INFLUENCE OF ELECTRODE DESIGN ON ELECTRIC FIELD DISTRIBUTION DURING ELECTROPORATION

N. Wilke*, J. O'Brien*, G. Casey**, T. Doody**, D. Soden**, A. Morrissey*
*Tyndall National Institute, Cork, Ireland
** Cork Cancer Research Centre, University College Cork, Ireland

nicolle.wilke@tyndall.ie

Abstract: In this paper we present the design of two kinds of silicon microneedle electrodes for electroporation. Finite Element Modelling (FEM) of the electric field in tumour tissue was carried out. The requirement for electroporation is the creation of pores in the cell membranes to allow the transport of ions through the cells. Impedance measurements were used to verify ion transport through cell membranes. An acupuncture needle device for the measurement of the voltage distribution as value of electric field strength was developed and tested as well. It shows the influence of isolating layers around tissue in terms of electric field strength. Our results demonstrate the significant improvement in the efficiency of electroporation using microneedle electrodes.

Introduction

In this work, we focus on one way of efficiency control during electroporation (EP). EP is the creation of microscopic pathways (pores) in a bio-membrane during short high electric voltage pulses. The pores enable larger molecules such as DNA, chemotherapeutic drugs and other substances to transfer into the interior of the cell. If EP is carried out correctly, the cell membranes will reseal after a certain time, leaving the transferred molecules inside the cell. A major difficulty during the treatment is the prediction of EP efficiency. It is known that a skin layer around the tissue causes a significant voltage drop, resulting in a very low electric field in the tissue. The electric field has an important role on the efficiency of the treatment, but a prediction is very difficult due to the fact that the electrical properties of tissue and skin vary from sample to sample, depending on inhomogeneity, anisotropy and permittivity. In order to overcome this problem, we incorporate a method to measure the voltage drop in tissue, exposed to a static electric field, in between a pair of parallel electrodes. Measurements are used to calculate the field distribution.

The presented acupuncture needle device can measure the voltage changes either in a static electric field or during short voltage pulses. It can therefore be used in combination with commercial EP-units. An electrode design of microneedle arrays is presented, which reduces the influence of the skin and allows a reduction in applied voltage. Impedance measurements before and after EP are used to estimate the creation of pores, resulting in resistance drop due to ion transport through permeable cell membranes generated.

Materials and Methods

Fundamental influence of electrical properties of tissue and skin were studied with electric field modelling using Maxwell software. The electrodes used to create the electric field are fabricated from silicon and covered with platinum. Microneedle electrodes have been fabricated by wet etching (Figure 1 left) using potassium hydroxide (KOH) [1] and dry etch technologies (Figure 1 right), using SF_6/O_2, which was alternately used with the BOSCH deep reactive ion etch process [2]. Planar electrodes have been compared with microneedle array electrodes on different kinds of tissue.

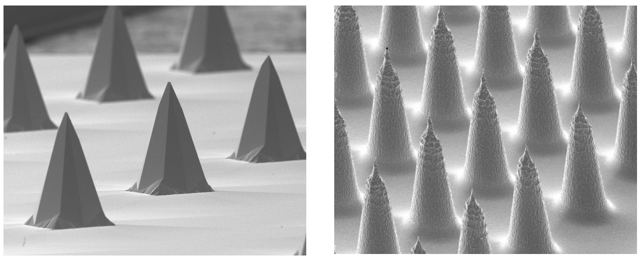

Figure 1: 230 µm tall wet etched needle with base diameter of 180 µm (left), 250 µm tall dry etched needle (bottom diameter 200 µm) (right)

In order to measure the voltage changes in the tissue, an acupuncture needle device was fabricated by generating 260 µm wide channel structures with 1.4 mm centre to centre distance onto a silicon wafer using thick photo resist (SU-8, MicroChem Corp.).

