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The Effect of Electroporation Type Pulsed Electric Fields on DNA in Aqueous Solution

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Electroporation is a physical phenomenon in which pulsed electric fields applied across a cell produce transient (reversible) or permanent (irreversible) permeabilization of the cell membrane. Irreversible electroporation is an important method of sterilization in the food industry and it is becoming an important minimally invasive tissue ablation technique in medicine. Motivated by recent observations of apoptosis like marker stains in irreversibly electroporated cells we performed a study on the effects of electroporation type electric pulses on the integrity of naked DNA in solution. Using gel electrophoresis analyses we show that pulses of the irreversible electroporation type have the ability to affect the naked DNA in solution. It is found that some electric parameters that lead to cell death by irreversible electroporation also cause changes in the naked DNA exposed to the same procedure. Our analysis tentatively suggests that some electroporation type electric pulses cause nicks in the DNA molecule. Therefore, it is possible that the mechanisms of cell death in irreversible electroporation also include damages to the DNA. However, this work did not investigate the possible effects of the electrode corrosion byproducts, such as Al^{3+} ions on DNA integrity; which should be also studied in the future. In general, since electroporation phenomena based applications are widely used in medicine and biotechnology, the current study suggests that further research into the effects of electroporation type electric pulses on the DNA are warranted.

Key words: Electroporation; Non thermal irreversible electroporation; NTIRE; DNA damage.

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Authors contribution
AG designed and performed experiments, analyzed data and wrote the paper, BR supervised the project and wrote the paper.

Introduction

Electroporation is a biophysical phenomenon in which the cell membrane permeability is increased due to application of pulsed electric fields (PEF) across a cell. PEF's from which the cell membrane permeabilization is temporary and the cells survive cause "reversible electroporation". PEF's which lead to cell death cause "irreversible electroporation". Reversible electroporation is one of the most important technologies in the life sciences armamentarium with such diverse applications as introduction of genes in cells for genetic engineering to study of drugs. Irreversible electroporation (IRE) has been considered for reduction of microbial load in food since the middle of the 20th century (1-5). Recently irreversible electroporation in a non-thermal mode (NTIRE) has shown promise in medicine for tissue ablation (6). The use of IRE in sterilization of drugs is also considered (7).

Cell death from IRE is usually attributed to change in intracellular homeostasis due to the permeabilization of the cell membrane and exposure of the cytosol to the extracellular solution (8). This study was motivated by the results of recent publications of fundamental studies on NTIRE by (9, 10). Those reports used apoptotic stains to examine the NTIRE treated tissues. They have shown that the nucleus is stained with the apoptotic stains after the treatment and that the stain slowly diffuses out from the cell over hours.

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The cell membrane is a dielectric insulator and acts as a capacitance. This is why, until now, it was thought that only high frequency nanosecond electric pulses can bypass the capacitance like effect of the cell membrane and affect the DNA (11, 12). However, it is known that after electroporation takes place and the cell membrane becomes permeabilized the electric field penetrates the cell *e.g.* (13). In view of the recent observations with apoptotic stains after irreversible electroporation in tissue it occurred to us that in addition to the permeabilization effect on the cell membrane, conventional electroporation type pulsed electric fields (ETPEF) might also directly affect the DNA, when they penetrate the permeabilized cell interior. Therefore, the goal of this study is to explore the possibility that ETPEF's may affect the DNA, directly. To this end we performed a study on the effects of ETPEF's on naked DNA in solution. We studied the effects of electric pulsed field strength ($E_{appl)}$, pulse duration (tp), number (N) and frequency (*f*) of pulses on the damage to the DNA molecule. The assessment of the treatment impact on DNA was done by agarose gel electrophoresis and fluorescence analysis of genomic DNA bands (14).

Materials and Methods

Naked DNA

Genomic DNA was extracted using a Qiagen blood mini kit (Qiagen, USA). Before treatment the DNA concentration was diluted in deionized water. The final concentration was 1 ng/µl.

Pulsed Electric Field Treatment

Pulsed electric field (PEF) treatment was performed using a BTX ECM830 electroporator (BTX, San-Diego, California, USA). 80 µl of the prepared solution, were placed in a commercial 1 mm gap cuvette with Al electrodes (BTX #610, San-Diego, CA, USA) and subjected to various electroporation type PEFs. Immediately after treatment the temperature in the cuvette was measured by a Reflex Signal Conditioner with a 0.7 mm probe covered with polyimide (Neoptix, Inc, Québec, Canada). The pH of the solution was measured immediately after the electroporation with Neutralit® pH 5.0-10.0 (MERCK, KGaA, Germany), pH-Indikatorpapier Spezialindikator pH 8.2-10.0 (MERCK, KGaA, Germany), Acilit® pH-Indikatorpapier pH 0.5-5 (MERCK, KGaA, Germany).

