REVIEW

Electroporation: theory and methods, perspectives for drug delivery, gene therapy and research

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Abstract

Electroporation designates the use of short high-voltage pulses to overcome the barrier of the cell membrane. By applying an external electric field, which just surpasses the capacitance of the cell membrane, transient and reversible breakdown of the membrane can be induced. This transient, permeabilized state can be used to load cells with a variety of different molecules, either through simple diffusion in the case of small molecules, or through electrophoretically driven processes allowing passage through the destabilized membrane – as is the case for DNA transfer. Initially developed for gene transfer, electroporation is now in use for delivery of a large variety of molecules: From ions to drugs, dyes, tracers, antibodies, and oligonucleotides to RNA and DNA. Electroporation has proven useful both *in vitro, in vivo* and in patients, where drug delivery to malignant tumours has been performed. Whereas initial electroporation procedures caused considerable cell damage, developments over the past decades have led to sophistication of equipment and optimization of protocols. The electroporation procedures used in many laboratories could be optimized with limited effort. This review (i) outlines the theory of electroporation, (ii) discusses factors of importance for optimization of electroporation protocols for mammalian cells, (iii) addresses particular concerns when using electroporation *in vivo,* e.g. effects on blood flow and considerations regarding choice of electrodes, (iv) describes DNA electrotransfer with emphasis on use in the *in vivo* setting, and (v) sums up data on safety and efficacy of electroporation used to enhance delivery of chemotherapy to tumours in cancer patients.
Keywords drug delivery, electrochemotherapy, electrogenetransfer, electroporation, genetherapy.

Based on theoretical studies and experiments with bilayer membranes in the 1960ies and 1970ies, the first publication of gene transfer into murine cells was authored by Neumann *et al.* (1982). That experiment was performed in a custom-built electroporation chamber. In this early stage, some researchers used short-circuited electrophoresis apparatuses as primitive electroporation equipment.

Exponentially decaying pulse electroporators were then developed, and electroporation, in particular, for DNA transfer to bacteria became widespread. When creating transfected clones, in principle, only one surviving, transfected bacteria is needed and the heap of dead bacteria next to it is not of importance. Therefore, bacterial transfection protocols focused little on cell viability after electroporation and this is likely to have caused electroporation to be conceived as a rather violent means of achieving gene transfer.

During the 1980s, several studies on the use of electroporation for delivery of molecules to eucaryotic

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cells in the *in vitro* setting were reported (e.g. Melvik *et al.* 1986, Mir *et al.* 1988); from the early 1990s, studies on *in vivo* electroporation also came forth (e.g. Okino & Mohri 1987, Belehradek *et al.* 1991, Mir *et al.* 1991, Salford *et al.* 1993, Heller *et al.* 1995). The first clinical study on the use of electroporation to increase uptake in tumours of a chemotherapeutic agent was reported by Belehradek *et al.* (1993), and since then several studies have been published on this aspect (e.g. Heller *et al.* 1998, Mir *et al.* 1998, Panje *et al.* 1998, Gehl & Geertsen 2000, Sersa *et al.* 2000a).

Over the last two decades, electroporation equipment has been refined. Notably, square wave pulse generators have been constructed. These generators modify the exponential pulse to a square pulse, where pulse amplitude and pulse length can be independently controlled, an important prerequisite for optimization. There are ongoing efforts to optimize delivery of molecules while preserving cell viability through various technical optimizations, resulting in new equipment now commercially available or under way.

Theory of electroporation

The process of electroporation

The transmembrane potential induced in a cell by an external field is generally described by the equation:

$$\Delta V_m = f E_{ext} r \cos \Phi \quad (1)$$

where V_m is the transmembrane potential, f a form factor describing the impact of the cell on the extracellular field distribution, E_{ext} the applied electric field, r the cell radius and Φ the polar angle with respect to the external field. The value for the factor f is listed by many authors as 1.5; however, this factor is dependent on a number of different factors (see, e.g. Kotnik *et al.* 1997, 1998).

