Expert Opinion

Electrodes for *in vivo* localised subcutaneous electropulsation and associated drug and nucleic acid delivery

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Background: Drug and nucleic acids can be delivered *in vivo* by an injection of the product followed by the application of a train of electric pulses. *Objective*: The success of the method is linked to the proper distribution of the electric field in the target tissue. This is under the control of the design of the electrodes. *Methods*: The field distribution can be obtained by computer simulation mainly by using numerical methods and simplifying hypothesis. The conclusions are validated by comparing the computed current and its experimental values on phantoms. A good agreement is obtained. *Results/conclusion*: Targeting the delivery to the skin can be obtained by using an array of very short needle electrodes, by pinching the skin between two parallel plate electrodes, or by using contact wire electrodes.

Keywords: applicators, DNA vaccine, drug delivery, electrodes, electroporation, electropulsation, skin

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1. Introduction

Delivery of drugs and nucleic acids in targets *in vivo* (skin, muscle, tumours) can be obtained by injecting the molecules in the target or intravenously (i.v.) and applying a well-defined electric pulse train on the tissue covering the target. This is now in clinical and preclinical applications in oncology (electrochemotherapy, irreversible electroporation) or to obtain gene expression (electrogenotherapy) or DNA vaccine expression. The technology is partly on the market and involves the design of safe and efficient electric applicators [1-3].

Cell membrane organisation is known to be very sensitive to short electric potential changes as observed when a cell is submitted to an electric field. Under suitable electrical conditions (strength, duration), a reversible membrane permeabilisation is triggered [4]. This was shown at the single cell level on isolated cell suspension but was present in cell assemblies (tissue). It was therefore possible to induce the cytoplasmic loading of non-permeant drugs in cells and in tissues. During the last 20 years, this has brought the development of a physical method to introduce therapeutic compounds in tissue for clinical applications. Electrochemotherapy is now a routine clinical practice in several European countries as a result of the European Union-funded Cliniporator and ESOPE projects [5-13]. Trials are also being performed in the US.

Another feature that is brought to the cell membrane surface on electropermeabilisation is the ability to give an electrotransfer of gene plasmids and to obtain their expression [14]. This again was first obtained with cells in culture, but was later shown to remain valid in tissues. This appears now to be one of the most effective non-viral approaches for gene therapy. Using naked plasmids to get the expression appears a safe method from the biological point of view [15-17].

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A key step for the clinical development of the method was obtained in 2008 when a group in Tampa was able to obtain significant clinical results by electrotransfer of IL12 coding plasmids against metastatic melanoma [18]. Besides its biological safety, electropulsation appears to be a rather straightforward medical technology. What is needed is, just after injection of the therapeutic compounds (drug, plasmid, siRNA), delivery of a carefully controlled electric field pulse train. This is not simple as there is still a lack of knowledge concerning the basic biophysical mechanisms involved in the membrane reorganisation associated with its permeabilisation. Such a reversible loss of cohesion remains poorly understood but can be empirically controlled [19].

Plasmid electrotransfer does not result from a direct transmembrane transfer but involves a slow membrane-mediated translocation to the cytoplasm. The intracellular events such as the nuclear internalisation are regulated by the cell. No experimental control on the intracytoplasmic steps is present yet [20].

As a rule of thumb, experimental results show that to obtain an effective permeabilisation, a field strength larger than a critical value must be present at the cell level. Permeabilisation (and gene transfer) increases with an increase of the pulse duration. One limit is that this may affect the cell viability. The definition of the field strength is easy in the case of diluted cell suspensions (the ratio of the voltage to the electrode gap, with plane parallel metal electrodes). When dealing with tissue, this is more complex as the field distribution is strongly affected by the packing of cells, as predicted by computer simulation and observed at the bench [21-24].

2. What should be done

A narrow window of effective field strengths is present to obtain permeabilisation and gene transfer and expression while keeping high the cell viability *in vitro*. *In vivo* (clinical) applications are facing the challenge that the technology must be chosen to make sure that a field that is as homogeneous as possible is present in the tissue to obtain an effective electropermeabilisation [25]. This is clearly a difficult task but it is needed to get an efficient delivery in tissues without any damage. It is clear that a proper design of the field applicators (electrodes) is required [1,26]. This is the purpose of this paper.