Electrophoresis

A load of 20 ng of each treated sample was analyzed with a commercial agarose gel electrophoresis system. We used E-Gel® EX 2% prestained agarose separation gel (Life Technologies, CA, USA). The DNA dye, incorporated in gel, has the excitation at 490 nm and emission at 522 nm. The separation was performed in an E-Gel® iBase™ Power System (Life Technologies, CA, USA). Gels were run for 10 min by the E-Gel® Ex gel program. After electrophoreses images were captured by an imaging system provided with the E-Gel® iBase™ Power System Transillumnator, consisting of a CMOS sensor (1.3 Mp, monochromatic, Aptina, US), objective lens (Myutron, Japan) and plastic amber filter. Gel analysis for signal to noise ratio was performed using a commercial available software TL-100 (Nonlinear Dynamics, UK).

Experimental Protocol

The goal of the study was to determine if typical ETPEFs affect naked DNA. The investigated pulse parameters were: electric field strength (E_{appl}) 0.25-25 kV/cm, delivered by applying potential with amplitude V (Volt) on the electroporation cuvettes, pulse duration (tp) from 1-100 ms, number of pulses (N) from 5-150 and pulse frequency (*f*) from 0.5-10 Hz. Untreated samples were used for controls. The end point of each experiment was a fluorescent image analysis of the electrophoresis gels loaded with the treated solutions. The temperature and pH were measured after each experiment, to remove the possibility that they may affect the results.

Results

Effect of Electric Field Strength on the DNA

There are obviously a large number of various ETPEF parameter combinations that could be used in a study such as this. To focus, we studied primarily experiments relevant to non-thermal irreversible electroporation as used in medicine for tissue ablation, because the apoptosis stain results were reported first for those conditions.

Effect of electric field strength (E_{appl}) is an important electroporation parameter. It is thought that a certain field strength threshold exists, below which no cell membrane permeabilization takes place (15, 16). Then, there is a range of field strengths through which reversible electroporation takes place (15, 16). Higher field strengths produce irreversible damage until thermal damage is reached (15-18). In the first part of the study we evaluated the effect of E_{appl} on the DNA, while keeping tp = 100 µs, *f* = 1 Hz and N = 50. We chose N = 50 in this part of the study because it is in the range of typical clinical irreversible electroporation protocols (10,19,20). The other ETPEF parameters are also typical irreversible electroporation parameters (10, 19-21). Example of an agarose gel image is shown in Figure 1 for the different values of E_{appl}. The figure also plots the fluorescence intensity, S_n, from each of the electric field treated DNA bands relative to the intensity from the untreated DNA sample (lane 1) as S_n/S_1, where, n refers here to the lane number. Figure 1

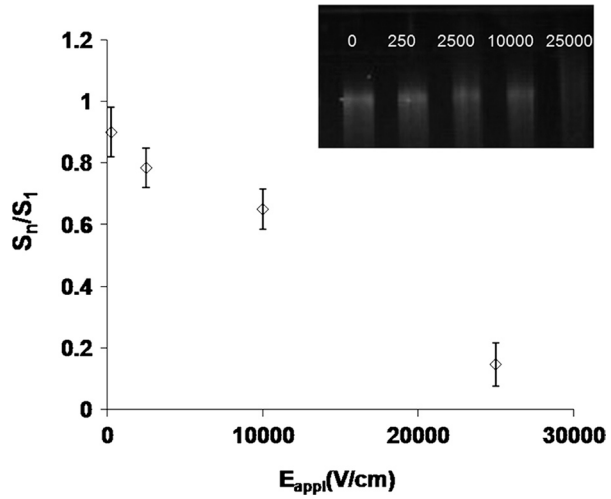

Figure 1: The effect of E_{appl} on DNA. Top right is a typical photograph of a gel. The diagram shows the ratio S_n/S_1 of treated samples, relative to the untreated control. E_{appl} (V/cm), is given over the corresponding lane. tp = 100 μs, *f* = 1 Hz and N = 50. Each experiment was repeated 9 times Error bars correspond to one standard deviation.

shows that the S_n/S_1 ratio decreases with an increase in electric field. Furthermore, a smearing in the treated DNA bands is observed at the high $E_{appl.}$ The effect is due to the electric field alone as the temperature rise is less than 1˚C. The pH of the solution did not change.

Effect of the Number of Electric Field Pulses on thSe DNA

In this part of the study we evaluated the effect of number of pulses (N) on the DNA. In this experiment E_{appl} was 2500 V/cm, tp = 100 μs and *f* = 1 Hz. These are typical ETPEF parameters used in NTIRE (10, 20). An N applied in experiment is also given in Figure 2 and was chosen to be in range

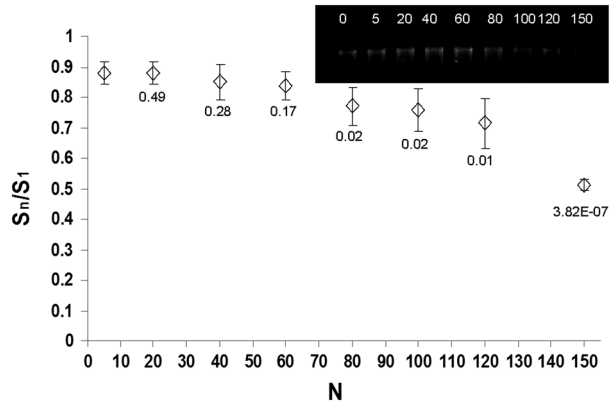

Figure 2: The effect of N on DNA. Top right is a typical photograph of a gel. The diagram shows the ratio S_n/S_1 from treated samples, relative to the untreated control. The number of pulses, N, is given above each relevant lane. V = 250 V, E_{appl} was 2500 V/cm, tp = 100 μs and *f* = 1 Hz. Each experiment was repeated 8 times Error bars correspond to one standard deviation. P-Values by one-tail Student t-test for differences between means and N = 5 mean are indicated for each point.

reported in (9, 10, 16, 19). The temperature raise is less than 4˚C, far below DNA thermal degradation (22). The pH of the solution did not change.

