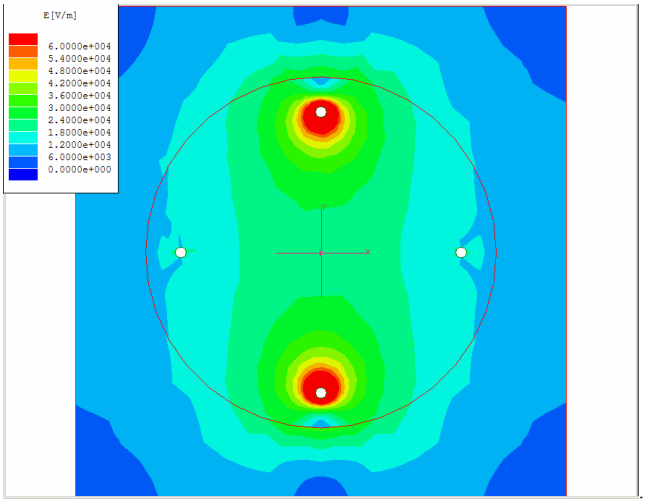

Fig 2.B.

Fig. 2. 2D plots showing electric fields on the surface of the culture dish. Darker blue represents 0 V/m; red represents values above 6e4 V/m or 600 V/cm. Fig. 2A. 2D computer simulation of cell culture dish including non-grounded rectangular elements for focusing electric fields. The non-grounded rectangular elements in the center are located in the center of the dish. Diameter of bottom of the culture dish is 5 cm, the diameter of the electrodes is 0.15 cm (1.6 mm). The separation of electrode and counter-electrode is 4 cm. Maximum electric field shown in red corresponds to the a threshold value of 600 V/cm (6 E 4 V/m). Fig. 2B. 2D computer simulation of cell culture dish. This simulation is representative of the control experiment; all other

TABLE I
ELECTRIC PROPERTIES OF MATERIALS FOR SIMULATIONS

Material	Conductivity Siemens / meter	Relative Permittivity Eps
Stainless Steel	1.1e+006	1
Phosphate Buffer Saline (PBS)	4	81
Air	0	1.0006

stainless steel rectangles in the center and four electrodes that were distributed in a circle with 4 cm of diameter at 45º of inclination with respect to the four non grounded rectangles. All electrodes were modeled as stainless steel probes. The media in the dish including the cells were modeled as phosphate buffer saline (PBS) solution at pH=7.4. The additional space surrounding the culture dish was modeled as air (see Table I for relevant material properties).

An electric potential of 1500 V was simulated across sets of two opposing electrodes by setting the electrode to +750 V and the counter electrode to -750 V (Per Fig. 1, electrode 1 with 2). Electric field intensity maps were obtained using the software package's post processor (figs. 2A, 2B). These simulation results showed higher electric fields in the area that corresponded to the space in between the rectangle geometries (center of the dish) and around the electrodes (Fig. 2A). These magnitudes were plotted and compared as as is shown in Fig. 3.

B. In vitro electroporation

The *in vitro* EP applicator was constructed to deliver the simulation suggested electric fields on the bottom of a 60mm culture dish containing adherent cells, and using the geometry in Fig. 1. The electrodes were constructed from stainless steel; the "passive" elements or non-grounded rectangular plates were stainless steel as well and were held into place using a Teflon™ rod which was not part of the 2D surface in contact with the cells in the culture dish. The electric fields were applied through the electrodes using an Electro square porator ECM 830 (BTX – Harvard Apparatus, Inc., Holliston, MA) pulse power generator.

The culture cells used for these *in vitro* experiments were B16F10 adherent murine melanoma cells (ATCC CRL-

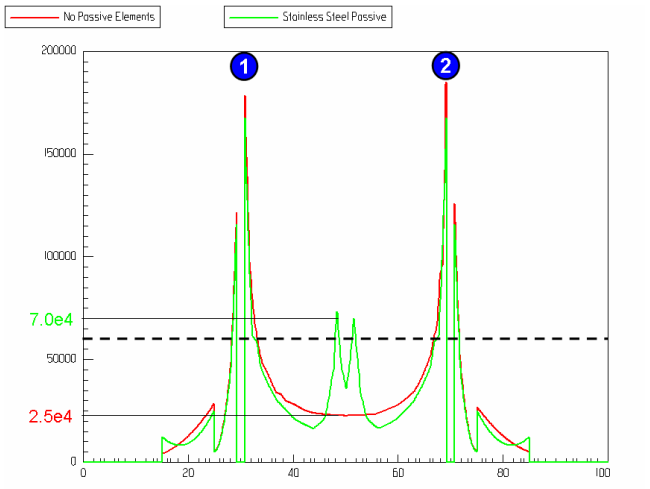

Fig. 3. Plot depicting electric field magnitude (units in graph are V/m) along vertical axis (passing through electrodes 1 and 2); horizontal axis units are mm. The green line represents the case in Fig. 2A, the point at which the non grounded elements are closer together, the electric fields are 700 V/cm as compared to 200 V/cm in the control case (red line – in Fig. 2B). The horizontal dashed line represents the threshold at which successful electroporation was achieved for this case (see next figure).