Electroporation is achieved when the ΔV_m superimposed on the resting transmembrane potential is larger than a threshold, ΔV_s As the bilayer membrane is a common feature for eucaryotic cells, ΔV_s is similar for various cell types. ΔV_s is generally reported to be in the order of 1 V (Kinosita & Tsong 1977a), although an experimental and theoretical study has later described it as being 200 mV (Teissie & Rols 1993).

A very general consideration is that the smaller the cell radius, the larger the external field needed to achieve permeabilization (eqn 1). For this reason, the electric fields necessary to permeabilize mammalian cells are considerably lower than those needed to permeabilize, e.g. bacteria. It is also evident that, e.g. mitochondria or other intracellular organelles will not be permeabilized by E-fields of the size sufficient to permeabilize the cell membrane.

A new aspect of electroporation is, however, that by using extremely short pulses in the nanosecond range at very high voltages, cellular organelles can be electroporated without the cell membrane being permeabilized. This is possible when using pulses so short, that the charging time of the cell membrane is not reached (see Schoenbach *et al.* 2001). Equipment for this type of electroporation is still in experimental set-up only.

The resting transmembrane potential is important not only for the threshold for permeabilization but also for the sequence of events. Permeabilization will initially happen at the pole of the cell facing the positive electrode, because owing to the negative interior of the cell this is where the capacitance of the membrane is first exceeded when an external field is applied (Fig. 1). The second event is permeabilization of the pole of the cell facing the negative electrode (Fig. 1). The *extent* of permeabilization (area of membrane which is permeabilized) on the pole facing the positive electrode can be controlled by pulse amplitude, i.e. the higher the pulse amplitude, the

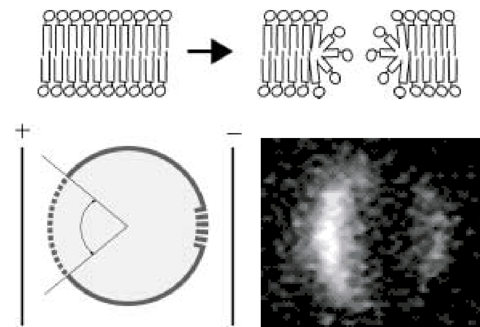

Figure 1 Top panel: Electroporation occurs when an applied external field exceeds the capacity of the cell membrane. It is proposed that water enters the cell membrane during the dielectric breakdown and that transient hydrophilic pores are formed (for reviews, see Weaver 1994, Neumann *et al.* 1999). The formation of permeable areas happens in the frame in less than a second whereas resealing happens over minutes. Bottom left: As the resting transmembrane potential is negative on the inside respective to the outside, the first part of the membrane that will be permeabilized is the pole facing the positive electrode. The area of the membrane permeabilized is larger the higher the pulse amplitude, whereas longer pulses will cause greater perturbation of the area permeabilized. See text for further details. Bottom right: The positive electrode should be imagined in the left of the picture and the negative electrode on the right. One 20-ms pulse of 1 kV cm^{-1} was delivered to a CHO cell suspended in medium containing propidium iodide. The image was recorded less than 40 ms after the pulses using a rapid ultra-low-light camera, as described by Gabriel & Teissie (1997). This experiment was performed by B. Gabriel, who kindly permitted reproduction.

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greater the area through which diffusion can take place (Gabriel & Teissie 1997). The *degree* of permeabilization can be controlled by the pulse duration (and also pulse number), i.e. the longer the pulse the greater the perturbation of the membrane in a given area (Gabriel & Teissie 1997). It has been shown that the area of the membrane being permeabilized is larger on the pole facing the positive electrode, but the degree of permeabilization is greater on the cell pole facing the negative electrode (Tekle *et al.* 1990). Thus, larger molecules will be able to diffuse into the cell at the pole facing the negative electrode, but the area over which diffusion can take place is larger by the positive electrode (Tekle *et al.* 1990).

For small molecules, diffusion alone is the cause of intracellular uptake, and uptake takes place regardless of charge (Neumann *et al.* 1998). With increasing molecule size, uptake is affected but molecules up to the size of antibodies and dextrans have been loaded by electroporation (see, e.g. Glogauer & McCulloch 1992, Verspohl *et al.* 1997).