A typical gel image is shown in Figure 2 for various N. The S_n/S_1 ratio decreases with an increase in the number of pulses. Furthermore, a smearing of the bands in the treated DNA gel lanes is observed. It is obvious that when N exceeds 50, a noticeable change in the DNA signature begins. Interesting, studies on the effectiveness of NTIRE have shown that a larger number of pulses is more effective for cell ablation (23).

Effect of Electric Field Pulse Frequency on the DNA

Here we evaluate the effect of the ETPEF delivery *f*. The parameters were E_{appl} = 2500 V/cm, N = 50 and tp = 100 μs, typical to irreversible electroporation. We tested 0.5 Hz ≤ *f* ≤ 10 Hz range.

A typical gel image is shown in Figure 3 for various cases of *f*. It is interesting that frequency has a measurable statistical effect. The temperature rise was less than 2˚C. The pH of the solution did not change.

Effect of Pulse Duration on the DNA

In these series of experiments we investigated the effect of tp on the DNA. The electric field parameters used were E_{appl} = 500 V/cm, N = 6, and the *f* = 1 Hz. These are conditions typical of the ETPEF parameters at the margin between reversible and irreversible electroporation conditions (24). The parameters area also similar to those used in transdermal

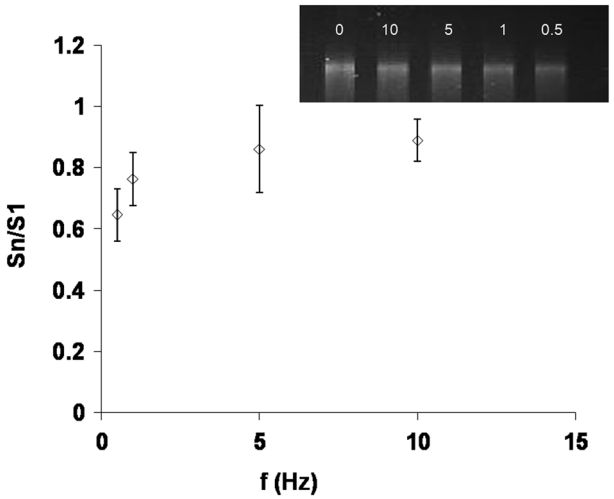

Figure 3: The effect of ***f*** on DNA. Top right is a typical photograph of a gel. The diagram shows the ratio S_n/S_1 of treated samples, relative to the untreated control. Applied *f* (Hz) is given above each relevant lane. V = 250 V, E_{appl} = 2500 V/cm, N = 50 and tp = 100 μs. Each experiment was repeated 8 times. Error bars correspond to one standard deviation.

reversible electroporation for drug delivery and DNA vaccination *e.g.* (25).

Typical gel images are shown in Figure 4 for the cases various tp. A strong correlation between tp and changes in the DNA is evident. We also tested tp shorter than 1 ms (data not shown) and found no effect on DNA molecule with these specific pulse parameters (E_{appl} = 500 V/cm, N = 6, f = 1 Hz). The effect is non-thermal since the temperature raise was less than 3.5˚C, and there was no measurable change in pH.

Treatment of Naked DNA with Electroporation Electric Pulse Parameters Reported in Literature

In the last series of experiments we tested the effect of ETPEF sequences from selected reports in the literature. Typical gel images are shown in Figure 5. Table I gives the treatment parameters and the appropriate reference.

It is interesting to observe that some NTIRE protocols reported in the literature to successfully ablate cells and tissues also affect the DNA.

Discussion

The first conclusion from the experiments is indisputable. Certain ETPEF's can affect the DNA.

Figure 1 shows that the ETPEF effect on the DNA increases with an increase in E_{appl}. The finding that irrerversible electroporation pulses are more effective at destroying cells with an increase in the E_{appl} was already made in the classic studies of Sale and Hamilton (2-4). However, here we show that E_{appl} in the range studied by Sale and Hamilton and others

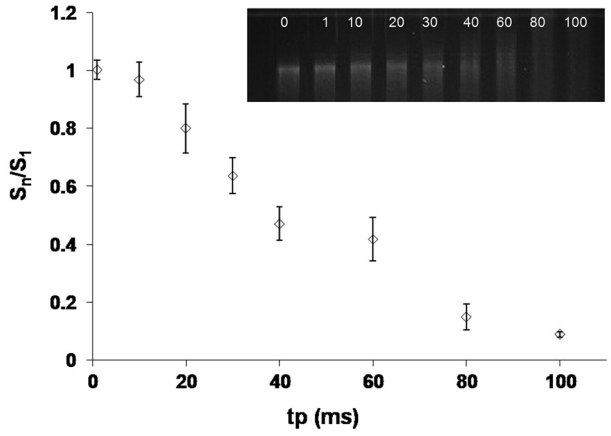

Figure 4: The effect of tp on DNA. Top right is a typical photograph of gel. The diagram shows the ratio S_n/S_1 from of treated samples, relative to the untreated control. The corresponding tp (ms) is given on top of each lane image. V = 50 V, E_{appl} = 500 V/cm, N = 6, f = 1 Hz. Each experiment was repeated 8 times. Error bars correspond to one standard deviation.