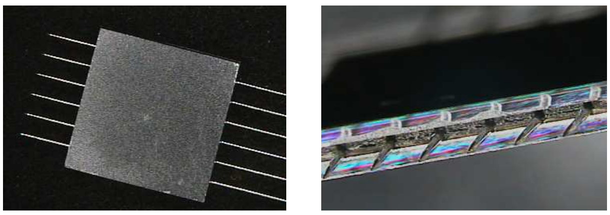

Figure 2: Acupuncture needle device to measure the voltage changes in tissue, top view (left), front view (right)

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Acupuncture needles of 250 µm diameter (asia-med Gesellschaft für Akupunkturbedarf mbH & Co. KG, Germany) were aligned in the channels. 2 silicon pieces with channel structures were then glued together using HTK Ultra Bond® 100 (HTK Hamburg® GmbH, Figure 2). The experimental setup is shown in Figure 3. Tissue with a surrounding resistant layer is placed between 2 parallel electrodes, packaged on printed circuit boards (PCB). The acupuncture needles are penetrated into the tissue, parallel to the electrodes, which apply the electric field. The potential of each acupuncture needle to the ground was measured continuously.

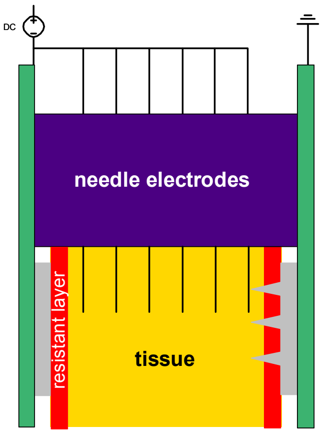

Figure 3: Experimental setup for 2 cases: planar electrodes (left) and needle electrodes (right); combinations of 2 kinds of electrodes possible, but 2 similar electrodes used for most experiments

Initial tests have been carried out with maximum applied voltages of 10 V, which generates an electric field (note: during EP applied voltages are higher). With the available data acquisition card (DAQ) of National Instruments Corporation, measurements were limited by 10 V. The impedance before and immediately after short voltage pulses was measured using the CythorLab™ unit (Aditus Medical).

Fundamental experiments have been carried out on potato tissue, due to the homogeneity and availability of higher amounts of tissue. It is known that the resistant upper layer of the around 100 µm thick skin, the stratum corneum, has a thickness of about 20 µm [3]. Cling film has more or less the same thickness and is elastic as well. We therefore used different layer thicknesses of cling film surrounding potato pieces of the same volume to demonstrate the influence of skin.

Results – Finite Element Modelling (FEM)
Fundamental considerations using planar electrodes

A principle structure of skin-tissue-skin was modelled for electric field simulation. Parameters have been adapted to cancer treatment. Typical applied voltages range between 50–300 V. The important parameter is the field strength in the tissue itself (V/cm), which depends on both the applied voltage and the electrode configuration [4, 5]. Due to the fact that the skin properties have the main influence on the electric field in the tissue, we examined the influence of skin thickness and permittivity of skin and tissue.

Chosen conductivity and permittivity are close to most of the literature values [6, 7]. They have been calculated using resistance measurements of tissue at a frequency of 1 Hz. The resistivity ρ of tissue is given by

$$\rho = \frac{R \cdot A}{d} \tag{1}$$

with the ohmic resistance R, the electrode area A and the electrode distance d. The ohmic resistance of 500 Ω for tumour tissue was measured using (5x15) mm^2 electrodes with a distance of 5 mm. The conductivity is the reciprocal value of the resistivity. The applied voltage is 150 V for all simulations; resulting in an electric field of 300 V/cm. Table 1 summarizes the chosen parameters for simulations.

Table 1: Chosen parameter for skin-tissue-skin model between 2 parallel electrodes

	Thickness µm	Conductivity S/m	Permittivity
Skin	100	0.0013	2
Tissue	4800	0.4	200

A voltage drop of around 60 V on both planar electrodes can be seen in Figure 4 for 100 µm thick skin. This leads to a reduction of the electric field in the tissue compared with the case where no skin is present. An increase of skin thickness results in a further electric field reduction, but the influence diminishes to a certain level. We conclude, that the presence of a skin layer, even as thin as 100 µm, causes a significant voltage drop.