6475, American Type Culture Collection, Manassas, VA). These cells were incubated using McCoy's 5A medium with 10% fetal calf serum and 90μg/ml of gentamycin in an incubator at 37ºC and 5% CO_2. The cells were detached using trypsin and placed in the 60mm culture dish. The cell count per plate was 4 million B16F10 cells on the surface of the 50mm diameter of the cell culture dish bottom. These cells were cultured for 18 hours to insure cell attachment to the bottom of the culture dish.

Calcein ($C_{30}H_{26}N_2O_1$, Sigma-Aldrich, Inc., St. Louis, MO) was used as a fluorescent tracer molecule. Calcein will effectively enter cells after the cells have been exposed to an electric field. Immediately before exposure to the desired field, the cell culture dish's surface was covered with 2ml of a 120 μM calcein PBS solution.

The applicator electrodes and rectangular geometries were put into contact with the surface of the of culture dish. Apparatus placement protocol dictated that the geometry in Fig. 1 was reproduced at the bottom of the dish where the cells were attached. Four pulses with a potential differential of 1,500 V (equivalent applied electric field strength of 375 V/cm), pulse length of 150 μ seconds and at interval of 500 milliseconds were delivered in the four directions (electrode 1 to 2, 2 to 1, 3 to 4, and 4 to 3; see Fig. 1 for electrode labeling).

After applying the pulsed electric fields and waiting for 30 minutes, the attached cells in the culture dish were rinsed three times using PBS. By this method, only those cells which were successfully electroporated (that is, reversible membrane permeabilization) will show fluorescence. This fluorescence is due to calcein trapped in cells that were permeabilized. Since the permeabilized cell membrane returned to the non permeable state before the expiration of the 30 minute wait period, only the calcein trapped in the cells did not get washed in the PBS rinse cycles.

Control experiments were run using the same experimental protocols. However, in this set of experiments the rectangular non grounded plates at the center of the culture dish were removed. The simulation for this configuration is shown in fig. 2B.

III. Results and Discussions

The control experiments equivalent to the geometries in the control simulation (Fig. 2B) did not exhibit calcein fluorescence in the center of the cell culture dish. These experiments did not use the rectangular elements in the center of the dish and the results showed fluorescence due to EP only at the electrodes. This is an indication that the electric field mediation effect that promotes EP was present but only being concentrated at the electrode locations as indicated in the simulations.

The non-control experiments produced a picture similar to the expectations projected by the computer simulations; that is, the presence of the non-grounded rectangular stainless steel geometries in the center of the dish redistributed the electric fields (Fig. 3). This new field focus produced adequate electric field mediated calcein delivery to demonstrate effective EP at the center of the cell culture dish in the *in vitro* experiments (Fig. 4). This new distribution of electric fields provided a focusing effect at the center of the dish as can also be seen in the simulation result in Fig. 3 where the rectangular elements caused the concentration of electric fields just above the threshold for electroporation (estimated at 600 V/cm for this case, parameters and conditions). Furthermore, for the rectangular elements when compared to the control (Fig. 3), the simulations show that the redistribution of electric fields lowered the electric field magnitude peaks near the electrodes. This will provide the additional bonus of reducing possible field related cell and tissue damage generated by the activated electrodes.

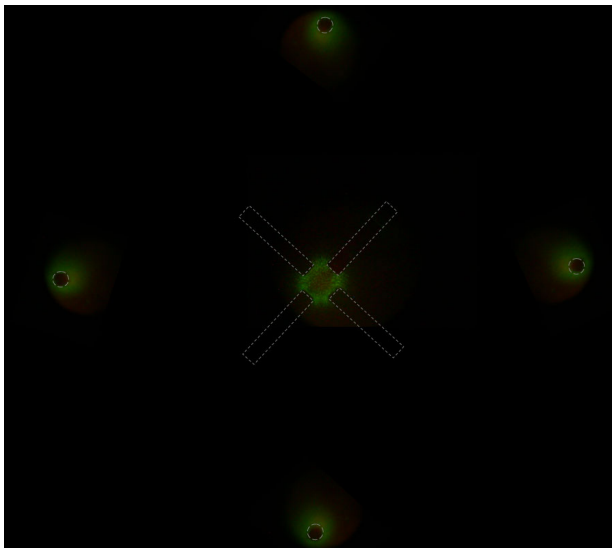

Fig 4. Composite image showing fluorescence (green) due to calcein uptake by cells on culture dish. Pulse electric fields were applied in both X and Y directions. Dotted white lines were added after to show location of electrodes (circles) and non-grounded elements (rectangles). This picture shows fluorescence at the places were the electrostatic simulations show highest electric fields.

IV. Conclusions and Future Work

We conclude that the electric field effective for EP can be distributed using different geometries and materials to guide and concentrate desired molecules into targeted areas or volumes. The use of these geometries and material combinations can effectively change the distribution of electric field intensities in such a way that higher electric fields can be achieved away from the electrodes and closer to desired treatment locations. The possible advantages of

this method include the reduction of problems that are typical at electrically active electrodes which might include Joule heating near the electrode and local electrochemical reactions at the electrode surface such as the release of H^+ or OH^- ions.

Continued research will include the use of different cell lines, the use of more materials that include changes in conductivity and permittivity throughout different geometries. These are all approaches that are being studied by the authors as means of manipulating electric fields for use in culture cells and live tissue applications. Further work will lead to *in vivo* applications to study the impact a focused field has on molecule delivery in tissue and tumors.

V. Acknowledgements

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VI. References

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