The uptake of DNA is not a result of diffusion, as evidenced by both *in vitro* studies (e.g. Golzio *et al.* 1998) and *in vivo* studies (e.g. Mir *et al.* 1999). It has already been shown that electrophoretic forces are important for successful DNA transfection by electroporation (Sukharev *et al.* 1992). Later, it has been shown that, in a study using DNA in suspension under an artificial bilipid layer, when applying the electric field, the presence of DNA facilitates pore formation through direct interaction with the membrane (Spassova *et al.* 1994).

An electrophoretic effect of the field causes the polyanion DNA to travel towards the positive electrode, and fluorescensce studies have shown that DNA enters the cell through the pole facing the negative electrode, where the membrane is more destabilized and where the field will drive the DNA towards the centre of the cell (Golzio *et al.* 2002). Once the DNA has entered the cell, there is active participation by the cell in the further processing of the DNA, as shown by ATP dependence of this step (Rols *et al.* 1998a).

In summary, because of the nature of electroporation, virtually *any* molecule can be introduced into cells. Consequently, electroporation has been used to introduce ions, dyes (Mir *et al.* 1988, Dinchuk *et al.* 1992, Gehl *et al.* 1998), radiotracers (Engstrom *et al.* 1999, Gehl *et al.* 1999), drugs (Belehradek *et al.* 1994, Sersa *et al.* 1995, Gehl *et al.* 1998, Jaroszeski *et al.* 2000a), oligonucleotides (Spiller *et al.* 1998), in addition to RNA (Teruel *et al.* 1999, Saeboe-Larssen *et al.* 2002) and DNA (Neumann *et al.* 1982, Heller *et al.* 1996, Rols *et al.* 1998b, Mir *et al.* 1999).

Membrane resealing

Whereas pore formation happens in the microsecond time frame, membrane resealing happens over a range of minutes.

Membrane resealing was first described in erythrocyte ghosts by Kinosita & Tsong (1977a). Since then, several authors have looked into membrane resealing *in vitro* (e.g. Rols & Teissie 1990, Lee *et al.* 1992). A very nice theoretical study based on empirical data has been published by Saulis (1997).

In vitro data have shown that resealing happens over the minute range (Rols & Teissie 1990), with variations depending on electrical parameters used and on temperature. It has also been shown that intact function of the cytoskeleton is of importance for pore closure (Teissie & Rols 1994).

In vivo data describing membrane resealing in mouse skeletal muscle tissue (Gehl *et al.* 2002) found the 63% resealing time (τ) to be approximately 9 min, which is similar to what was found in a previous *ex vivo* study of rat skeletal muscle (Bier *et al.* 1999).

A subject for discussion is the issue of irreversible permeabilization. It is clear that irreversible permeabilization ensues with the use of extremely strong pulses. But whether there are possibilities for slow recovery of cells after extensive permeabilization and loss of intracellular components is unclear. There are indications that cell death from electroporation is more likely to occur *in vitro* than *in vivo,* most likely because of the fact that loss of intracellular molecules would be larger when there is abundant extracellular volume than when the extracellular space is comparatively small, as is the case in tissues (Gehl *et al.* 1999).

Interestingly, it has been shown that the surfactant poloxamer 188 can increase the rate of resealing. It has been established that electroporation of muscle tissue plays an important role in the pathology of electric trauma (Lee *et al.* 1992). Efforts to alleviate the sequels of electric trauma have led to the search for 'membrane-sealing compounds', and the surfactant poloxamer 188 has a possible role in the management of electric trauma victims (Lee *et al.* 1992).

Optimization of *in vitro* electroporation protocols

Whereas cell survival is generally not a problem when working with bacterial transfections, this is a concern for transfection of mammalian cells. An important improvement was the construction of square wave pulse generators, where pulse duration and amplitude can be controlled separately. Optimization of protocols has led to a situation where high cell viability can be combined

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with high percentages of cell permeabilization and/or gene transfection.

