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Electroporation Delivery of DNA Vaccines: Prospects for Success

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Summary

A number of noteworthy technology advances in DNA vaccines research and development over the past few years have led to the resurgence of this field as a viable vaccine modality. Notably, these include - optimization of DNA constructs; development of new DNA manufacturing processes and formulations; augmentation of immune responses with novel encoded molecular adjuvants; and the improvement in new *in vivo* delivery strategies including electroporation (EP). Of these, EP mediated delivery has generated considerable enthusiasm and appears to have had a great impact in vaccine immunegenicity and efficacy by increasing antigen delivery upto a 1000 fold over naked DNA delivery alone. This increased delivery has resulted in an improved *in vivo* immune response magnitude as well as response rates relative to DNA delivery by direct injection alone. Indeed the immune responses and protection from pathogen challenge observed following DNA administration via EP in many cases are comparable or superior to other well studied vaccine platforms including viral vectors and live/attenuated/inactivated virus vaccines. Significantly, the early promise of EP delivery shown in numerous pre-clinical animal models of many different infectious diseases and cancer are now translating into equally enhanced immune responses in human clinical trials making the prospects for this vaccine approach to impact diverse disease targets tangible.

Introduction: The Promise of DNA Vaccines

The concept of using DNA to immunize people was first advanced in the early 1990s and immediately gained widespread recognition due to its apparent simplicity and elegance [1–3]. What could be simpler than simply injecting a DNA plasmid encoding the antigen of interest into host cells and letting the host-cellular machinery carry out the tasks of protein translation and antigen processing and presentation *in vivo*? Indeed the simplicity of this concept for eliciting meaningful immune responses was exemplified by the rapid translation of molecular biology constructs *in vivo* into immune responses and protection in some challenge models in small animals – notably mice [4,5].

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The DBW laboratory notes possible commercial conflicts associated with this work from consulting fees, stock ownership, Advisory Board or Review Board Service, speaking support among others which may include the following companies: Pfizer, Inovio, BMS, VGXI, Virxsys, Ichor, Merck, Althea, Aldevron, Novartis, and possibly others.

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Over the years other advantages of DNA vaccination came to the fore. DNA remains the only vectored platform that does not induce anti-vector immunity making it suitable for vaccine regimens that include both priming as well as boosts. Additionally, manufacturing of plasmid DNA is considerably faster and easier than most other vaccine platforms and relies primarily on bacterial hosts for production. Indeed manufacture of small-scale non-GMP research grade plasmid material has become a commodity business and the difficulties associated with manufacturing and handling live/attenuated viral vaccines as well as large variability in potency from lot-to-lot are largely not an issue with DNA. Furthermore, DNA is relatively stable at room temperature making the requirement for maintaining the vaccine cold-chain less critical compared to other vaccine platforms. In addition, manufacturing of DNA can be done extremely safely especially as compared to killed pathogenic vaccine platforms.

From the vaccinologists' perspective, DNA, due to its ability to combine the power of genomics with *in vivo* antigen expression, provides a tantalizing opportunity to easily customize vaccines through the use of molecular biology. Indeed it can be said that DNA vaccines bring to fore the strengths of molecular biology and genetic engineering to harness the potential of the immune system. The ability to easily combine multiple plasmids or disparate gene products into a single formulation without apparent loss of potency allows the possibility to formulate multi-component vaccines targeting multiple antigens or even multiple pathogens simultaneously [6,7]. Similarly, a seasonal flu vaccine combining DNA plasmids targeting influenza A/H1N1, H3N2, and influenza B strains can be readily contemplated and coupled for *in vivo* delivery with an A/H5N1 vaccine thus allowing for the simultaneous targeting of both seasonal and pandemic strains [8]. Just as important, such vaccine can be designed to increase the breadth of the immune responses and potentially increase pathogen coverage. Thus approaches such as the use of synthetic consensus immunogens and mosaics – both approaches available simply in a DNA based platform - are expanding the notion of vaccine design to focus on developing "universal" vaccines to simultaneously target multiple divergent but related strains of given pathogens [9–13].