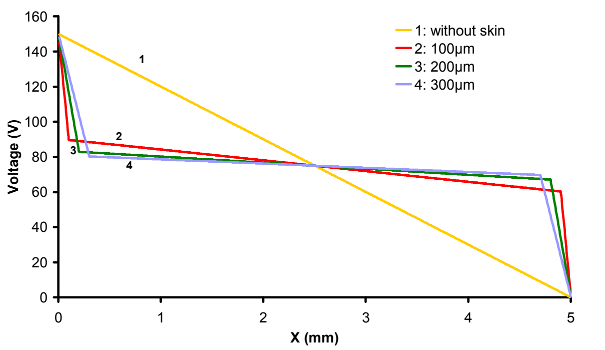

Figure 4: Voltage distribution modelling; Voltage for different skin thicknesses, X is the distance between the electrodes, parameter Table 1

The voltage drop in the skin layer has a significant influence on the electric field in the tissue, which is presented in Figure 5. In fact, the electric field of 300 V/cm (applied field) will be reduced to 62 V/cm with a 100 µm thick skin and further down to 35 and

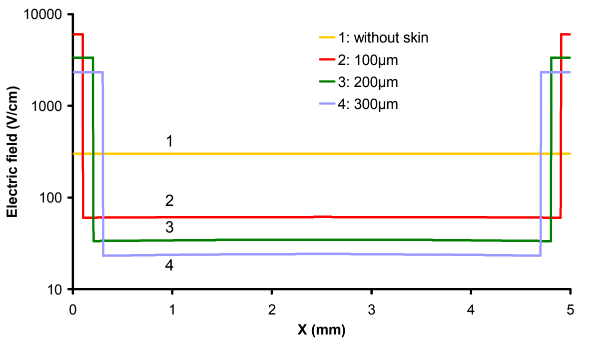

Figure 5: Electric field modelling; Voltage for different skin thicknesses, parameter Table 1

25 V/cm with 200 and 300 µm respectively. Such a field reduction would result in very low efficiency of EP. Results in Figure 6 show that the influence of permittivity changes of the skin is significant. Changing the permittivity from 2 to 1 (vacuum) causes a further voltage drop of around 7 V in the skin on both electrode sides. Increasing the value by one order of magnitude (20), results in a very small voltage drop. A prediction of that influence is very difficult. Literature values of skin permittivity vary in some orders of magnitude, depending on frequency, skin thickness and other parameters. Permittivity changes in tumour result vice versa. Higher permittivity of the tumour tissue further reduces the electric field in the tissue. The voltage drop in tumour increases with increasing permittivity.

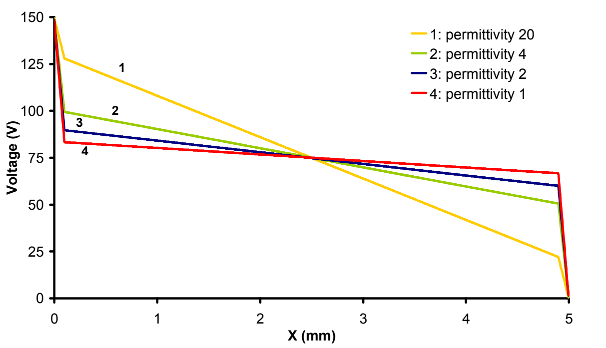

Figure 6: Voltage distribution modelling; Voltage for different skin permittivity, parameter Table 1

Effect of influence in electrode design

Figure 7 illustrates the main idea of using microneedle electrodes to penetrate through the high resistant skin layer. Ignoring all electrical parameters and values of electric field strength, one can clearly see that the influence of skin thickness is minimal to a certain point. The skin is normally around 100 µm thick. Using 300 µm tall microneedles, the high resistant stratum corneum does not reduce the field strength in the tissue, even with a thickness of 200 µm. For cancer treatment it results in much lower applied voltages to achieve the desired electric field. It will therefore increase the efficiency dramatically.

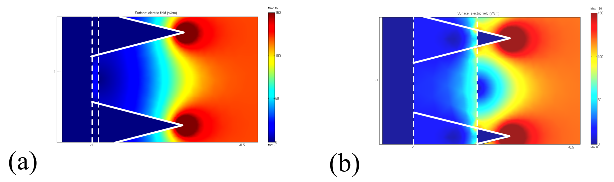

Figure 7: Finite Element Modelling (FEM) of microneedle electrodes penetrated into tumour tissue surrounded by skin (clarified by white lines) using FEMLAB software; needle height 300 µm; skin thickness 30 µm (a) and 200 µm (b)

The difference between planar and microneedle array electrodes (needles 300 µm tall) are presented in Figure 8. The voltage drop in a skin layer of 100 µm thickness, for the given electrical properties is 60V for planar electrodes and 22V for microneedle array electrodes (150V applied).