It is achievable to have over 90% of cells permeabilized with less than 5% cell death (e.g. Gehl *et al.* 1998). Conditions traditionally used to achieve this are eight square wave pulses of 100 μs with a frequency of 1 Hz, using 1.2 kV cm^{-1} (e.g. 480 V if the electrode gap is 4 mm).

There are some issues that should be considered when performing *in vitro* electroporation:

1. *Cell size*. According to eqn 1, cell size is inversely correlated to the size of the external field needed to create permeabilization. Therefore, optimization for each cell type is necessary. From this also follows that the likelihood of a good result increases with homogeneity in cell size and conversely that for cell lines with very large variations in cell size (e.g. Ehrlich ascites cells) it is difficult to obtain good results. Likewise, cell orientation matters for cells that are not spherical.
2. *Temperature*. It has been shown that membrane resealing is strongly temperature dependent, with slow closure at low temperatures (Kinosita & Tsong 1977b). For DNA transfer, it has been shown that cooling at the time of permeabilization and subsequent heating in an incubator increases transfer efficacy and cell viability (Rols *et al.* 1994).
3. *Post-pulse manipulation*. Cells are vulnerable when in the permeabilized state, and it has been shown that waiting 15 min after electroporation in order to allow resealing before pipetting cells increases cell viability (Gehl *et al.* 1998). Cultured cells can as well be permeabilized while growing in, e.g. petri dishes with a simple set of steel plates inserted down into the dish (Zerbib *et al.* 1985); in this way manipulation is avoided.
4. *Composition of electrodes and pulsing medium*. For short pulses there is probably little need for concern about release of metal from the standard aluminium electrodes used in standard disposable cuvettes. However, when using long pulses this can be a concern. Some authors advocate the use of low-conductivity media for DNA transfer in order to increase viability and increase transfection efficacy (Rols *et al.* 1994). It is generally considered wise to be careful with calcium in the medium during electroporation in order not to cause sudden high intracellular levels of this electrolyte. However, some authors advocate the use of, e.g. calcium or magnesium in the buffer when performing DNA transfer as this will act as a positively charged 'glue' between the negatively charged exterior of the cell and the polyanion DNA, approximating the DNA molecules to the membrane prior to the electroporation procedure (Neumann *et al.* 1996).
5. *Heating – is not a problem*. It has been shown that when using standard electroporation conditions, heating is less than 1 °C (Bhatt *et al.* 1990).

Optimizing *in vitro* electroporation of cells in suspension can be done quite simply by using, e.g. propidium iodide (Sersa *et al.* 1995, Gabriel & Teissie 1997) or lucifer yellow (Mir *et al.* 1988, Dinchuk *et al.* 1992), and by using, e.g. eight pulses of 100 μs and increasing the voltage until the optimum is found. Such an assay should be accompanied by an investigation of cell death at appropriate voltages.

Some groups have designed particular electroporation systems to cover special needs. Examples of such innovative developments include the use of slides with inbuilt metal strips so that some but not all cells grown on the slide can be electroporated and the function of gap junctions be examined (Raptis *et al.* 1994). Also in culture electroporation of single nerve bodies has been described (Teruel & Meyer 1997).

In vivo electroporation

In vivo electroporation has been performed in a number of different species, either using plate or needle electrodes; an example of such electrodes is shown in Figure 2. *In vivo* drug and gene delivery has been carried out in liver (e.g. Heller *et al.* 1996, Ramirez *et al.* 1998), bladder (Kubota *et al.* 1996), brain (Salford *et al.* 1993, Nishi *et al.* 1996), muscle (e.g. Aihara & Miyazaki 1998, Gehl *et al.* 1999, Mir *et al.* 1999) and skin (e.g. Prausnitz *et al.* 1995, Dujardin *et al.* 2001). A recent book reviews various methods (Jaroszeski *et al.* 2000b).

Two important features of *in vivo* electroporation are discussed below.