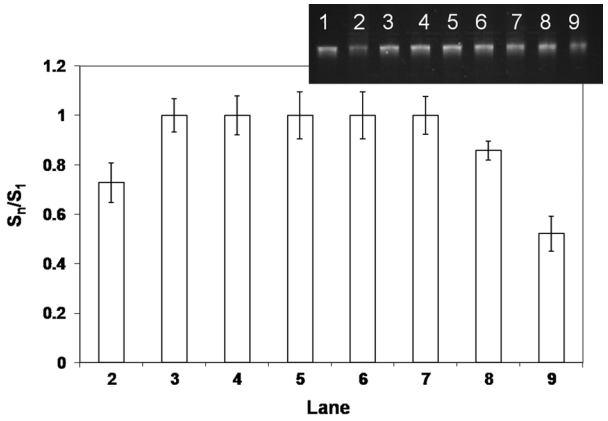

Figure 5: The effect of electroporation protocols from the literature on the DNA. Top right is a typical photograph of a gel. The lane numbers given in the photograph corresponds to the lane number listed in Table 1. The diagram shows the ratio S_n/S_1 from lanes 2 to 9, of the treated samples, relative to the untreated control, lane 1. Each experiment was repeated 5 times. Error bars correspond to one standard deviation.

lead to changes in the DNA signature on agarose gels. Figure 2 shows that an increase in N has the effect of increasing the changes in the DNA gels. The important effect of N on the increase in the effectiveness of the irreversible electroporation was reported in several recent publications (20, 23, 26). Indeed, N exceeding 50 has been shown to produce more cell death and at the same time have shown here to affect the DNA signature. Thus the increased N used in NTIRE may have an effect on the DNA itself, in addition to their impact on cell membrane.

The effect of f on the DNA signature, shown in Figure 3, is surprising. It appears that lower f in the range studied have a greater effect on the DNA integrity. While substantially more work needs to be done to determine if the observation

Table I

Literature reported parameters for various electroporation based applications. The table gives the electrical field parameters used for each of the gel lanes in Figure 5. The last column gives the reference from which this protocol was taken.

	Treatment					
Lane	V (V)	E_{appl} (V/cm)	N	tp	f (Hz)	Reference
1	Not treated (control)					
2	20	200	2	60 ms	5	(58)
3	160	1600	6	1 ms	0.5	(59)
4	150	1500	6	99 µs	1	(60)
5	380	3800	1	3.6 ms		(61)
6	50	500	1	40 ms		(62)
7	100	1000	1	20 ms		(21)
8	1000	10000	20	100 µs	1	(7)
9	250	2500	80	100 µs	0.3	(10)

is valid, a possible explanation could be related to the time of relaxation of the affected DNA molecule.

The effect of tp shown in Figure 4 suggests that ms long pulses have a stronger effect on the DNA. Indeed ms long pulses have been shown to be effective in non-thermal irreversible electroporation and their effectiveness increases with the tp, *e.g.* (23, 27, 28). Since the range of tp here is similar to that used in those studies, it is indeed possible that the effects on cell death may be also related to effects on the DNA. Furthermore, considering that ms pulses are now considered in gene therapy in the reversible electroporation mode, this topic may indeed require further and more detailed analysis.

Last, the results in Figure 5 show that indeed some irreversible electroporation parameters reported in the literature to be effective for cell death also affect the DNA.

While the fact that ETPEFs can affect the DNA is indisputable, the mechanism is not obvious. We will suggest a tentative explanation based on the analysis of the electrophoretic gel appearance. The ETPEF induced changes in the DNA are expressed by gel electrophoresis in two forms, as retardation and as decrease in DNA fluorescent band intensity. These types of changes are observed in all the experiments (Figures 1–5). They are illustrated at higher magnification by Figure 6. The arrow points to the location of the original DNA band. The signal intensity of the treated DNA fluorescent band is evidently lower than the untreated DNA band. The evidence for retardation is the smear in the gel on lanes 5 to 9, in Region 1. Usually, Region 1 indicates the location of molecules of changed DNA conformation. No fluorescent signal is seen in Region 2. Region 2 usually indicates the location of molecules with lower than original DNA mass, which appear when the DNA is fragmented.