One of the advantages and limits of the electropulsation approach is that only targets that are close to the skin can be easily treated without any surgery. Access to deeply seeded tumours is possible after surgery or by sophisticated methods (very long partially insulated electrodes under ultrasound guidance) [27].

Other technical problems are present. The electrical parameters (pulse shape, duration, delay, number) must be under control to get efficient transfer and to avoid dramatic side effects (due to burning when high currents are delivered). The distribution of the drug (plasmid) in the target tissue must be properly achieved to bring an efficient delivery within the pulsed cells. This is clearly a problem when injecting plasmids within tumours owing to the extracellular matrix. Another problem is that the speed of injection plays a decisive role in the case of plasmid electrotransfer in muscles or in the liver [28].

Tissue organisation appears to be very sensitive to electropermeabilisation. A vascular lock is observed in muscles electropulsed with plate non-invasive electrodes, bringing a reduced blood flow. This may be an advantage for drug delivery if an i.v. procedure is operated. The drug remains trapped in the electropulsed tissue and is not washed away [29-31].

3. Computer-assisted simulation of the field distribution

To obtain a safe and efficient electropulsation in tissues, electric field distributions should be computed in advance by means of modelling. Owing to tissue complexity, analytical solution of such problems is almost impossible. Numerical modelling techniques give a fair evaluation. Mostly, finite element and finite difference methods are applied [32]. Both numerical methods have been used successfully. They were validated by comparison of computed measured electric field distribution and associated consequences in tissues [33]. Finite element model validation by computed reaction current was used only very recently [34]. Current measurement is much faster than the imaging method, which can be used to obtain electric field distribution in tissue. Experimental current measurements are indeed a fast and easy to handle method of model validation.

A drawback is that numerical methods are computationally demanding. Simplifications in the modelling process are needed, which can decrease computational efforts and at the same time preserve the accuracy of the result, that is, electric field distribution or current. Such simplifications can be performed either on the description of electrodes or on the physical properties of tissue. Simpler shapes for electrodes are selected (polygonal rather than cylindrical for needles). Tissues are assumed to have a homogeneous conductivity and soft gels prepared in saline buffers can be used as phantoms. This is indeed in fair agreement with *ex vivo* direct observations, where large domains with homogeneous conductance are present in tumours. This is valid in clinical applications under the assumption that the skin in contact with the electrodes is bypassed within a few microseconds after the pulse onset when using non-implanted electrodes [35,36].

The recent development of studies on the time-dependent changes in tissue local electropermeabilisation has brought a new approach into the simulation. The local change in the tissue electrical conductivity due to its position-dependent permeabilisation induces a new field distribution during the pulse. New models are under development, taking into account this non-homogeneous conductance [37-39].

One major problem in the non-homogeneous electrical conductivity is caused by the skin. The stratum corneum is a good insulator, which is brought to a conductive state by a pulse delivered by contact (external) electrodes [40-42] This

electrically induced shunt remains only partly conductive and strongly decreases the voltage present under the skin. This leads to the design of the needle electrodes, which are inserted through the skin to be in direct contact with the tissue. Another approach to bypass the skin problem is to use a surgical approach and cut the skin to have a direct contact to the tissue [43].

The main limit in this simulation approach is that the conductance of a tissue is not homogeneous. This was clearly illustrated in the case of tumours [44]. Therefore, there is a need to validate all conclusions from simulation by an experimental approach. This can be obtained by an evaluation of the current that is delivered along the voltage pulse [34]. Therefore, for safety in treatment, there is a need to measure the conductance change during discharge [45]. The conclusions of other groups were that electropulsation should be operated at constant current, not at constant field [46,47].

4. Electrode designs

The most common electrode arrays comprise surface contact electrodes (typically 'plate' style) or tissue-penetrating electrodes (typically 'needle' style). More recently other designs have been reported, such as wire contact models or soft electrodes.

4.1 Needle electrodes

To gain access deep in the target tissue, the use of needle electrodes is clearly an advantage, but the major conclusion of the simulation is that a complex heterogeneous field distribution is present. It is not only under the control of the gap between the needles of different polarities, but was also predicted to be dependent on the diameter of the electrodes. This was confirmed by experiments in rabbit liver tissue [33]. In this study, three diameters of electrodes were evaluated. The electric field intensity was very high at the electrode surface but decreased rapidly with distance from the electrodes. This decrease was very steep with small diameter electrodes, meaning that a highly heterogeneous field distribution was therefore present. The second conclusion (and experimental observation) was that the current density was higher (at given electrode gap and voltage). This meant that the local Joule heating (and potential burning) was higher; but the tissue damage linked to the needle insertion was less than when electrodes with larger diameters were used.