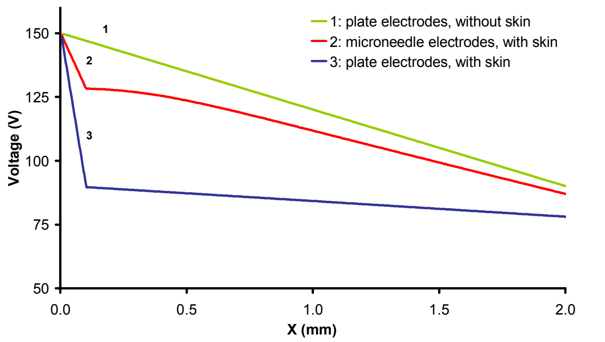

Figure 8: Voltage distribution modelling; plate electrodes (1 and 3), microneedle array electrodes (2), needle height 300 µm; parameter Table 1

The resulting electric field in the tissue volume is approximately 4 times higher with microneedle array electrodes compared to planar electrodes. Thus the electric field in the tissue is around 77% with microneedle electrodes and around 20% with planar electrodes (reference of 100% is without skin and planar electrodes). The electric field for needle electrodes in tissue without skin is slightly higher compared to planar electrodes, because the needle tips are 600 µm closer together.

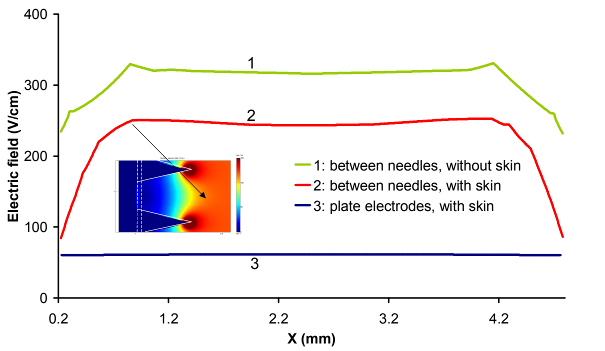

Figure 9: Electric field distribution modelling; microneedle array electrodes without and with skin (1 and 2), needle height 300 µm; plate electrodes for skin-tissue-skin model (3), parameter Table 1

For the experiments, electrodes were mounted on a digital calliper parallel to each other. Such a setup is used to clamp a volume of tissue. In certain cases, electrodes need to be placed parallel to the skin (for example for skin cancer treatment). Figure 10 shows that the electric field under the skin layer is much higher using microneedle electrodes with a penetration depth through the resistant skin layer compared to flat electrodes on the skin. Electric carriers can easily move between the needle tips. The skin will minimally affect the electric field.

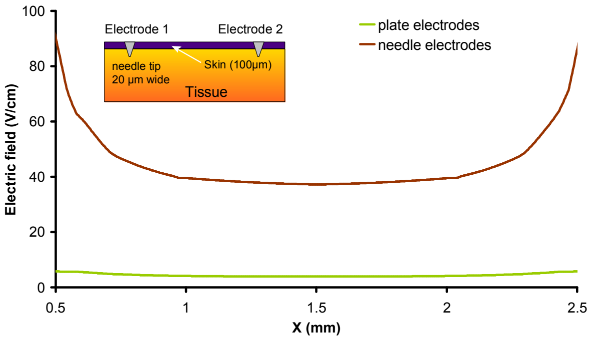

Figure 10: Electric field modelling 50 µm under the 100 µm thick skin layer; plate and microneedle electrodes parallel to skin; microneedles 300 µm tall; needle tip to tip distance 2.5 mm, applied voltage 10 V; parameter Table 1

Results-Voltage measurements
Potato tissue

Experiments were performed measuring the voltage drop in potato tissue surrounded by cling film. Figure 11 verifies the effect of voltage drop in the cling film layer using planar electrodes. The maximum amount of voltage drops on both electrodes side, resulting in no electric field in the tissue. Increasing the applied voltage by one order of magnitude could cause a low electric field. Measurements show that the tissue is very homogeneous.