Electric field distribution

When performing *in vitro* electroporation in cuvettes, there is a reasonably even electric field (E-field) distribution. Plate electrodes can also been used in the *in vivo* setting, but often needle electrodes are necessary. When using needle electrodes it is very important to take a look at the field distribution (see, e.g. Miklavcic *et al.* 1998, Gehl *et al.* 1999). As electroporation is done using fields of, e.g. 1 kV cm^{-1}, it is evident that 'whole-body electroporation' would be a rather dangerous idea. However, as the pulses are very short lasting, several applications can be used to cover a desired area.

Vascular effects

A particular feature of *in vivo* electroporation is that it causes hypoperfusion in the pulsed areas. We have suggested (Gehl *et al.* 2002) that this vascular effects is

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Figure 2 An example of plate and needle electrodes, as constructed at our institution. The plate electrodes are constructed of copper plates mounted on non-conductive material. The screw fixes the two plates in parallel position and different gaps can be obtained by combining plates mounted at different widths. The needle electrodes consist of parallel arrays of hypodermic needles inserted into a custom-made platform that ensures good electrical contact as well as stability and parallelism. We have used this type of electrodes both for *in vivo* work and in the treatment of patients. When performing *in vivo* electroporation, particular attention should be paid to E-field distributions. E-field calculations for the electrodes shown are presented in Gehl *et al.* (1999).

caused by (i) a reflexive constriction of resistance vessels leading to the electroporation area and (ii) a phase of interstitial oedema in interplay with reduced intravascular pressure because of permeabilization of endothelial structures. The reflexive constriction of arterioles last in the order of 1–2 min whereas the interstitial oedema resolves with membrane resealing. Interestingly, this vascular effect seems to be particularly prominent in tumours where the endothelial structures are less mature and the interstitial pressure is higher, and this can, in fact, be turned to an advantage in cancer treatment (Sersa *et al.* 1999, Gehl & Geertsen 2000, Cemazar *et al.* 2001).

The consequences of these vascular effects are given below.

The vascular lock. If a drug is present in the tissue before electroporation (e.g. after intratumoral or intramuscular injection), then the vascular effect of the electroporation will mean that *just at the time when the cells in the tissue are permeabilized,* the drug is retained in the area. We term this the vascular lock (Gehl *et al.* 2002).

Optimization of gene transfer. It has been shown that short perfusion delays are linked to reversible permeabilization, and that expression of transferred genes is optimal in this window. Therefore, determining perfusion delays can be a quick method to determine the approximate window of optimal gene transfer (Gehl *et al.* 2002).

An explanation for post-defibrillation cardiac ischaemia? DC-shocks used for defibrillation and cardioversion can cause electroporation of cardiac muscle cells (Tung *et al.* 1994). Interestingly, electrocardiogram ST-segment depressions, which are a diagnostic hallmark of cardiac ischaemia, can also be seen after ventricular defibrillation (Tung *et al.* 1994, Bardy *et al.* 1996). A randomized study on defibrillation equipment compared damped sine wave shocks and truncated biphasic shocks for transthoracic defibrillation (Bardy *et al.* 1996). The defibrillation success rate was equal for the two wave forms, but ST-segment depressions 10 s after the shock were significantly more prominent with the damped sine wave shocks, which utilized an applied voltage twice as high as the truncated biphasic shocks. The cause of defibrillation-induced ST-segment depression has not been established previously. From the data presented here, it would be logical to suggest that ST-segment depressions seen after defibrillation are related to vascular reactions to electroporation. Electroporation of cardiac cells may lead to arrhythmias because of transmembrane potential changes and ionic imbalances, an unfortunate event in critically ill cardiac patients. Assuming that there is a link between ST-segment depressions and vascular reactions to electroporation, optimization of defibrillation procedures could be performed by simply looking at the severity and duration of ST-segment depressions.

Relation to electrical injury. Electroporation of muscle and nerve play an important role in electrical injury (Lee & Kolodney 1987). Electroporation-induced ischaemia may also play a part in this.