To improve the homogeneity of the field distribution, the choice was to use not a single pair of electrodes, but an array. Different designs have been proposed, as one limit was the electrical power demanded from the pulse generator when multiple electrodes were present [1]. The solution appeared to be consecutive switches to the different needles to generate field pulse in the different parts of the tissue volume to be treated. It should be mentioned that no simulation has been described taking into account that the electrical conductivity of the tissue was locally affected by the previous pulses when such successive pulse trains were applied. Efforts in simulation are now needed to solve these problems to gain an accurate description of the tissue modifications.

Intradermal electropermeabilisation can be obtained by using microelectrode arrays. The length of the 'puncturing' electrodes and the gap between them are ~ 0.5 mm. This gives a good field distribution in the dermis, a positive feature to obtain a high level of DNA vaccine expression and immune response. The method involves skin electroporation using plasmid DNA-coated 80 microneedle arrays [48]. An array of 11 $\times$ 11 alternately connected electrodes gives an efficient pain-free drug electrodelivery on a 6 mm^2 skin surface [49].

The penetration of needles is easy in soft tissues. Bending of small diameter electrodes may occur when the tissue is harder (as in the case of some veterinary applications). This may be a problem in the distribution of the field as it will change the interelectrode gap. This is a great concern in the use of the needle electrodes as it is difficult to control this mechanical event and to check whether it takes place. It can be detected only on electrode removal.

An advantage of the needle system is the opportunity to use the needle not only as an electrode, but also as a means to inject the drug (or DNA) [50]. A more homogeneous distribution of vectors can be obtained in the vicinity of the electrode by pushing out the product along the insertion of the needles. Industrial developments are now on the market taking advantage of this technological feature [3,51,52]. One concern is that the plasmid is going to be present close to the electrode surface, that is, the tissue area which is the target of electrical damages (burning, electrochemistry).

Recently, a system has been developed where only one single needle is present to get plasmid delivery and expression in the thymus in anaesthetised mice. The needle, whose size is optimised with the animal (a few millimetres long), is inserted between the two first ribs following the angle of the paw parallel to the benchtop. This stimulating syringe delivers plasmid DNA solution and acts as the anodic electrode during electric pulses. A crocodile clip attached to the paw of the opposite side closes the electrical circuit [53]. Simulations predict that a volume close to the needle is submitted to a field suitable for a proper delivery and expression.

Needle electrodes make treatment deep in the tissue possible. A limitation in the use of needle electrodes is that only a limited *trans*-surface is treated, except if an array with a large number of needles is used. As the insertion is not easy, even if commercially automatised devices are now on the market, repetitive insertions on different localisations, which allow a large area to be treated, is technically difficult. Furthermore, it is painful, even if the patient is under (local) anaesthesia.

4.2 Parallel plate electrodes

Electrodes are two parallel metal plates at a fixed distance. The tissue is carefully pinched between the electrodes. An improved electrode-to-skin contact is provided by a conductive gel. More sophisticated systems are obtained with an adjustable gap by using a calliper or tweezers to hold the

plates [54]. A key advantage of this electrode configuration is that the orientation of the field in the tissue can be easily obtained by a rotation of the electrode set around the target tissue. As electropermeabilisation and electrotransfer are vectorial processes, a significant improvement of the effects is observed [55,56].

A numerical analysis of electric field distribution was based on two-dimensional models for electrodes and electrode configurations by means of the finite element method [33,35]. The conclusion was that the field was present only in the volume trapped between the two plate electrodes. A clearcut definition of the pulsed volume was theoretically achieved. In the authors' experiments (**Figure 1**) (unpublished data), rectangular rods of a conductive gel (15 mS/cm) with different lengths but with a given thickness were inserted between the plate electrodes. The shortest length was just as long as the electrode width (1 cm). It was observed that the current for a given voltage was not changed significantly by increasing the length of the rod. This was direct experimental confirmation that the current was flowing almost exclusively between the electrodes. The field was geometrically confined at the interelectrode space.

