

# Ultrashort high voltage Research and

by Roland Glaser

It has been known for a long time that electric field pulses in the magnitude of order of some kV/m and microsecond duration lead to a disruption of the cell membrane in cell suspensions. This method, in literature called 'electric breakdown', 'electroporation', or 'electropermeabilization', can be explained by the short-term increase in the membrane potential of cells to amounts of over one volt. It is widely used in different areas of biotechnology and therapy as a method for the infiltration of molecules into cells. Some years ago, the laboratory of **U. Zimmermann** in Würzburg began to apply nanosecond pulses of higher field strengths in order to influence also smaller structures, including cell organelles (Müller, K.J., Sukhorukov, V.L., Zimmermann, U.: Reversible electropermeabilization of mammalian cells by high-intensity, ultra-short pulses of submicrosecond duration. *J. Membrane Biol.* 2001; **184**, 161-170). This shortening of pulse length and the simultaneous increase of field amplitude to amounts of more than one MV/m open whole new possibilities for the application of this method in biotechnology.

This year's BEMS meeting gave much attention to this research area. It was addressed in a plenary lecture of K.H. Schönbach (Center for Bioelectrics, Old Dominion Univ., Norfolk, USA) titled: "Pulsed HV Research" (HV = High Voltage), as well as in Session I: "Pulsed HV Research & Applications" where 6 lectures dealt with it. In addition, three lectures of Ses-





# pulses: applications

sion V, titled: "THz Applications in Biology and Medicine" addressed the issue of nano pulses. Different posters basically presented in extended form the results introduced in the lectures. Moreover, there was a "Tutorial Session" hosted by the U.S. Air Force, a side event of the congress taking place in the afternoon of June 24 where more than a dozen referees reported on the results of their research work within the AFOSR-MURI program (MURI = Multidisciplinary University Research Integration) sponsored by the military, in lectures strictly limited to 5.5 min (!). In the following, there was a non-public consultation on further activities within this program. In this report, we will try to summarize the foci of this research field being of interest here, based on the results of the different working groups that were presented more than once, but in different detail and with different emphasis.

What is the military's interest in this kind of research? A representative of the Air Force tried to answer this obvious question at the beginning of the MURI symposium, a bit evasively, by using the following analogy: Way back in the fifties of the past century, the military had promoted research into laser technology too without there being a direct benefit for the military. But there is a clear difference with the present situation which was addressed by the chairman of Session I in his introduction to the lectures: Pulsed HV research is about effects of direct voltage pulses,

not pulsed microwave radiation. Cells are positioned at millimeter distances or even narrower gaps between metal electrodes, and more or less rectangular pulses are applied. This is very different from microwave kV-pulses which can be radiated over great distances. In contrast to microwave pulses, a military use of HV pulses really is difficult to imagine.

In his instructive lecture, K.H. Schönbach demonstrated the basic idea and the technical realization of this 'supraelectroporation', as it was also called. If pulse length is large compared to the time constant required by the membrane to act as a condensator for electrical charging, then the change in the membrane potential is proportional to the applied external field strength and the radius of the cell. This is true for rectangular pulses longer than approx. one microsecond. In this stationary state, the membrane is polarized, whereas the inner cell mostly is field-free. It is different with nanosecond pulses. During this time span, the membrane condensator just starts to charge. At first, the cell membrane potential is increasingly affected. On the other hand, cell organelles, such as the nucleus, mitochondrias and other membrane-enclosed areas, are affected by the electric field within the cell. These small vesicles have a smaller time constant of capacitative charging, on the other hand they are obviously more susceptible to electric breakdown, due to the membrane bending being more pronounced. Of course, the field strength of the

pulse has to be higher than at a simple 'breakdown' of the cell membrane. At the same time, we have to keep in mind that the monopole input pulse transforms into a bipolar pulse in the inner cell, due to the capacitive coupling of the inner milieu with the external field via the cell membrane. Schönbach depicted the relation between pulse length and pulse height being required for the manipulation of the different membrane systems. Due to the short length of the pulse, temperature changes of more than 1° K are not to be expected. The actual field strength inside the cell can be made evident by ultrashort laser-fluorescence photometry applying corresponding dyes.

