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Irreversible Electroporation in Medicine

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This is a brief introduction to the emerging field of irreversible electroporation in medicine. Certain electrical fields when applied across a cell can have as a sole effect the permeabilization of the cell membrane, presumable through the formation of nanoscale defects in the cell membrane. Sometimes this process leads to cell death, primarily when the electrical fields cause permanent permeabilization of the membrane and the consequent loss of cell homeostasis, in a process known as irreversible electroporation. This is an unusual mode of cell death that is not understood yet. While the phenomenon of irreversible electroporation may have been known for centuries it has become only recently rigorously considered in medicine for various applications of tissue ablation. A brief historical perspective of irreversible electroporation is presented and recent studies in the field are discussed.

Keywords: cell membrane, irreversible electroporation, tissue ablation, oncology

Certain electrical fields, when applied across a cell, have the ability to permeabilize the cell membrane through a process that was named in the early 1980's "electroporation" (1). The mechanism through which the cell membrane is permeabilized is not yet fully understood. It is thought to be related to the formation of nano-scale defects or pores in the cell membrane, from which the term "poration" was derived. Some electrical fields permeabilize the cell membrane temporarily after which the cells survive and then the process is known as "reversible electroporation". Other fields can cause the cell membrane to become permanently permeabilized, after which the cells die, in a process referred to as "irreversible electroporation".

Reversible electroporation has become an important tool in biotechnology and medicine. Among the many uses are the permeabilization of the cell membrane to molecules that normally do not penetrate the membrane, such as genes (1), fusion of cells (2) , introduction of drugs into cells (3, 4), electrochemotherapy for treatment of cancer (5), gene delivery in tissue (6), transdermal delivery of drugs and genes. Numerous reviews, books, issues of journals and thousands of publications were published on the various features of reversible electroporation, such as, (7-19). The various applications of reversible electroporation require cells to survive the procedure and therefore the occurrence of irreversible electroporation, following which cells die, is obviously undesirable. Therefore, during the last three decades, when the field of electroporation has become dominated by reversible electroporation applications, irreversible electroporation was viewed as an undesirable side effect and was studied only to define the upper limit of electrical parameters that induce reversible electroporation. However, during the last few years irreversible electroporation is beginning to emerge as an important medical technology in its own right. This issue of "Technology in Cancer Research and Treatment" is a compilation of the latest studies on

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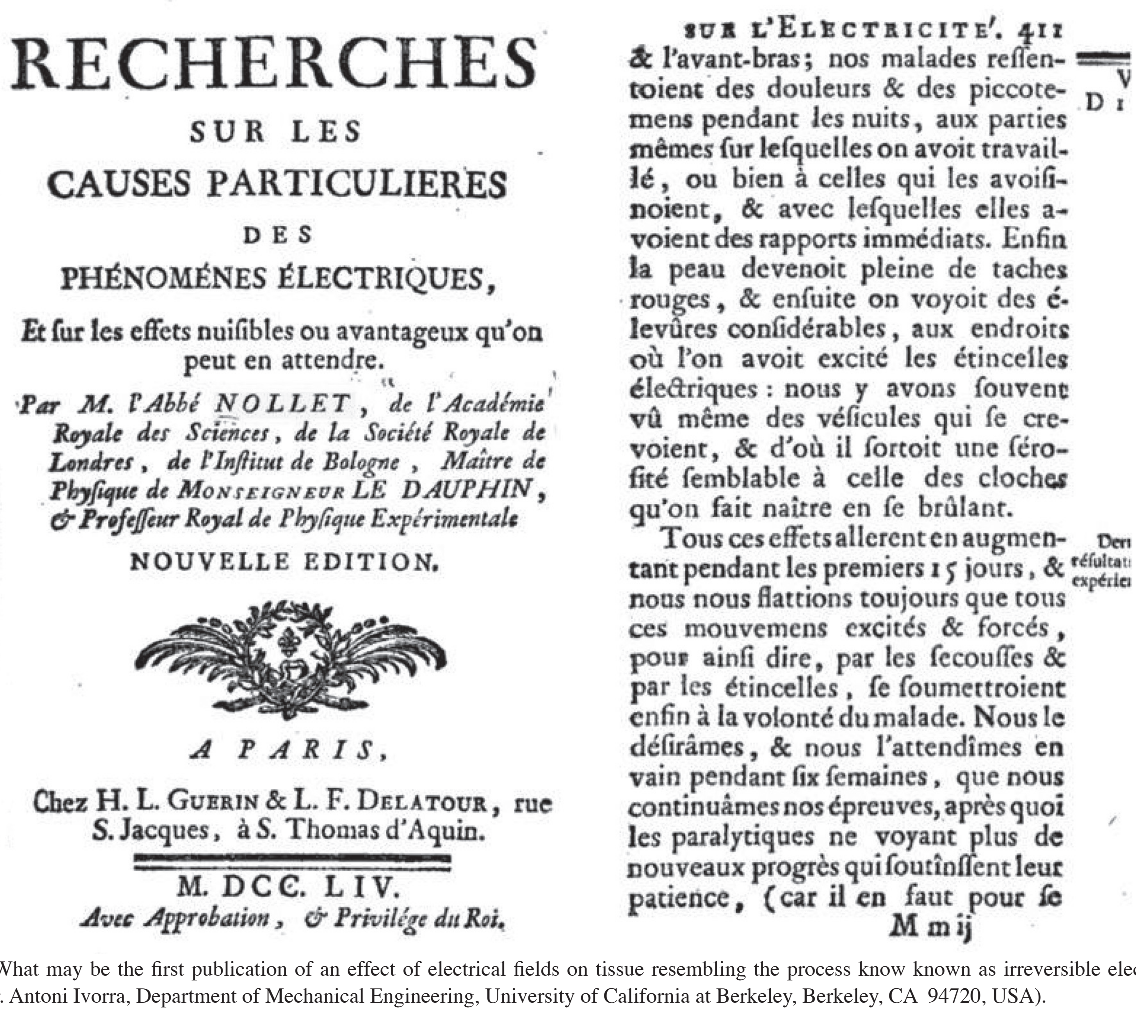
irreversible electroporation in medicine and represents the state of the art in this promising and emerging field.