One prediction of the simulation was that sharp angles at the edges of the plate must be avoided to limit the occurrence of high field values at their positions. If the skin is the target, it must be pinched between the electrodes. Some local heterogeneities are therefore present at the edges of the plate [42].

Two recent papers reported the importance of a homogeneous contact between the tissue and the electrode surface. The conclusion was that the target tissue should be embedded in a commercial conductive gel (5 mS/cm). This conductivity is supposed to match that of the tissue [57,58].

The risk with the plate electrodes is that the field inside the tissue is high only when the stratum corneum is affected and becomes highly conductive. The resulting Joule heating (as a current is delivered) must remain under control to avoid burns on the skin and irreversible damage [59]. Spatula electrodes used for electropulsation of mouse muscle have been shown to induce less tissue damage compared with plate and needle electrodes, but minor surgery is needed, with high risk of contamination [60].

Large size object electropulsation is possible. Plate electrodes are routinely used for drug delivery in tumours (so-called electrochemotherapy, which is a routine practice in many European clinics) and for electric gene transfer in muscles; but as the gap between the plates can be adjusted, it can be wide enough to pinch the thorax (to obtain field effect on the lungs) [61] or the head (to target the brain) [62]. When a proper preinjection of the plasmids had been operated before pulsing, effective electrotransfer was observed.

4.3 Grid parallel electrodes

The use of expensive materials (gold, platinum) has been proposed for the construction of electrodes to reduce the potential electrochemical contamination that was observed with the delivery of repetitive trains of unipolar pulses (see below) [63]. The use of grids rather than plates should reduce the cost [2]. No numerical simulation has been performed to check whether the field distribution was under the control of the grid geometry. Different shapes of grids were therefore experimentally evaluated to check whether the field distribution was altered (**Figure 2B**) (unpublished results). The ohmic behaviour was taken advantage of to compare the different systems (**Figure 2C**). The plate parallel electrodes were taken as a system where a homogeneous field distribution between the electrodes was observed as just described. By comparing the currents for different applied voltages, the equivalent resistance of the system, that is, the field distribution was obtained. This resistance is dependent on the grid geometry. The lowest value was obtained with the plate electrodes, where a homogeneous field distribution was present, as just described. When the grid stitch was very small (**Figure 2B**) the resistance was close to the previous case, indicating that the homogeneity of the field distribution was not strongly affected. The two other models where either the grid was coarser (**Figure 2B**) or where the wire, which built the grid, was thicker (**Figure 2B**), gave higher resistances. This means that the field distribution was more heterogeneous. Grids are associated with a poor homogeneity in the field distribution, except if the stitch is very small. The diameter of the wire in the grid needs to be very small and makes the electrodes rather fragile.

4.4 Contact wire

The skin is one of the most important targets for gene expression (for DNA vaccine) and the localisation of many solid tumours (melanomas). Electrotransfer at its level requires a targeted field distribution on a thin flat layer to be obtained. This is predicted from simulations with the new concept of contact cylindrical wire electrodes [64]. Field distribution was observed to be very localised and highly homogeneous. This allowed the field effects to be focused along the surface of the tissue to induce a controlled release of drugs or plasmids. The electrical contact with the skin is improved by spraying a conductive gel on the electrodes. Non-invasive (contact) electrodes can be moved rapidly on the body and avoid puncturing the skin and the tissue. The orientation of the field can be changed very rapidly by a rotation of the holder. As the electrode gap can be 1 cm wide, they can be used for large surface effects, to treat the skin and subcutaneous tumours. The use of contact electrodes after drug or DNA intradermal injection was validated by clinical treatment of large surface equine tumours [65] and by *in vivo* imaging of permeabilisation or of gene expression [66]. A similar approach but on a smaller scale is a meander design [1]. Meander contact electrodes have been proven to be effective for gene delivery and expression in the skin [67].

4.5 Specific electrodes

Adjusting the shape of the applicator to the target has been approached by empirical methods [2]. This is strongly needed when the target is a sensitive organ, as in the case of eye.