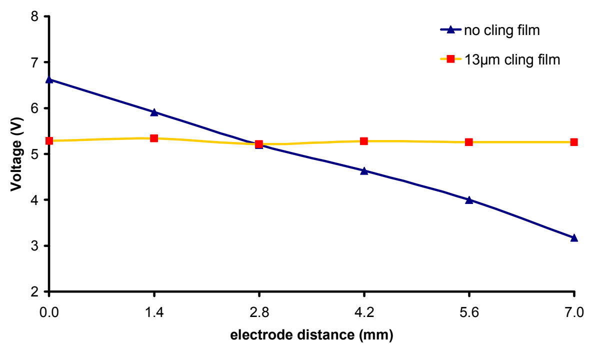

Figure 11: Voltage distribution in potato tissue surrounded by cling film layers of different thicknesses on both electrode sides; electrode distance 10 mm, planar electrodes

The voltage drop for tissue without cling film is comparable to planar electrodes (Figure 12). With increasing cling film thickness the slope attenuates till approximately zero for a thickness of 154 µm. If the acupuncture needle device is not exactly in the middle of the 2 electrodes that apply the electric field, a shift of the turning point will occur, but this does not effect the measurements of electric field.

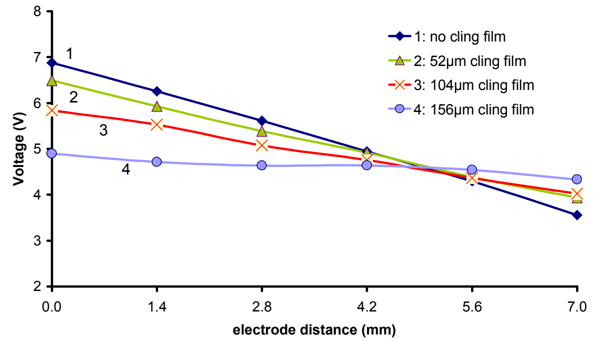

Figure 12: Voltage distribution in potato tissue surrounded by cling film layers of different thicknesses on both electrode sides; electrode distance 10 mm, microneedle electrodes

Another issue rises when a non-conductive layer is only on one electrode side or when the thicknesses are different. In the case of two similar layers on both electrode sides, the voltage will drop with the same amount on ground and voltage. The voltage in the middle is therefore half of the applied voltage. If the resistant layer is only on one electrode side, no voltage drop occurs on the counter electrode. Figure 13 illustrates that the configuration of tissue-resistant layer has a significant influence on the electric field.

It can be clearly seen, that the electric field drops at around 15% having 13 µm thick cling film on both sides. Between 26 and 78 µm thickness the field levels at around 80 % and is still quite high. A significant decrease to less than 50 % was measured for thicknesses of more than 100 µm. Those results present the efficiency of microneedle electrodes, which are penetrated through non-conductive cling film, without a significant degradation of the electric field up to 80 µm thickness.

One can see, that the field strength is approximately the same until 80 µm thick cling film, having the cling films either on both electrodes or only on the ground electrode. The field strength decreases when the cling film, only on the ground electrode, is thicker than 130 µm. Better results can be achieved when the voltage drop occurs on the voltage electrode. More than 90% of the maximum electric field was measured for thicknesses up to 80 µm.

Regarding the thickness of the stratum corneum, which is less than 20 µm, a loss of less than 20 % can be guaranteed with microneedle electrodes.

Tumour tissue

Voltage measurements in tumour tissue have been carried out using a digital calliper with mounted electrodes parallel to each other. The tumour tissue from

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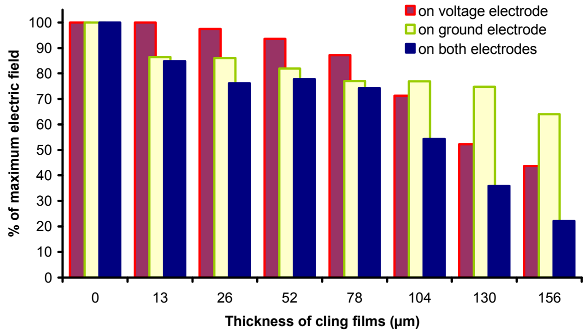