A historical review shows that irreversible electroporation may have been observed as early as 1754 when Nollet studied the discharge of a static electrical generator on the skin (20) (Fig. 1). Other studies that are possible candidates for a designation as the first studies on irreversible electroporation are the 1898 publication of Fuller, in which it is reported that multiple high voltage discharges have bactericidal effect on a water sample (21), or the 1903 study of Rockwell who finds that "Under the discharges of the Leyden jar the red corpuscles change their shape and lose their color," which may be hemolysis induced by irreversible electroporation (22). Some of the first systematic studies that describe phenomena typical of irreversible electroporation were done on myelinated nerve tissue such as the 1956 work of Frankenhauser and Widen (23), who base their work on the 1898 work of Biedermann (24), and whose results were confirmed by Stampfli and Willi in 1957 (25). The seminal study on irreversible electroporation is a series of three papers by Sale and Hamilton who demonstrate a non-thermal lethal effect of high electrical fields on organisms in suspensions (26-28). In their papers, they also conclude that the cell membrane is damaged when trans-membrane voltages of around 1V are reached. They base this calculation on the theoretical potential of an insulating sphere in a conducting medium in an analysis that has become classical in the field of electroporation. They also observe, probably for the first time, leakage of intracellular contents following irreversible electroporation.

While ignored by medicine, until recently, irreversible electroporation has been used in the food industry for sterilization and preprocessing of food since the 1961 work of Doevenspeck (29). A recent review of the state of the art of this important use of irreversible electroporation was published in 2006 (30). Interestingly, it also appears that most of the medical applications which engage thermal effects of electrical fields in tissue ablation also produce electroporation. For instance, in 1978 Belov, most likely not aware of earlier work on electroporation, has proposed that coagulation induced by electrosurgical devices is related to cell membrane breakdown caused by excessive voltage (31). Lee shows, in


RECHERCHES SUR LES CAUSES PARTICULIERES DES PHÉNOMÉNES ÉLECTRIQUES, Et ſur les effets nuiſibles ou avantageux qu'on peut en attendre.