Combinations of short-sequence pulses of different lengths applied in are of interest too. If the cell membrane at first is disrupted by a longer pulse of smaller field strength, a subsequent short pulse will find it easier to affect the inner organelles of the cell. This has been confirmed in exemplary experiments investigating calcium release from intracellular vesicles or permeation of fluorescent dyes through the double membrane of the nucleus. The opening of the nucleus membrane is assumed to be caused by the electrical impact on pore proteins rather than by the disruption of the lipid double membrane. However, the details of this process are still unclear.

The first lecture of the session devoted to this topic, held by **J.C. Weaver**, introduced a model allowing the presentation of field distribution within the cell at application of different types of pulses. Repeatedly applied by the author, it is a two-dimensional model consisting of a network of very small RC circuits (transport lattice approach) simulating membranes and the electrolyte milieu of the cell and its internal structure. In contrast to the comments made by Schönbach, Weaver thinks that ns pulses can also disrupt the cell membrane. Due to the extremely high field strengths, they are sufficient for a breakdown to take place, despite polarization of the cell membrane just

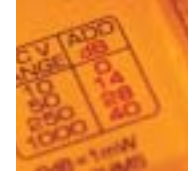
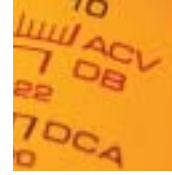
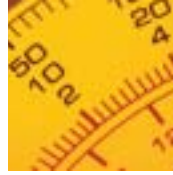
beginning in the nanosecond range. Obviously, the membranes of mitochondrias are relatively resistant to external pulses, since their capacity is much larger than that of the nucleus and other vesicles, due to its high degree of folding.

The other contributions of the sessions basically emphasised aspects addressed in the plenary lecture, not surprisingly so, as Schönbach was the co-author of most other referees. So **Tseng** et al. reported on gene expression in human HL-60 cells after 3 pulses of 60 ns length, with a field strength of 1.5 MV/m. 603 of the 20,498 examined genes were up- or down-regulated at least by two-fold. However, this did not result in cell apoptosis. And there were neither changes in cell characteristics in series.

**Beebe** et al. examined the intensity threshold of pulses leading to cell apoptosis. They too could detect the triggering of different signalways by the applied pulses.

**Pakhomov** et al. dealt with the question of quantitative evaluation of pulse treatment as a function of duration, amplitude and number of applied pulses. Apart from the wide biological variability of cells, dependent on their actual stage in the cell cycle, cells of different cell lines naturally can be distinguished from each other. At least, typical dose-effect curves could be recorded by comparing survival rate of cells to the absorbed dose in J/g. Though the mechanisms of cell death caused by HV pulses are still unclear, the author draws at least formal parallels to effects of ionizing radiation.

Various posters and short reports of the **MURI symposium** addressed the physical characterisation of the applied pulses and the electrochemical processes occurring at the electrodes. Of course we cannot expect pulses to be really rectangular, due to the switch mechanisms (MOSFET switch, mechanical devices), electrode kinetics and, not last, the dielectric properties of cells themselves. Instead, Fourier analysis demonstrates frequency summation - though usually



not exceeding the 10 MHz limit. So sometimes the term 'ultrawideband electromagnetic radiation' is applied here (= UWB-EMR; Natarajan et al.). Of course, it means the same as the term 'ns pulse'. But in these studies, only electronically detectable pulse forms were analyzed. When considering that even measurement electrodes have their own impedance kinetics, one realizes that the actual temporal field course at the respective membrane can scarcely be detected with precision. At the same time, field distribution across the applied systems, as well as expected temperature leaps were analyzed theoretically. The optimization of electric and electrochemical systems for the application of these pulses is one of the foci of future research.

In this research program, much relevance is of course given to the question of actual interaction mechanisms of these pulses. On the one hand, this is a question of appropriate methods being fast enough to detect processes occurring during or immediately after the pulse. On the other hand, it is about reactions triggered by the pulse, but manifesting only in the course of hours and days after the treatment. The MURI symposium impressively showed how to cooperatively address a current issue on a broad multidisciplinary basis, when sufficient funds are available. We can be sure that this will help to promote sustained development of the nanosecond-pulse technique and respective biotechnological applications.