Figure 13: Electric field proportions in potato tissue surrounded by cling film layers of different thicknesses and configurations (cling film only on voltage or ground side or on both electrode sides) using microneedle electrodes; electrode distance 10 mm

the JBS cell line, which is a murine fibrosarcoma, with a volume of around (10x8x8) mm, was harvested from Balb/c mice.The electrodes were placed on the skin sides with surrounding pelt. The acupuncture needle device (presented in Figure 2) had 3 needle electrodes in a distance of 1.4 mm. A voltage of 10 V was applied to the parallel electrodes by a pulse generator for every experiment.

The results in Figure 14 show a wide range of values, which was caused by inhomogeneous tissue and penetration difficulties due to the softness and pelt of the tissue. We observed that once the needles are penetrated, maximum fields could be achieved. The penetration of frustum shapes (electrode 2) could not be assured at all times, because higher forces were needed to penetrate through pelt and skin. Interesting to mention is that taller dry etched microneedles (electrode 4) did not show significantly better results compared to smaller once with the same shape (electrode 5). Dry etched needles, which are much closer together than wet etched microneedles (ca. 3 times), do not penetrate as well as wet etched needles. Such an effect is caused by insufficient space between needles for tissue displacement.

Surface roughness can also have an influence on penetration force. Results (not presented) show, that maximum field strengths of 100 % can be achieved with all microneedle and frustum shapes, once they are penetrated into the tissue. The average drops down due to lower measurements when microneedles were not totally penetrating into the tumour skin. We assume that the penetration into human skin is better, expecting the tissue is harder than the tumour tissue used. The electric field using planar electrodes was less than 50 %.

Impedance measurements

An alternative measurement of EP efficiency is the documentation of impedance changes during the pulses. The impedance was measured before and straight after the pulses. Initial tests have been carried out on potato tissue, due to very good reliability of measurements. Further tests were done on tumour tissue.

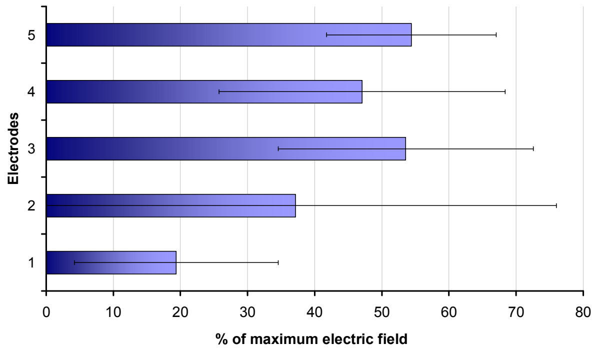
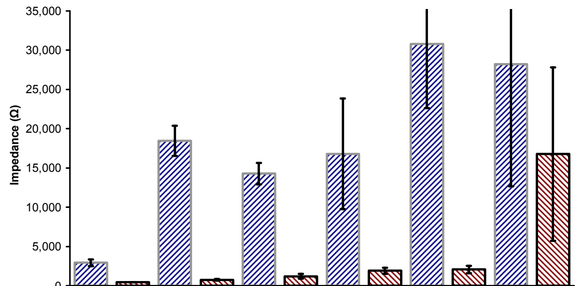


Figure 14: Electric field proportion in tumour tissue surrounded by skin and pelt for different electrodes; Electrodes: (1) planar, (2-5) microneedles, (2) wet etched frustums (height 260 µm, width on top 100 µm), (3) wet etched (height 270 µm), (4) dry etched (height 400 µm), (5) dry etched (height 260 µm)

Figure 15 shows the measurements of the impedance at a frequency of 1 Hz. One can see that the impedance drops down significantly until cling film thicknesses of around 50 µm. Even the impedance rises dramatically with increasing cling film thickness before EP, the impedance values after the pulses stay low. The standard deviation of impedance measurements increases dramatically with increasing thickness of cling film. The same effect was measured on tumour tissue surrounded by skin. The impedance varies ±420 Ω before EP and less than 20 Ω after EP without cling film layer. It can be seen in Figure 16 that the impedance after EP is in the same order of magnitude for 13 µm thick cling film for wet and dry etched microneedles.