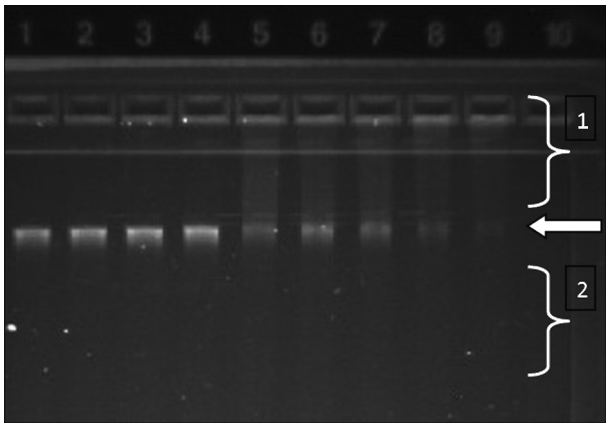

Figure 6: Example of electroporation pulse induced DNA retardation and fluorescence reduction. Gel load and treatments were done as described for Figure 4. The arrow points to the position of the original, DNA molecule. Region 1 indicates the location of molecules of changed DNA conformers. Region 2 indicates the location of molecules with lower than original DNA mass, which appear when the molecule is fragmented.

The treated DNA retardation in agarose gel as well as loss of signal intensity in the original DNA band tentatively suggest that one possible explanation of our results is that the DNA molecule is nicked by the pulsed electric field. Retardation is consistent with the nicked DNA movement in agarose gels electrophoresis described in (29). Single strand nicking was previously reported in literature, as an event which leads to DNA conformation shifts, changing supercoiled DNA to linear and relaxed forms, which, in turn, can be detected by agarose gel electrophoresis (30). The migration velocity of the relaxed form is significantly lower than that of the supercoiled and linear isoforms (31). The proposed mechanisms by which DNA isoforms separation and trapping occurs in gels were previously discussed in literature (30, 31).

The loss of signal in the DNA band may be explained by multiple nicks formation on the DNA molecule, as suggested by (32, 33). Zipper *et al.,* (32) investigated the mechanism and structure–property nature of SYBR type dyes interaction with DNA molecule. In his work it was shown that SYBR dye both intercalates and binds to the surface of DNA molecule (32). Thus, intercalation may not take place in the nicked region and the fluorescence intensity will decrease.

Nicks in the DNA can be induced by other mechanisms also such as various chemical, enzymatic and physical methods. Indeed, site specific enzymatic nicking by endonucleases lead either to the formation of an additional band (part of the original molecules which were cleaved) or to the retardation of the entire original band, if all molecules were cleaved (34-36). Studies on irradiation effects on DNA show that at specific, low dozes, DNA nicking takes place and the results can be detected by agarose electrophoreses either as smear (similar to our results) or as a separate bands (37-39).

All these suggest that one possible explanation for the results of this study is that certain ETPEF's affect naked DNA molecules, which may be nicked. This is obviously a preliminary observation. However, in order to confirm this hypothesis substantial additional experiments that will show the specific nick positions and number should be performed in order to prove the idea on the single molecule and single DNA base pare level.

Previously it was reported that DNA in cells undergoes fragmentation under the applications of much higher than ETPEF nanosecond pulses (40-42). Our finding may indicate that also conventional ETPEF can affect the DNA.

A main focus in the development of pharmaceutical DNA medicine is the stability of injected DNA, which is mainly reflected by the DNA topology (43). For instance, Schleef

and Schmidt (43) focus on the importance of the plasmid conformation stability for gene therapy. Analyzing the work of Lee *et al.,* (44), Smith proposed that DNA nicks can stimulate homologous recombination (45). Accordingly, they may lead to translocations and other potentially dangerous genome rearrangements in uncontrolled situations (45). The results of our work show that certain ETPEF may affect the DNA topology. This is not unique to electroporation. Indeed, other DNA introduction techniques affect the DNA. For instance, Walther *et al.,* (46) reported DNA conformational changes due to gene-gun or jet-injection method. Nevertheless, extrapolating from the parameters we have used in our study, care may be warranted in the development of electroporation protocols for gene and drug delivery by this method. On the other hand, electric pulse induced nicks during electroporation procedures, if correctly planned, may increase the efficiency of inserted DNA integration in the host genome and may not necessarily have a totally negative effect for certain applications.

The model of DNA damage by PEF has certain limitations. Although we showed a correlation between DNA retarding in gel electrophoreses and florescence signal decrease with various parameters of the applied PEF, an additional effect of pulse application – electrode material release – is still to be investigated. Currently, two types of electrodes are used for electroporation applications. Aluminum electrodes, as used in this study and stainless steel electrodes, which are popular in large scale PEF systems for food treatment. Studies on PEF in the food industry and cell electro-transfection revealed that electrode material is released to the electrolyte solutions due to the application of high voltage pulses (47, 48). The nature of DNA-metal binding has been recently reviewed in the literature (49, 50). Moreover, Mustak (2002) investigated the impact of various metals on DNA conformation changes in relevance to neurological disorders (51). Specifically, it was shown that Al^{3+} and Fe^{2+} ions not only bind to DNA, but also induce confirmation changes in the DNA molecule, including DNA nicking (51). Furthermore, additional works showed that aluminum and iron ions cause to DNA damage and topology changes (52-57). Hence, future studies should address a possibility that DNA damage, in addition to applied electric fields, is caused by released electrode materials.

