

Tumor Ablation with Irreversible Electroporation

Bassim Al-Sakere^{1,2}, Franck André^{1,2}, Claire Bernat^{1,2}, Elisabeth Connaert^{1,2}, Paule Opolon^{1,2}, Rafael V. Davalos³, Boris Rubinsky^{4,5,6}, Luis M. Mir^{1,2,6}

1 CNRS UMR 8121, Institut Gustave-Roussy, Villejuif, France, **2** University Paris-Sud, UMR 8121, Villejuif, France, **3** School of Biomedical Engineering and Sciences, Virginia Tech-Wake Forest University, Blacksburg, Virginia, United States of America, **4** Department of Bioengineering, University of California at Berkeley, Berkeley, California, United States of America, **5** Department of Mechanical Engineering and Graduate Program in Biophysics, University of California at Berkeley, Berkeley, California, United States of America, **6** Center for Bioengineering in the Service of Humanity and Society, School of Computer Science and Engineering, Hebrew University of Jerusalem, Givat Ram, Jerusalem, Israel

We report the first successful use of irreversible electroporation for the minimally invasive treatment of aggressive cutaneous tumors implanted in mice. Irreversible electroporation is a newly developed non-thermal tissue ablation technique in which certain short duration electrical fields are used to permanently permeabilize the cell membrane, presumably through the formation of nanoscale defects in the cell membrane. Mathematical models of the electrical and thermal fields that develop during the application of the pulses were used to design an efficient treatment protocol with minimal heating of the tissue. Tumor regression was confirmed by histological studies which also revealed that it occurred as a direct result of irreversible cell membrane permeabilization. Parametric studies show that the successful outcome of the procedure is related to the applied electric field strength, the total pulse duration as well as the temporal mode of delivery of the pulses. Our best results were obtained using plate electrodes to deliver across the tumor 80 pulses of 100 μ s at 0.3 Hz with an electrical field magnitude of 2500 V/cm. These conditions induced complete regression in 12 out of 13 treated tumors, (92%), in the absence of tissue heating. Irreversible electroporation is thus a new effective modality for non-thermal tumor ablation.

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INTRODUCTION

Minimally invasive tissue ablation has become of central importance in the modern surgery armamentarium. In the treatment of benign or malignant tumors it is important to achieve ablation of the undesirable tissue in a well-controlled and precise way without affecting the surrounding healthy tissue. As an alternative to surgical resection, a number of minimally invasive methods have been developed to destroy specific areas of undesirable tissues. Most of these techniques are thermal using cold, e.g. cryosurgery [1–3] or heat, e.g. radiofrequency [4,5].

Electroporation, also known as electroporation, is a term used to describe the permeabilization of the cell membrane as a consequence of the application of certain short and intense electric fields across the cell membrane, the cells or the tissues. The permeabilization can be temporary (reversible electroporation) or permanent (irreversible electroporation) as a function of the electrical field magnitude and duration, and the number of pulses [6]. Reversible electroporation is commonly used *in vivo* to facilitate the penetration of various otherwise non-permeable macromolecules across the cell membrane [7–9]. Irreversible electroporation, the ability of certain electrical pulses to permanently permeabilize the cell membrane, has been known for over three decades. For most of this period irreversible electroporation (IRE) was used primarily for ablation of microorganisms and cells *in vitro* and studied only as an upper limit of electrical parameters for reversible tissue electroporation applications. Our group has pursued the understanding of the electrical fields and processes that produce IRE with single cell micro-electroporation technology [10,11].

The study of Davalos, Mir and Rubinsky, which showed that IRE can ablate substantial volumes of tissue without inducing a thermal effect and therefore serve as an independent and new tissue ablation modality, opened the way to the use of IRE in surgery [12]. Subsequently, Eddi *et al.* demonstrated tissue ablation with IRE *in vivo* in the normal liver of rats. [13]. Complete ablation of the targeted liver tissue was achieved by exposing the tissue to

electrical parameters that do not induce thermal damage [13]. Massive blood vessel congestion was observed in the sinusoids of the treated volume, which should significantly enhance the treatment. The study concluded that IRE produces precisely delineated ablation zones with cell scale resolution between ablated and non-ablated areas and the ability of mathematical modelling to precisely predict the ablated area. A more recent study was performed to evaluate the long term effects of IRE in a large animal model [14]. The results demonstrated the ability of electroporation to ablate large volumes of tissue using electrical parameters that while substantially above those conventionally used in reversible electroporation do not induce substantial thermal effects. The histology has reconfirmed the results in Davalos *et al.* [12] and Eddi *et al.* [13] showing that mathematical modeling of electrical and thermal fields are a powerful tool in designing IRE ablation protocols, that IRE can be used to ablate tissue with cell scale resolution and that indeed IRE affects only the cell membrane and therefore spares connective tissue. Another

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Competing Interests: R. Davalos and B. Rubinsky have potential interests in the revenues of pending patents held by Univ. of California at Berkeley (e.g. No 10/371,162, Tissue Ablation with Irreversible Electroporation). B. Rubinsky also has a financial interest in Excellen Life Sciences and in Oncologic, companies in the field of electroporation. L. M. Mir was a consultant for Oncologic.

* To whom correspondence should be addressed. E-mail: lmir@nrg.fr

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