Electroporation for DNA transfer

DNA electrotransfer

As previously mentioned, the transfer of DNA to cells is a process where the cell is permeabilized; subsequently DNA will be moved by electrophoretic forces and enter the cell cytosol during the following pulses. As a natural consequence of this, it has been shown that whereas small molecules can diffuse into permeabilized cells in the minutes before membrane resealing, there is no gene transfer if the DNA is added immediately after the pulse (Mir *et al.* 1999).

Optimization

Some effort has been put into optimization of conditions for DNA electrotransfer, and the latest develop-

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ment in equipment opens possibilities to design various pulse combinations. There are (at least) three ways to deliver pulses for DNA transfer:

1. Just short, high-amplitude pulses. Using a protocol similar to that used for delivery of small molecules (e.g. six pulses, 100 μs and 1.4 kV cm^{-1}), it is possible to obtain a reasonable efficacy and a low mortality (Heller *et al.* 1996).
2. Just long, low-amplitude pulses (e.g. eight pulses, 20 ms, 200 kV cm^{-1}) (Mir *et al.* 1999). The longer pulses have a better electrophoretic effect and therefore transfection rates can be increased.
3. Short, high-amplitude pulse followed by long, low-amplitude pulses (e.g. Bureau *et al.* 2000). This pulse combination is based on the concept that after permeabilization with a high amplitude pulse, long-duration low-voltage pulses can then drive the DNA across the destabilized membrane.

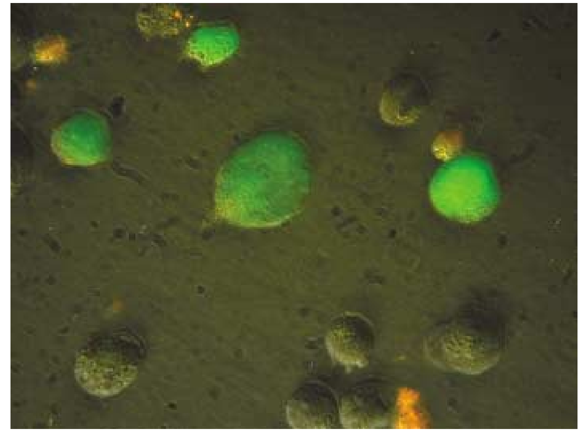

Figure 3 K562 cells transfected with the plasmid phGFPS65T (Clontech) encoding a modified Green Fluorescent Protein – the plasmid concentration was 25 mg mL^{-1}, and the electroporation parameters: six 99-ms pulses of 1.4 kV cm^{-1}. The picture was taken 48 h after transfection using a fluorescence microscope equipped with standard FITC filters (by Jens Eriksen, see acknowledgements).

Electrogenetransfer in vitro

The classic use of electroporation for gene transfer is transfection of bacteria – a topic which is not discussed in the present review.

Some important points for *in vitro* gene transfer to eucaryotic cells were described in the section on 'Optimization of *in vitro* electroporation protocols'.

Numerous papers deal with electrogenetransfer to mammalian cells, and a good description of methods can be seen in Jaroszeski *et al.* (2000b).

Electrogenetransfer in vivo

Use of electroporation for *in vivo* electroporation is rapidly attracting interest for several reasons:

1. The use of viral vectors is avoided, reducing safety problems and cost and avoiding the problems caused by antibody formation against the viral vector.
2. Electroporation for gene transfer *in vivo* has proven *highly efficient* (see Figs 3 and 4). In particular, in muscle tissue, where the myofibers neither die nor divide, the electroporation-induced gene transfers that are, in fact, to be considered as transient transfections become functionally permanent with *long-term expression* (Mir *et al.* 1999). Several authors describe up to two log increase in gene expression when comparing direct injection with electrogenetransfer (e.g. Aihara & Miyazaki 1998, Mir *et al.* 1999).
3. The area to which the gene is transferred is clearly delineated by the electrodes. Therefore, gene transfer can be targeted to a certain area, e.g. in the liver. *In vivo* electrogenetransfer has been reported in skin (Titomirov *et al.* 1991, Heller *et al.* 2001), muscle (Aihara & Miyazaki 1998, Mir *et al.* 1999), liver (Heller *et al.* 1996), brain (Nishi *et al.* 1996) and tumours (Rols *et al.* 1998b).