Summarizing, while this is only a preliminary study, it shows that conventional electroporation type pulsed electric fields affect the DNA, tentatively, through the formation of nicks. Since commercial electroporation technology was used in this study – the finding that DNA is damaged during electroporation pulses requires further research. Therefore, more detailed studies on the effects of electroporation pulses and of the means for electroporation pulse delivery on the naked DNA are warranted as advances in molecular surgery, including gene therapy, DNA vaccinations and non-thermal irreversible electroporation for tissue ablation occur.

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Competing financial interests

Authors declare not competing financial interests with the work contents.

References

1. Doevenspeck, H. Influencing cells and cell walls by electrostatic impulses. *Fleischwirtschaft 13*, 968-987 (1961).
2. Sale, A. J., Hamilton, W. A. Effect of high electric field on microorganisms. I. Killing of bacteria and yeast. *Biochim Biophys Acta 148*, 781-788 (1967a).
3. Sale, A. J., Hamilton, W. A. Effect of high electric field on microorganisms. II. Mechanism of action of the lethal effect. *Biochim Biophys Acta 148*, 788-800 (1967b).
4. Sale, A. J., Hamilton, W. A. Effects of high electric fields on microorganisms. III. Lysis of erythrocytes and protoplasts. *Biochim Biophys Acta 163*, 37-43 (1968).
5. Lelieved, H. L. M., Notermans, S., de Haan, S. W. H. *Food Preservation by pulsed electric fields. From Research to Application.*, (CRC, 2007).
6. Lavee, J., Onik, G., Mikus, P., Rubinsky, B. A novel nonthermal energy source for surgical epicardial atrial ablation: irreversible electroporation. *Heart Surg Forum 10*, 162-167 (2007).
7. Golberg, A., Belkin, M., Rubinsky, B. Irreversible Electroporation for Microbial Control of Drugs in Solution. *AAPS Pharm Sci Tech*, DOI: 10.1208/s12249-12009-19277-12243 (2009).
8. Esser, A. T., Smith, K., C, Gowrishankar, T. R., Weaver, J. C. Towards Solid Tumor Treatment by Irreversible Electroporation: Intrinsic Redistribution of Fields and Currents in Tissue. *Tech Cancer Res Treat 6*, 261-273 (2007).
9. Lee, E. W., Loh, C. T., Kee, S. T. Imaging guided percutaneous irreversible electroporation: ultrasound and immunological correlation. *Tech Cancer Res Treat 6*, 287-294 (2007).
10. Al-Sakere, B., André, F., Bernat, C., Connault, E., Opolon, P., Davalos, R. V., Rubinsky, B., Mir, L. M. Tumor Ablation with Irreversible Electroporation. *PLoS ONE 2*, e1135 (2007).
11. Beebe, S. J., White, J., Blackmore, P. F., Deng, Y., Somers, K., Schoenbach, K. H. Diverse Effects of Nanosecond Pulsed Electric Fields on Cells and Tissues. *DNA Cell Biol. 22*, 785-796 (2003).
12. Nuccitelli, R., Chen, X., Pakhomov, A. G., Baldwin, W. H., S, S., Pomicter, J. L., Ren, W., Osgood, C., Swanson, R. J., Kolb, J. F., Beebe, S. J., Schoenbach, K. H. A new pulsed electric field therapy for melanoma disrupts the tumorw's blood supply and causes complete remission without recurrence. *Int J Cancer 125*, 438-445 (2009).
13. Huang, Y., Rubinsky, B. Micro-electroporation: improving the efficiency and understanding of electrical permeabilization of cells. *Biomedl Microdevices 2*, 145-150 (1999).
14. Sutherland, J. C., Monteleone, D. C., Trunk, J. G., Bennett, P. V., Sutherland, B. M. Quantifying DNA damage by gel electrophoresis, electronic imaging and number-average length analysis. *Electrophoresis 22*, 843-854 (2001).
15. Dev, S. B., Rabussay, D. P., Widera, G., Hofmann, G. A. Medical Applications of Electroporation. *IEEE T Plasma Sci 28*, 206-223 (2000).