### **THz or T radiation and its application in medicine, biology (and security!)**

The Bioelectromagnetics Society has widened its scope of interest to higher frequency spectrum of electromagnetic fields. Whereas the upper limit of 'non-ionizing radiation' before was set at 300 GHz and the range above, up to ionization by ultraviolet light, was left to photobiologists, the range of interest was elevated by two powers of ten at this year's conference. Thus, the BEMS follows up on a worldwide trend in

technology and research entering this terra incognita as documented in two plenary lectures and a session titled: "THz Application in Biology and Medicine".

Due to the newness of this issue, there was a need for broad information on the properties of this type of radiation, the technology to produce and detect it, its application in biology and medicine and, eventually, its potential biological effects. It was provided by the lectures of **M. Chamberlain**, University of Durham (UK): "Application of Tetrahertz-Frequencies Radiation in Medicine and Biology", and of **R. Osiander**, John Hopkins University, Laurel, USA: "THz Research: An Overview".

Also in this context, **G.P. Gallerano** (ENEA-UTS Tech-Fisiche Avanzate, Frascati, Italy) presented the "THz BRIDGE" project of the European Union bundling the cooperation of 10 laboratories in 5 EU countries and Israel. The name BRIDGE does not only stand for the bridging function of this frequency range between GHz fields and infrared, but is also an acronym for: "THz radiation in biological research, investigation on diagnostics and study on potential genotoxic effects". Chamberlain reminded the attendees that the wish to build a bridge between the oscillations of electro-technics and the frequencies of optics is quite old. He mentioned a publication titled: "Heat Ray of Great Wavelength" (Phys. Rev.) of **Rubens** and **Nichols** published as early as in 1897. The authors complained that, despite many tests, electric oscillations were not fast enough and wavelengths of infrared light were not large enough to bridge this gap within the spectrum of electromagnetic waves. In fact, according to the referee, you cannot exceed 300 GHz, i.e. 0.3 THz, by transistors or similar components of solid state physics, whereas lasers scarcely reach the range below 30 THz.

Therefore, this frequency range has remained largely unknown for a long time. Then, about 10 years ago, THz pulses could be beat out of semiconductor crystals by applying fast and intensive laser pulses – a

huge effort Chamberlain illustrated by a cartoon showing a Porsche driven against a wall to beat a few bricks out of it. In fact, these are huge installations filling up much space. A breakthrough was achieved by **A. Tredicucci** from Pisa, Italy, who succeeded some years ago, in cooperation with **G. Davis** of the University of Cambridge, to generate radiation in the 4.4 THz range by means of a THz laser composed of several nanolayers. However, this small device requires the temperature of liquid nitrogen for functioning.

At present, besides generation also detection and measuring of THz fields are an area of intense research and development. In this context, Osiander mentioned the bolometer as a classic indicator based on heat development in a broad frequency range, electronic detectors, photoactive dipoles and electro-optical crystals.

The first THz range scans were sufficient to obtain research funds from anti-terror programs - an aspect especially dealt with in the lecture of R. Osiander. Actually, the emission maximum reached over the frequency course of 300 K body heat radiation is several 10 THz. THz radiation emitted by the human body penetrates clothes so that a fully clothed looks naked in the THz image. This way, objects concealed under clothes not visible to currently used methods can be identified.

But let's return to the area of bioelectromagnetics. What are the characteristics of this radiation? How can it be used in biomolecular analysis? Its most important property certainly is the strong absorption of radiation by water. On the one hand, this limits its application in communications and, of course, in biology and medicine. But on the other hand, THz spectroscopy obviously is useful to examine the water content of cells and e.g. of leaves. However, for spectroscopic analyses and cellular studies water-free preparations or extremely thin layers of biological matters are required.