Par M. l'Abbé NOLLET, de l'Académie Royale des Sciences, de la Société Royale de Londres, de l'Inſtitut de Bologne, Maître de Phyſique de MONSEIGNEUR LE DAUPHIN, & Profeſſeur Royal de Phyſique Expérimentale

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SUR L'ELECTRICITÉ. 411

& l'avant-bras; nos malades reſſentoient des douleurs & des piccotemens pendant les nuits, aux parties mêmes ſur leſquelles on avoit travaillé, ou bien à celles qui les avoiſinoient, & avec leſquelles elles avoient des rapports immédiats. Enfin la peau devenoit pleine de taches rouges, & enſuite on voyoit des élevûres conſidérables, aux endroits où l'on avoit excité les étincelles électriques : nous y avons ſouvent vû même des véſicules qui ſe crevoient, & d'où il ſortoit une féroſité ſemblable à celle des cloches qu'on fait naître en ſe brûlant.

Tous ces effets allerent en augmentant pendant les premiers 15 jours, & nous nous flattions toujours que tous ces mouvemens excités & forcés, pour ainſi dire, par les ſecouſſes & par les étincelles, ſe ſoumettroient enfin à la volonté du malade. Nous le déſirâmes, & nous l'attendîmes en vain pendant ſix ſemaines, que nous continuâmes nos épreuves, après quoi les paralytiques ne voyant plus de nouveaux progrès qui ſoutînſſent leur patience, (car il en faut pour ſe

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Figure 1: What may be the first publication of an effect of electrical fields on tissue resembling the process know known as irreversible electroporation (courtesy Dr. Antoni Ivorra, Department of Mechanical Engineering, University of California at Berkeley, Berkeley, CA 94720, USA).

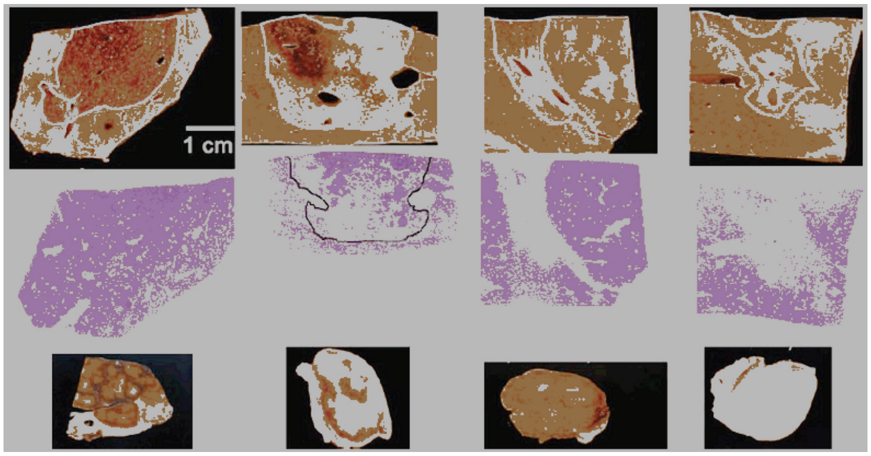

Figure 2: Experimental results from the application of irreversible electroporation showing the treated tissue as a function of time after the application of the treatment. Each colums shows: top macroscopic gross histology, middle macroscopic H&E histology and bottom lymph node. The areas marked in the top raw are those affected by irreversible electroporation. It is interesting to note that the affected areas are continuous to the margin of large blood vessels. The columns are from left to right in days after electroporation: one, three, seven, and fourteen. It is interesting to note that rapid resolution of the lesion without scars. The bottom row shows the respective lymph nodes. It is evident that the first day after electroporation the node is active and inflamated and it resolved and returns to normal the fourteenth day. From (37).

series of studies, that electrical fields produced tissue damage, which in the past was attributed only to thermal effects, is also affected by irreversible electroporation that occurs in superposition with the thermal effects, *e.g.*, (32, 33).

The fact that every electrical field produces a thermal effect, the so-called Joule effect, is not disputed. It is also undisputable that certain electrical field can produce irreversible electroporation. Studies have shown that certain electrical fields which produce thermal damage can also induce irreversible electroporation. The question that was raised in an analytical study by Davalos, Mir and Rubinsky is whether irreversible electroporation can be isolated from thermal effects and used by itself to produce substantial volumes of tissue ablation in vivo, with negligible thermal effects (34). Their finding that irreversible electroporation can be used as an independent modality for ablation of substantial volumes of tissue was subsequently confirmed in studies on cells (35), small animal models (36) and in large animal models in the liver (37) and the heart (38). Perhaps some of the most important aspects of the findings in these papers are that irreversible electroporation produces a well defined region of tissue ablation, without areas in which the extent of damage changes gradually as during thermal ablation. A cell is either destroyed by irreversible electroporation or not. Furthermore, it is observed that irreversible electroporation affects only the membrane of living cells and spares the tissues scaffold. Irreversible electroporation does not cause the denaturation of proteins typical to thermal ablation and is not affected by blood flow. The work of Rubinsky *et al.* (37) was the first to highlight some of the essential clinical features of irreversible electroporation such as the ability to spare large blood vessels and bile ducts, rapid tissue regeneration because of the intact large blood vessels scaffolds, rapid activation of the immune system, no scarring and the potential ability to treat tumors near large blood vessels. Figure 2 shows some of the results of IRE in the liver from reference (37). The present issue of TCRT further expands on the use of irreversible electroporation as a new method for tissue ablation and highlights important areas of research and applications in this field as well as the state of the art in those areas.