16. Miklavcic, D., Semrov, 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. *Biochim Biophys Acta 1523*, 73-83 (2000).
17. Davalos, R. D., Mir, L. M., Rubinsky, B. Tissue ablation with Irreversible Electroporation. *Ann Biomed Eng 33*, 223-231 (2005).
18. Zimmerman, U. in: *Electromanipulation of cells* edited by U. Zimmerman & G. A Nail) 1-106 (CRC Press, 1996).
19. Maor, E., Ivorra, A., Rubinsky, B. Non Thermal Irreversible Electroporation: Novel Technology for Vascular Smooth Muscle Cells Ablation. *PLoS ONE 4*, e4757. doi:4710.1371/journal.pone.0004757 (2009).
20. Al-Sakere, B., Bernat, C., André, F., Connault, E., Opolon, P., Davalos, R. V., Mir, L. M. A Study of the Immunological Response to Tumor Ablation with Irreversible Electroporation. *Tech Cancer Res Treat 6*, 301-305 (2007).
21. Edd, J. F., Horowitz, L., Davalos, R. V., Mir, L. M., Rubinsky, B. In Vivo Results of a New Focal Tissue AblationTechnique: Irreversible Electroporation. *IEEE T Biomed Eng 53*, 1409-1415 (2006).
22. DNA Fragmentation, Kreatech protocols, http://www.kreatech.com/Default.aspx?tabid=119, Accesed Sptember 16.
23. Rubinsky, J., Onik, G., Paul Mikus, P., Rubinsky, B. Optimal Parameters for the Destruction of Prostate Cancer Using Irreversible Electroporation. *J Urology 180*, 2668-2674 (2008).
24. Miklavcic, D., Semrov, 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. *Biochim Biophys Acta 1523*, 73-83 (2000).
25. Roos, A. K., Eriksson, F., Timmons, J. A., Gerhardt, J., Nyman, U., Gudmundsdotter, L., Brave, A., Wahren, B., Pisa, P. Skin electroporation: effects on transgene expression, DNA persistence and local tissue environment. *PLoS ONE 4*, e7226 (2009).
26. Onik, G., Mikus, P., Rubinsky, B. Irreversible electroporation: implications for prostate ablation. *Technology in cancer research & treatment, 6*, 295-300 (2007).
27. Miller, L., Leor, J., Rubinsky, B. Cancer Cells Ablation with Irreversible Electroporation. *Technol Cancer Res Treat*, 699-705 (2005).
28. Edd, J. F., Horowitz, L., Davalos, R. D., Mir, L. M., Rubinsky, B. In vivo results of a new focal tissue ablation technique: irreversible electroporation. *IEEE T Biomed Eng 153*, 1409-1415 (2006).
29. Johnson, P., Grossman, L. I. Electrophoresis of DNA in Agarose Gels. Optimizing Separations of Conformational Isomers of Double- and Single-Stranded DNAs. *Biochemistry (Mosc) 16*, 4217-4225 (1977).
30. Mickel, S., Arena, V., Bauer, W. Physical properties and gel electrophoresis behavior of R12-derived plasmid DNAs. *Nucleic Acids Res 4*, 1465-1482 (1977).
31. Akerman, B., Cole, K. D. Electrophoretic capture of circular DNA in gels. *Electrophoresis 23*, 2549-2561 (2002).
32. Zipper, H., Brunner, H., Bernhagen, J., Vitzthum, F. Investigations on DNA intercalation and surface binding by SYBR Green I, its structure determination and methodological implications. *Nucleic Acids Res 32*, e103 doi:110.1093/nar/gnh1101 (2004).
33. Even-Chen, S., Barenholtz, Y. DOTAP cationic liposomes prefer relaxed over supercoiled plasmids. *Biochim Biophys Acta, Biomembr 1509*, 176-188 (2000).
34. Kuhn, H., Protozanova, E., Demidov, V. Monitoring of single nicks in duplex DNA by gel electrophoretic mobility-shift assay. *Electrophoresis 23*, 2384-2387 (2002).
35. Xu, Y., Lunnen, K. D., Kong, H. Engineering a nicking endonuclease N.AlwI by domain swapping. *Proc Natl Acad Sci USA 98*, 12990–12995. (2001).
36. Morton, B. S., Matson, S. W. Escherichia coli DNA helicase I catalyzes a site- and strand-specific nicking reaction at the F plasmid oriT. *J Biol Chem 266*, 16232-16237 (2001).
37. Boudaiffa, P., Cloutier, P., Hunting, D., Huels , M. A., Sanche, L. Resonant formation of DNA strand breaks by lowenergy (3 to 20 eV) electrons. *Science 287*, 1658-1660 (2000).
38. Ito, T., Saito , M., Taniguchi, T. A survey of photoproducts of an irradiated oligodeoxynucleotide by monochromatic photons with the energy ranges from 6.5 to 22.5 eV. *Photochem Photobiol 46*, 979-984 (1987).
39. Smialek, M. A., Moore, S. A., Mason, N. J., Shuker, D. E. G. Quantification of Radiation-Induced Single-Strand Breaks in Plasmid DNA using a TUNEL/ELISA-Based Assay. *Radiat Res 172*, 529-536 (2009).
40. Hall, E. H., Schoenbach, K. H., Bebe, S. J. Nanosecond pulsed electric fields induce apoptosis in p53-wildtype and p53-null HCT116 colon carcinoma cells. *Apoptosis 12*, 1721–1731 (2007).
41. Hall, E. H., Schoenbach, K. H., Bebe, S. J. Nanosecond Pulsed Electric Fields Have Differential Effects on Cells in the S-Phase. *DNA Cell Biol 26*, 160-171 (2007).
42. Nuccitelli, R., Chen1, X., Pakhomov, A. G., Baldwin, W. H., Sheikh, S., Pomicter, J. L., Ren1, W., Osgood, C., Swanson, R. J., Kolb, J. F., Bebe, S. J., Schoenbach, K. H. A new pulsed electric field therapy for melanoma disrupts the tumors blood supply and causes complete remission without recurrence. *Int J Cancer 125*, 438-445. (2009).
43. Schleef, M., Schmidt, T. Animal-free production of ccc-supercoiled plasmids for research and clinical applications. *J Gene Med 6*, S45-S53 (2004).
44. Lee, G. S., Neiditch, M. B., Salus, S. S., Roth, D. B. RAG Proteins Shepherd Double-Strand Breaks to a Specific Pathway, Suppressing Error-Prone Repair, but RAG Nicking Initiates Homologous Recombination *Cell 117*, 171-184 (2003).
45. Smith, G. R. How Homologous Recombination Is Initiated: Unexpected Evidence for Single-Strand Nicks from V(D)J Site-Specific Recombination *Cell 117*, 146-148 (2004).
46. Walther, W., Stein, U., Fichtner, I., Voss, C., Schmidt, T., Schleef, M., Nellessen, T., Schlag, P. M. Intratumoral low volume jet-injection for efficient non-viral gene transfer. *Mol Biotechnol 21*, 105-115 (2002).
47. Roodenburg, B., Morren, J., Bergb, H. E., de Haan, S. W. H. Metal release in a stainless steel Pulsed Electric Field (PEF) system Part I. Effect of different pulse shapes; theory and experimental method. *Innovative Food Science and Emerging Technologies 6*, 327-336 (2005).
48. Loomis-Husselbee, J. W., Cullen, P. J., Irvine, R. F., Dawson, A. P. Electroporation can cause artefacts due to solubilization of cations from the electrode plates. Aluminum ions enhance conversion of inositol 1,3,4,5-tetrakisphosphate into inositol 1,4,5-trisphosphate in electroporated L1210 cells. *Biochem J 277 (Pt 3)*, 883-885 (1991).
49. Anastassopoulou, J. Metal–DNA interactions. *Journal of Molecular Structure 651-653*, 19-26 (2003).
50. Hadjiliadis, N., Sletten, E. *Metal Complex-DNA Interaction*. (Wiley-Blackwell, 2009).
51. Mustak, B. *Studies on metal DNA interaction induced DNA conformations in relevance to neurological disorders* PhD thesis, Central food technology research institute, (2006).
52. Kumar, V., Bal, A., Gill, K. D. Aluminium-induced oxidative DNA damage recognition and cell-cycle disruption in different regions of rat brain. *Toxicology 264*, 137-144 (2009).
53. Lankoff, A., Banasik, A., Duma, A., Ochniak, E., Lisowska, H., Kuszewski, T., Gozdz, S., Wojcik, A. A comet assay study reveals that aluminium induces DNA damage and inhibits the repair of radiation-induced lesions in human peripheral blood lymphocytes. *Toxicol Lett 161*, 27-36 (2006).
54. Magisetty, O., Rao, D. M., Sundar, S. Studies on Genomic DNA Stability in Aluminium-Maltolate Treated Aged New Zealand