In moist tissue, the penetration depth of T radiation is about 5 to 6 mm. In dry tissue, there is a maximum of 11 mm, and in fat layers penetration depths of 20 to 30 mm can be reached. Osiander – surely hoping for larger funds from American anti-terror programs – presented further data on the penetration of different types of clothes by THz fields. Both plenary lectures, as well as the contribution of **Crawley** et al., demonstrated possibilities to screen teeth and obtain images of dental caries of much higher resolution than that of x-rays. However, so far the technique has been practised only on extracted teeth. The practical application of this method in dentistry is still in the future, as long as neither THz sources nor imaging devices have the small size required to provide images of teeth in the mouth of patients.

Of particular interest are various methods of THz spectroscopy of organic molecules. The referees mentioned the high specificity of vibrational spectra of amino acids, proteins and ribonucleic acids. This aspect was not only mentioned in the plenary lectures but also by **A. Oka** et al. from Japan, in the first lecture of the THz session. There is even talk of 'fingerprint' rotational spectra, especially in gas phases. Also here, Osiander emphasized the possibility to find explosive traces (anti-terror programs!) and ground mines. They should be visible from distances up to 10 m. He pointed to the mined rice fields in Cambodia, the 300 people killed or injured by mines per month in Afghanistan, etc.

Especially the application of THz Time Domain Spectroscopy (TDS) not only promises new insights into the detection of specific amino acids and possibly even DNA sequences without specific markers being required, but also important information on protein interaction and their conformational changes. This is mainly due to the fact that the oscillation frequency of hydrogen peroxide bridge bindings is within the range of THz frequencies enabling proof and the study of dynamics by TDS. A problem remains of course the



necessity to eliminate disturbances due to the water content. It is one of the foci of the European THz BRIDGE Project to create a file of spectroscopic parameters of selected biomolecules in the frequency range between 0.1 and 20 THz.

There were detailed reports on possible future applications of THz radiation in cancer diagnostics. **Chamberlain** e.g. presented the possibility to detect melanoma and basal cell carcinomas. There are 50,000 new patients with these diseases every year in the United Kingdom. Evidence given by THz would be fundamental in early diagnostics and in the imaging of the area actually to be removed in surgery up to a depth of 1 cm. He announced that also other types of epithelial carcinomas could be identified this way. This method could also help in diagnosis of mamma carcinomas. Obviously, the water content of tissue is crucial when trying to distinguish healthy from cancerous tissue. But differences in the absorption and diffusion of THz radiation between healthy and damaged tissue have yet to be exactly determined. In this context, Chamberlain mentioned the chance for development of THz microscopy.

What would the effect of this type of radiation on biological tissue be? This question naturally is closely linked to the issue of limits and safety standards. These issues are given much attention in the European project. There is of course the question of specific frequency windows associated with the measured absorption spectra. Chamberlain referred to the Fröhlich hypothesis from 1977 which then prompted a frantic search for sharp windows of efficiency in the microwave range. As we all know, this theory predicted such frequency specifics for the high GHz range. But despite initial positive appearing results, the existence of such frequency windows in the GHz range could not be confirmed. Will they perhaps be found in the frequency spectrum of T radiation? Further research will show. But thinking back to the objections raised against the Fröhlich hypothesis which are op-

posed to, the probability of finding such windows in this frequency range. In spite of that, the old question about non-thermal effects in this frequency range will be raised anew. The BRIDGE project therefore puts the emphasis on studies investigating potential genotoxic effects of this type of radiation. There are only preliminary results yet that will have to be verified.

The safety recommendations of ICNIRP and corresponding laws and decrees of single countries on non-ionizing radiation refer to the frequency range up to 300 GHz. In the face of the new development, they have to be extended giving special attention to the characteristics of THz pulses. The BRIDGE project plans to investigate doses of exposure of staff being involved in the development of THz-emitting devices and their application.

Overall, this seems to be a whole new area of research, which is perhaps less relevant for communications technology than for medicine and biotechnological analyses. In any case, the technological progress made in the development of smaller, cheaper and more powerful THz sources, as well as of detection devices, is important. It is impressive to see how biomedical research is right on the heels of technological development.