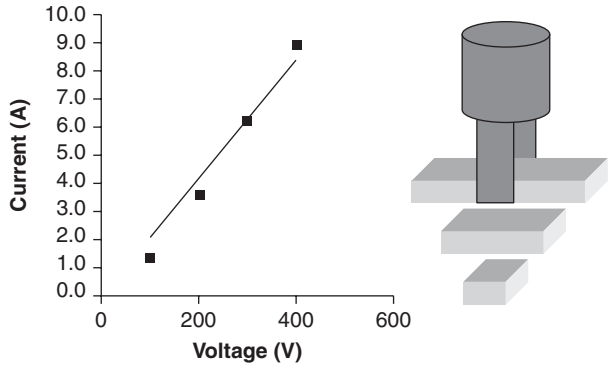

Figure 1. Simulations and experiments on slabs of gel with plate parallel electrodes. Slabs of a conductive gel with different lengths were put between the electrodes, as pictured on the right. The electrode gap is kept to 4 mm and the width is 10 mm. On the current–voltage plot, different gel lengths (10, 20, 40 mm) are used, the shortest being 10 mm, that is, the electrode width. Their thickness is 4 mm. The continuous line is the simulation prediction assuming an ohmic behaviour (conductivity 15 mS/cm), whereas the squares are the experimental results. No difference could be found for the different lengths. Field simulation can be found in [35].

Ciliary muscles can be treated. A very small needle, where only the tip is not insulated, is inserted through the corneal tunnel and may be used to inject the plasmid, while the counter electrode can be a large plate electrode to fit the rat scleral surface overlying the ciliary body [68].

Electrotransfer can be obtained in the retina after a targeted injection. Two platinum-iridium 20 gauge wire loops (diameter 2 mm) were positioned on the eye, one directly underneath the retina injection site on the scleral surface of the mouse globe and the other positioned diametrically opposite from the injection site [69]. Getting access to buried tumours can be achieved by adjusting electrodes at the tip of an endoscope [70-72].

4.6 Nanosecond pulsed electric field (PEF)

The new development of irreversible electropermeabilisation (IRE) by using ultra-short (nanosecond) but high intensity (tens of kilovolts per centimetre) was introduced recently for the eradication of cutaneous tumours. Two new technical problems are present: the need for a high voltage to get the high field in the tumour volume and the flashover between electrodes during the pulsed field application (in humid air, sparks are induced with 10 kV/cm). Two needle array designs are used. In one model, 30 gauge hypodermic needles (300 μm diameter) extending 2 mm from a Teflon base were used in a square configuration where the central needle was the anode and the 4 surrounding needle electrodes all forming the cathode were at a distance of 4 kilovolts per centimeter. The skin was coated with vegetable oil to avoid the sparks. A highly heterogeneous field distribution was of course obtained [73,74]. In the other one, there was the central conductor pin (0.8 mm in diameter) and an array of 4 thinner gold-plated brass pins at a distance of 1.8 mm all penetrating 5 mm deep in the tissue [75]. A parallel plate electrode design was more efficient in murine tumour eradication. The tumour was pinched at a separation of 0.5 – 1 mm between two stainless steel electrodes (diameters of 3 – 5 mm) coated with a 0.5-mm-thick layer of conductive agar (1 M NaCl in 2% agar). A highly efficient eradication was obtained with this device. Owing to the need for a high field, only small tumours can be treated by a single trained shot.

5. Electrochemistry

Biocompatibility of electrodes during the treatment is of course a key request. This is a problem with needle electrodes where a direct contact between the metallic surface and the tissue is present. The conductive gel that is used with plate electrodes plays a protective role and improves the safety of the treatment. The large majority of electrodes (plate as well as needle) are built of stainless steel. The advantage of this is a reduction of the electrochemically mediated release of toxic ions (nickel, chromium) or the formation of non-conductive oxides (aluminium). In a few cases, it was proposed to use gold-coated electrodes. The problem is more serious for DNA (electrogenotherapy [EGT]) than with drug (electrochemotherapy [ECT]) delivery as the electric charges associated to the pulses are larger. The problem remains open with nsPEF, but again the safety is improved owing to the use of gel-coated plate electrodes.

6. Conclusion

The need for a homogeneous field distribution is a key feature for proper clinical use of electropulsation (electroporation). A large volume needs to be treated. The different designs for electrodes must be evaluated by using computer simulations (finite element approach) using relevant assumptions. The main limit is that the tissue has, up to now, always been considered to be homogeneous as far as its electric conductivity is concerned. The conclusion can nevertheless be validated at the bench by measuring the current detected when pulsing phantoms. This allows the assertion that the simulations provide a fair evaluation of the field distribution.