A fundamental understanding of the cellular scale phenomena in the permeabilized tissue during irreversible application is important in the design of optimal electroporation protocols. Esser *et al.* in this issue present a first mathematical model that describes the electrical fields and the dynamics of pore formation during irreversible electroporation in a multi-cellular system composed of irregular cells at a 100 micron spatial scale and at a 200 mm spatial scale (39). This study provides insight into the cellular scale phenomena during irreversible electroporation, which was lacking until now. Practical application of irreversible electroporation will most likely require treatment planning, which requires finding the electrical fields in the treated region as well as the temperature distribution. Edd and Davalos employ continuum models to illustrate and instruct in the design of irreversible electroporation treatment protocols (40).

The potential clinical applications of irreversible electroporation are numerous. It is likely that irreversible electroporation will become an important tissue ablation tool in surgeon's armamentarium. Lee *et al.* present image guided percutaneous irreversible electroporation in the liver, as a promising method for treatment of liver cancer (41). Their results illustrate the hallmarks of irreversible electroporation. It is technically a simple procedure, requiring only the insertion of electrode needles, it is extremely fast, microseconds to milliseconds, can be monitored with ultrasound, produces a sharp demarcation between treated and untreated regions and affects only cells while sparing the connective structure. Onik *et al.* show the use of irreversible electroporation in the treatment of the prostate, which is a potentially important application of irreversible electroporation (42). Some of the highlights of their findings are that the margins of the treated are very distinct with a narrow transition from complete necrosis in the treated region to the normal tissue. There was rapid resolution of the treated area and structures such as the urethra, larger blood vessels, nerves and the rectum were not affected by the parameters used for irreversible electroporation in this study.

Some of the unique aspects of irreversible electroporation are addressed by Maor *et al*. (43) and Al-Sakere *et al*. (44). The observation that irreversible electroporation spares the vascular structure gives hope to the use of this technique in treatment of tumors abutting large blood vessels. The study of Maor *et al*. shows that a major blood vessel, the carotid artery, remains structurally intact and the animal survive for 28 days (until the termination of the experiments) the application of extremely large irreversible electroporation pulses applied directly on the blood vessel. Al-Sakere *et al*. confirm that the immune system is not instrumental in the irreversible electroporation efficacy and therefore may be a treatment modality of interest to immune-depressed cancer patients.

Irreversible electroporation devices present technical challenges in designing devices for the delivery of the appropriate electrical fields. Bertacchini *et al*. address this technological challenge in their description of the first irreversible electroporator, its architecture, design choices and safety considerations (45).

Irreversible electroporation is a new tool in the surgeon's armamentarium. It has some unique advantages and present new challenges. Irreversible electroporation is technically simple to use and suitable for minimally invasive surgery. The procedure is extremely rapid, micro to milliseconds, and the ablated area is continuous and well defined. It affects only the cell membrane and has the potential of sparing structure. It lends itself to treatment planning and unlike thermal ablation techniques is not affected by blood flow. Many challenges remain. Fundamental understanding of the mechanism of irreversible electroporation and cell damage as well as mathematical models need to be further advanced. Numerous clinical applications that take advantage of the special attributes of irreversible electroporation can be developed and much work remains in optimizing the applications studied so far. For instance, could the structure sparing ability and the well defined area of application of irreversible electroporation be used in treatment of elusive cancers such as in the prostate, the lung and the brain? New device technologies will need to be developed to fit new applications. While much research and work remains to be done on irreversible electroporation, the future of this field, as illustrated by this issue of "Technology in Cancer Research and Treatment", appears promising.

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