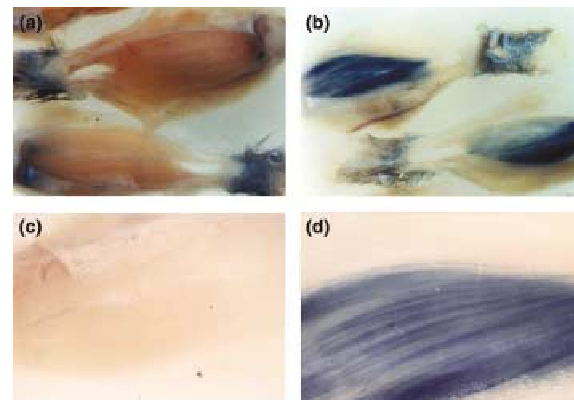

Figure 4 Several studies have now shown that *in vivo* gene transfer is simple and efficient. Although gene transfer by electroporation generally gives rise to transient transfections, gene transfer to skeletal muscle can lead to long-term expression as the muscle cells do not die or divide. An example is given of transfection of the tibial cranial muscle in mice with the gene encoding for beta-galactosidase (with permission, from Mir *et al.* 1999). Legs from mice injected with DNA without (a) or with (b) exposure to electric pulses (eight pulses, 200 V cm^{-1}, 20 ms, 1 Hz); c and d correspond to a and b, shown at larger resolution.

Use of electrogenetransfer in the clinical setting

Perspectives for gene therapy Given the above-mentioned advantages of electrotransfer, it is likely that this

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method will soon be put to use also in the clinical setting. Several uses of this technology can be envisaged, e.g. production of 'missing' proteins in various deficiency syndromes, production of cytokines in the treatment of malignant tumours or production of antigens from tumour cells, enhancing an antitumoural host response.

DNA vaccines. One possible use of *in vivo* gene transfer by electroporation is for vaccination (Glasspool-Malone *et al.* 2000, Somiari *et al.* 2000, Drabick *et al.* 2001, Liu & Huang 2002). It can be anticipated that with small electrodes local gene transfer can be performed giving rise to antigen production either in skin or skeletal muscle. A present problem in vaccination is the need to keep stores of vaccines fresh and to transport them cold, also in warm climates and under difficult conditions. As electroporation equipment can easily be transported and as DNA is very stable, this may facilitate vaccination programmes.

RNA transfection. Electroporation is also increasingly being used for RNA transfer. One particular cell type where RNA transfer has proven to be much more successful than DNA transfer is dendritic cells. Both on murine and human dendritic cells, high transfection efficiencies have been shown (Saeboe-Larssen *et al.* 2002, Van Meirvenne *et al.* 2002).

Clinical use of electroporation in the treatment of cancer

After the first report of the clinical use of electroporation (Belehradek *et al.* 1993), several studies have reported the use of electroporation for delivery of chemotherapy (electrochemotherapy) to cancer patients.

It stands to reason, that for lipophilic drugs there is no gain from permeabilizing the cell membrane (Gehl *et al.* 1998, Jaroszeski *et al.* 2000a). The two drugs that have been used in the clinical setting are bleomycin and cisplatinum. Bleomycin is a hydrophilic and charged molecule, which under normal circumstances, is only internalized in limited amounts (Mir *et al.* 1996). It is, however, a very cytotoxic drug. Acting as an enzyme each bleomycin molecule creates several DNA double strand breaks (Tounekti *et al.* 1993). With the use of electroporation to introduce bleomycin directly into the cell cytosol, its cytotoxicity can be increased 300–5000-fold (Orlowski *et al.* 1988, Gehl *et al.* 1998, Jaroszeski *et al.* 2000a). In the case of cisplatinum, the increase in cytotoxicity is limited to 2–13-fold (Sersa *et al.* 1995, Gehl *et al.* 1998, Jaroszeski *et al.* 2000a), which is, however, still a considerable gain.