Two designs are the most popular: needle and plate electrodes. The calculated e-field distribution is more homogeneous for plate than for needle electrodes [76]. Field effects can be delivered deeper in the tissues with inserted needles but with a high risk of local burns.

7. Expert opinion

Electropulsation of cutaneous targets is no longer limited to academic laboratory applications. Companies are now providing different sets of equipment (see **Table 1**). The needle approach is limited to the microelectrodes array to obtain a specific targeting of the skin, but brings the advantage of the colocalisation of injection and field application. Low voltage

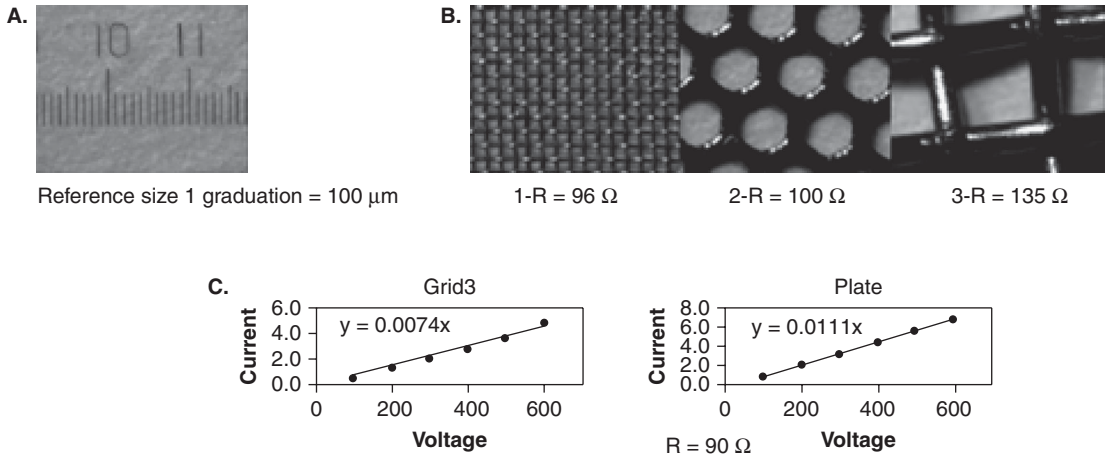

Figure 2. Grid electrodes. A. Size, one small division is 0.1 mm. **B.** 1 – grid with a narrow mesh (R = 96 Ω); 2 – platinised grid with a hexagonal perforated array (R = 110 Ω); 3 – grid with a large mesh (R = 130 Ω). **C.** Current–voltage plots with plane plate electrodes and with a large mesh. The same 12-mm-thick gel (15 mS/cm) was between the two electrodes (section 90 mm^2). The linear plot gives the value of the resistance (90 Ω, 130 Ω). A higher current is present with the plate. No field simulation can be obtained owing to the sophistication of the electrode design.

Table 1. *In vivo* electropulsation electrode providers' websites.

Nepa gene	http://www.nepagene.jp/E/Eindex.htm
IGEA	http://www.igea.it/interno.php?p=7&voce=137&id=377&t=0
BTX Harvard	http://www.btxonline.com/products/electrodes/invivo/
Cytopulse	http://www.cytopulse.com/Datasheet%20CC%20In-vivo%20Electrodes.pdf
Inovio (VGX)	http://www.inovio.com/technology/intramusculardelivery.htm
	http://www.inovio.com/technology/intradermaldelivery.htm
Ichor	http://www.ichorms.com/techoverview.shtml

can be used. The skin must be soft to prevent electrode bending during the insertion. The plate electrodes need to pinch the skin in the electrode gap, meaning that again it is limited to a soft skin. The wire contact electrodes offer a more easy way to treat large surfaces of the skin. Strong skin (as in the case of horses) can be treated with no difficulty. Low voltage can be used with the meander design and the microneedle array but plate or wire electrodes are compatible with all square wave electropulsators available on the market; but there is a need for a prepulse localised injection of the drug or nucleic acids

All approaches are associated with bearable pain for the patient. Drug delivery to cutaneaous tumours is now routine clinical practice in several European countries as a consequence of the successful Cliniporator and Esope projects. Promising results for DNA vaccines have been reported for immunisation of large animals and should be a field of further development for human health.

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Declaration of interest

The authors state no conflict of interests and have received no payment in the preparation of this manuscript.

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