Molecular Therapy
Nucleic Acids
Commentary

DNA electroporation in a vacuum: A "shocking" innovation for vaccines

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https://doi.org/10.1016/j.omtn.2023.102110

The manuscript by Generotti et al. in this issue of *Molecular Therapy Nucleic Acids* describes a new DNA vaccination method based on combining non-invasive intradermal electroporation with the application of vacuum at the vaccination site.[1] This method, which the authors "baptized" as ID-VEP (intradermal-vacuum electroporation), was able to induce potent humoral and cellular immune responses in a guinea pig model injected with a plasmid expressing the Middle East respiratory syndrome coronavirus (MERS-CoV) spike protein. Interestingly, this method induced stronger immune responses than those raised by DNA electroporation without vacuum.

The use of DNA for vaccination was proposed many years ago as a cheaper and easier-to-manufacture alternative to vaccines based on recombinant proteins or attenuated/killed pathogens. The rationale behind a DNA vaccine is that when taken up by cells, including antigen-presenting cells, the gene contained in the vaccine will express the antigen of interest. This antigen will either be secreted, eliciting humoral responses, or degraded and presented in the context of major histocompatibility complexes, inducing cellular responses. However, despite promising data in animal models, DNA vaccines have not shown satisfactory results in human clinical trials, with only one vaccine being recently approved in India against COVID-19 (ZyCoV-D).[2] This is in contrast to vaccines based on messenger RNA (mRNA), several of which have shown remarkable efficacy against COVID-19 and are approved worldwide.

One key issue for the success of nucleic acid vaccines is the use of a good delivery system, given that naked DNA and mRNA are not efficient at entering cells due to their large size and high polarity. The use of cationic lipid nanoparticles has revolutionized the field of mRNA vaccines since these compounds are able to neutralize the negative charges of RNA and at the same time provide it with the hydrophobicity needed to cross cell membranes. Although the same principle holds true for DNA, this molecule faces an additional obstacle: the need to reach the cell nucleus to be functional, a process that is usually inefficient.

A very interesting alternative to cationic compounds is the use of electroporation to deliver DNA *in vivo*.[3] Electroporation is a process that creates transient pores in cell membranes, allowing diffusion of nucleic acids, and other molecules, inside cells. Typically, electroporation is performed by inserting several electrodes in the skin or muscle, where the DNA is also injected, followed by the application of electric pulses of different intensity and duration. One caveat of this procedure, which is called invasive electroporation, is that the insertion of the electrodes usually induces pain and discomfort. To avoid these problems, several groups have developed less invasive electroporation methods using contactless or minimally invasive electrodes, such as the CELLECTRA-3P device,[4] which targets dermal and subcutaneous layers of the skin with mild electroporation conditions and minimal tissue damage. In fact, this device has been used in several clinical trials to deliver DNA vaccines against MERS-CoV, Ebola virus, and, more recently, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).

In order to improve the performance of non-invasive DNA electroporation, Generotti et al. combined the CELLECTRA-3P device with a vacuum chamber that generates negative pressure in the area to be electroporated.[1] The ID-VEP system functions by pulling a small, and controllable, volume of skin tissue into a vacuum and applying electric pulses after intradermal DNA injection (Figure 1, top). The authors developed several prototypes of this device using vacuum chambers of different sizes produced with a 3D printer. When they tested these prototypes in the skin of guinea pigs by applying different voltages and vacuum strengths, they observed an inverse association between the ID-VEP chamber size and the electric field intensity, with the best outcomes using chambers of 8–10 mm in diameter. In these initial experiments, they also noticed that the electric field intensity increased with voltage and vacuum strength, obtaining the best results with 200 V and 70 kPa.

Based on these results, they analyzed GFP expression *in vivo* by electroporating a DNA plasmid containing this reporter gene into guinea pigs under different vacuum strengths. Again, a higher GFP expression in the skin correlated with a stronger vacuum. However, increasing the voltage from 100 to 200 V decreased expression, in contrast to what was observed previously when determining the electric field intensities. Interestingly, animals electroporated without vacuum (ID-EP) or that received vacuum without electroporation (ID-vacuum) showed lower GFP expression, which validated the higher efficacy of ID-VEP.