	0 µm	13 µm	26 µm	39 µm	52 µm	104 µm
before EP	2935	18464	14275	16786	30787	28226
after EP	454	763	1202	1913	2073	16767

Figure 15: Impedance measurement on potato tissue with cling film layer thicknesses, using wet etched microneedle electrodes; parameter: 250V/cm, 8 pulses, pulse length 20 ms, frequency 1 Hz

The impedance rises dramatically with a resistant layer between electrode and tissue, but the level after EP is equal. That means that the microneedles penetrate sufficiently through the layer. The impedance after EP rises slightly with wet etched microneedles. The variation in measurements using dry etched microneedles is a result of penetration difficulties, which were mentioned before. The penetration depth decreases and penetration forces rise respectively when needles are very close to each other. Those results show

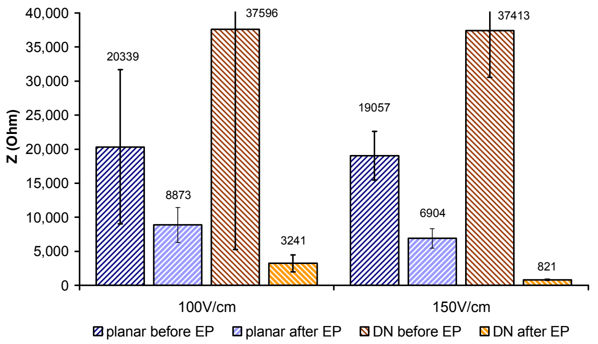


Figure 16: Impedance measurement on potato tissue with different cling film layer thicknesses, using wet and dry etched microneedle electrodes (designs Figure 1); parameter: 250V/cm, 8 pulses, pulse length 20 ms, frequency 1 Hz

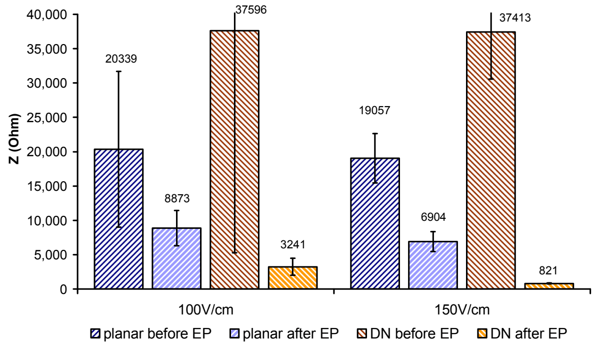


Figure 17: Impedance measurement before and after EP (EP) on tumor tissue using planar and dry etched microneedle electrodes (DN); parameter: 4pulses, pulse length 50 ms, frequenzy 1Hz, 2 different field strengths

that the penetration efficiency plays a very important role in EP. The electric field between wet and dry etched microneedles is comparable, but wet etched microneedles penetrate easier into the tissue, due to smoother surfaces and wider distances between the tips. Impedance measurements on tumour tissue are presented in Figure 17. One can clearly see that the impedance is much lower after EP using dry etched microneedle electrodes compared to planar electrodes.

Even with very high impedance measurements before EP, good efficiency with very low standard deviation was achieved at 150 V/cm. To achieve such low impedance values after EP with planar electrodes, more than 200 V/cm are necessary to apply. The use of microneedle electrodes can therefore reduce the applied voltages dramatically. Due to very low deviations, we conclude that much more reliable results can be achieved compared to planar electrodes.

Conclusions

FEM of electric field distributions in tumour tissue has shown, how the field strength can be improved using microneedle electrodes. The design and effectiveness of silicon microneedle electrodes was demonstrated compared to planar electrodes. A novel acupuncture needle device was used to measure the voltage distribution in tissue, to show the influence of isolating layers (such as skin) around the tissue. Measurements of the impedance, which are still ongoing, show that the applied voltage during EP can be reduced dramatically using microneedle electrodes. The reliability of impedance measurements improves once microneedles are penetrated through the skin. Our results therefore demonstrate the significant improvement in EP. This will lead to future optimisation of EP as a requirement for treatment of cancer. Further tests results on tumour tissue will be presented at the conference.

Acknowledgements

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