Rabbit: Relevance to the Alzheimer's Animal Model. *J Clin Med Res 1*, 212-218 (2009).
55. Rao, K. S., Rao, B. S., Vishnuvardhan, D., Prasad, K. V. Alteration of superhelical state of DNA by aluminium (Al). *Biochim Biophys Acta 1172*, 17-20 (1993).
56. Bharathi, Jagannatha Rao, K. S., Stein, R. First evidence on induced topological changes in supercoiled DNA by an aluminium D-aspartate complex. *J Biol Inorg Chem 8*, 823-830 (2003).
57. Yaffee, M., Walter, P., Richter, C., Muller, M. Direct observation of iron-induced conformational changes of mitochondrial DNA by high-resolution field-emission in-lens scanning electron microscopy. *Proc Natl Acad Sci USA 93*, 5341-5346 (1996).
58. Wallace, M., Evans, B., Woods, S., Mogg, R., Zhang, L., Finnefrock, A. C., Rabussay, D., Fons, M., Mallee, J., Florian Schödel, M., Musey, L. Tolerability of Two Sequential Electroporation Treatments Using MedPulser DNA Delivery System (DDS) in Healthy Adults. *Mol Ther 17*, 922-928 (2009).
59. Wells, J. M., Li, L. H., Sen, A., Jahreis, G. P., Hui, S. W. Electroporation-enhanced gene delivery in mammary tumors. *Gene Ther 7*, 541-547 (2000).
60. Heller, R., Jaroszeski, M., Atkin, A., Moradpour, D., Gilbert, R., Wands, J., Nicolau, C. In vivo gene electroinjection and expression in rat liver *FEBS Lett 389*, 225-228 (1996).
61. Prausnitz, M. R., Corbett, J. D., Gimm, J. A., Golan, D. E., Langer, R., Weaver, J. C. Millisecond measurement of transport during and after an electroporation pulse. *Biophys J 68*, 1864-1870 (1995).
62. Canatella, P. J., Black, M. M., Bonnichsen, D. M., McKenna, C. M., Prausnitz, M. R. Tissue Electroporation: Quantification and Analysis of Heterogeneous Transport in Multicellular Environments. *Biophys J 86*, 3260-3268 (2004).

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