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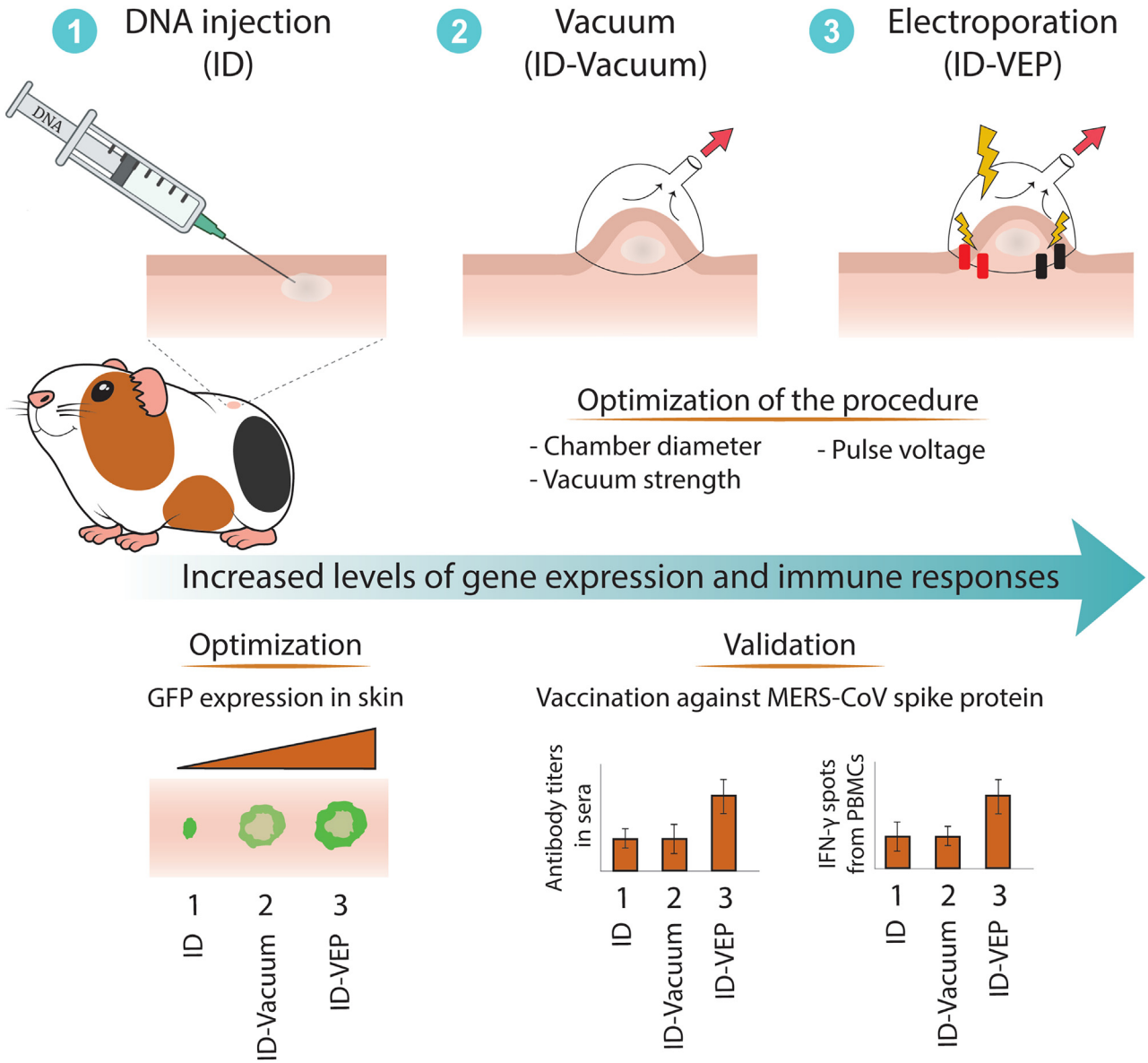

Figure 1. Intradermal-vacuum electroporation (ID-VEP): A non-invasive method to increase DNA delivery *in vivo*

This strategy involves three subsequent steps: (1) intradermal DNA administration, (2) precise application of negative pressure at the injection site, and (3) electroporation using needleless electrodes. Various variables, including chamber diameter, vacuum strength, and electric pulse parameters, exert substantial influence on gene expression and merit careful optimization. While vacuum alone increased gene expression compared to intradermal DNA administration, only the combination of vacuum and electroporation enhanced the immunogenicity in vaccination studies, inducing cellular and humoral responses of significant magnitude. This technique holds promise as a non-invasive, reliable, and quick procedure, potentially reducing variability in DNA delivery.

Finally, they tested the use of ID-VEP to vaccinate against MERS using a plasmid expressing the MERS virus spike protein (Figure 1, bottom). The authors showed that although they were able to generate similar humoral and cellular immune responses against MERS with ID-VEP and ID-EP, these were raised more quickly in the first case. ID-vacuum was also able to induce immune responses but at a much lower level than ID-VEP and only after boosting.

Although the use of vacuum for tissue electroporation is not completely novel, it had only been used until now to enhance the delivery of small molecules, such as chemotherapeutic drugs, recently shown to be a safe procedure in humans.[5] Here, Generotti et al. showed that vacuum can also enhance DNA uptake by electroporation. The authors claim that the added benefit provided by the vacuum is likely a result of its ability to disrupt cell membranes in the skin. This might allow the injected plasmid to be redistributed laterally through

the skin, potentially reaching more cells than in the absence of vacuum. However, further experiments will be required to determine the precise mechanism of this combined strategy. It had also been previously proposed that electroporation could function as a physical adjuvant by inducing a localized tissue damage that triggers the recruitment of immune cells. However, since the tissue damage or cell death caused by electroporation may not be highly immunogenic, the incorporation of immunogenic molecules such as poly IC, STING agonists, or nucleic acids coding for cytokines into the procedure could create a more favorable environment for the activation of antigen-specific immune responses.[6] These responses could be potentially enhanced in the presence of vacuum, due to a higher tissue disruption.

Regarding the safety of this procedure, the authors only observed a transient and localized redness on the skin in the spot where the vacuum or electroporation was applied. Although the skin of guinea pigs is more similar to human skin than that of other rodents, it still has strong differences like a lower thickness, a higher hair follicle density, and a looser attachment.[7] Hence, conducting experiments in larger animal models, such as pigs, the skin of which is more similar to that of humans, would be preferable before clinical trials are performed.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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