Several different cancer types have been treated: basal cell carcinoma (Glass *et al.* 1997), malignant melanoma (Heller *et al.* 1998, Gehl & Geertsen, 2000, Sersa *et al.* 2000b), adenocarcinoma (Mir *et al.* 1998), squamous cell carcinoma (Belehradek *et al.* 1993, Panje *et al.* 1998), transitocellular carcinoma (Kubota *et al.* 1998) and renal cell carcinoma (Sersa *et al.* 2000c).

In all of these histologically different cancer types, electrochemotherapy was highly efficient. As an example, in one study of skin tumours of various histologies, the complete disappearance of tumours using once-only electrochemotherapy was 90% (Heller *et al.* 1998).

What matters in electrochemotherapy is actually the anatomy that restricts the placement of the electrodes and thus the E-field, rather than chemosensitivity of the tumours, which are subject to overkill given the hundreds-of-folds increase in cytotoxicity induced.

Side-effects to electrochemotherapy are modest. With adequate local anaesthesia, brief contractions of the underlying musculature are the main side-effect. Although up till now principally skin tumours have been treated, it is anticipated that electrode systems for deeper situated tumours will be developed. As electrochemotherapy can be performed very quickly and as a once-only treatment, it would also be possible to do electrochemotherapy during open surgery.

Immunological effects of electroporation

Several studies have shown that there is an immunogenic effect of electroporation. The weak immunogenic effect of electroporation alone can be strengthened by the addition of bleomycin and there is a synergistic effect when combined with interleukin-2 or other cytokines. The nature of this immunogenic effect is not established, but release of antigens from the cytosol or changes in the cell membrane are possible candidates. Animal studies have shown that the combination of electrochemotherapy and interleukin-2 in a synergistic way:

1. enhances the local anti-tumour effect in the treated area (Mir *et al.* 1995);
2. also affects tumours implanted in other sites of the same animal (Mir *et al.* 1995);
3. can reduce the frequency of metastases, leading to longer survival (Orlowski *et al.* 1998, Ramirez *et al.* 1998);
4. can induce a memory effect, so that treated animals did not develop tumours of a given type when re-challenged at a later time (Mir *et al.* 1995);
5. finally, it has been shown that blocking of the immune system can block the systemic effect of electrochemotherapy (Mir *et al.* 1992).

One of the human cancers known to respond to immunotherapy is malignant melanoma. We have, therefore, initiated a Phase II clinical study where patients with advanced malignant melanoma are treated

with electrochemotherapy of cutaneous metastases followed by local, subcutaneous injections of interleukin-2. Preliminary results (Gehl *et al.* 2001) show that this treatment has clinical efficacy with modest side-effects. Paraclinical studies of the immunoreactivity of circulating lymphocytes show that responses against known melanoma antigens are induced (Andersen *et al.*, manuscript submitted). These results point out that electrochemotherapy may be used not only as a treatment for localized tumours, but also in combination with interleukin-2 and/or other cytokines, and also in the treatment of systemic disease.

Drug delivery through skin by electroporation

Beyond the scope of this review, but not to be left unmentioned, is that a considerable amount of literature describes the use of local electric pulses to deliver drugs through the skin, and this is likely to hold future for parenteral controlled delivery of a number of otherwise non-permeating molecules. For reviews, see Prausnitz (1999), Vanbever & Preat (1999), Weaver *et al.* (1999) and Jaroszeski *et al.* (2000b).

Perspectives

The perspective of electroporation is that a wide range of molecules, e.g. ions, drugs, dyes, DNA, proteins, oligonucleotides, can be introduced directly into the cell cytosol *in vitro, in vivo* and in patients. The electroporation technology has been in development over the past decades, both regarding equipment and sophistication of electroporation protocols based on increased knowledge of the processes involved in electroporation. Clearly, this opens many perspectives both for researchers and physicians in various fields.

Jens Eriksen, PhD, Department of Oncology, Herlev Hospital, performed experiments shown in Figure 3.

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