And yet for all the promise, the early DNA vaccine human clinical trials failed to meet immunogenicity end points. The translation of results from preclinical models to humans was largely ineffective bringing into question the scalability of induction of immune responses from small animals to humans. Was this inability due to limitations of vaccine dose (delivery on a weight by weight basis)? Or vaccine potency? Or due to differences in the immune systems of animals versus humans to recognize DNA based antigens differently? Or possibly a combination of these factors?

Research in these areas led to important discoveries on the role of DNA to activate innate immunity and the identification of potential receptors and/or intracellular sensors for double stranded DNA including TLR9 [14,15], DAI (DNA-dependent activator of interferon regulatory factors)[16] [17], AIM2 [18,19], and HMGB (high mobility group box) proteins[20]. Similarly, research into improving the potency of DNA vaccines through the use of conjugates to recruit T-cell help [21,22] or inducing effector T-cells [21,23] or through the use of molecular adjuvants [24–27] has resulted in a better understanding of the immune system and its response to DNA based vaccines. These approaches are very interesting and have shown recent early promise in small clinical trials [28,29].

Electroporation Enhanced DNA Vaccine Delivery

Electroporation (EP) is a method to introduce macromolecules such as nucleic acids into cells, either in vivo or in vitro, via the application of brief electric pulses to induce transient and reversible permeabilization of the cell membrane (Figure 1). Over the last decade the

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technique has evolved from an experimental technique to now being used in several clinical trials to deliver nucleic acids as well as drugs to a variety of target tissues [30]. A number of mechanistic studies have helped develop the hypothesis that during EP, transient pores are formed as a function of the transmembrane voltage [31,32]. During the period of membrane destabilization (on the nano to milli second timescale), macromolecules present in the extracellular medium surrounding the target cells gain access to the intracellular milieu [33]. After the EP pulses, a slow resealing of the membrane occurs on the second to minutes time scale. Although the exact mechanism of translocation of DNA across the membrane pores is debated (electrophoretic facilitation versus passive diffusion) the end result of the process is that upwards of 100–1000 fold enhancement of plasmid delivery and gene expression can be achieved relative to delivery of DNA alone without electroporation (see references below).

Translating *In vivo* Expression to Immunogenicity and Efficacy

The observation that *in vivo* electroporation can dramatically improve gene delivery has led to a great deal of interest to assess the consequences of this delivery to enhancing immunogenicity and effectiveness of DNA vaccines in specific model systems. While it has been difficult to quantitatively measure directly the enhancement in plasmid delivery and its translation to increased transgene expression, and consequent induction of immune responses in the same animals, a number of experiments have measured this correlation indirectly. Increased expression following DNA delivery via EP has been measured quantitatively by assessing reporter gene products (GFP and SEAP) at the injection site or their circulating levels in the sera [53]. In parallel, experiments with vaccine antigens comparing delivery with or without EP have shown increased immune responses (cellular and humoral) at significantly lower doses (dose-sparing). Indeed data from several vaccine candidates spanning cancer and infectious disease published in the last two years has demonstrated that a 10–100 fold enhancement of immune responses as well as protection from pathogenic challenge is routinely achievable in various animal models of disease including SIV/HIV[34–39], malaria [40,41], HCV[42], HBV [43–46], Botulinum toxins A, B, E [47], HPV[48,49], Anthrax [50], influenza [51,52].

Much of the early work for EP mediated delivery of DNA vaccines was conducted using intramuscular (IM) delivery. Recently however, there has been an increasing shift towards developing intradermal (ID) delivery systems to complement delivery to the muscle [6,53–55]. The skin is the most accessible organ of the human body, is easily monitored as well as being a highly immunocompetent target organ [56,57]. A variety of experimental EP devices have been reported in the literature to target the skin. Some use plates or webs which deliver the electrical charge to the skin. In addition devices which target through the skin and access deeper dermal layers have also been studied. The immune responses with many of these have been variable, however the ability to transfect in small animals has been reported. We recently reported the development of a minimally invasive device that merely scratches the surface of the skin [54]. Indeed studies from our group have shown that electrodes that target different depths and varying degrees of skin/muscle invasion induce unique immune phenotypes by contacting different and unique immune compartments of the skin/muscle. These range from non-contact (piezoelectric assisted delivery[58]) to minimally invasive (ID/SQ delivery) to IM delivery (Figure 2) [53,54,59]. Studies in this area illustrate a superior level of immune control that can be demonstrated by specific immune compartment targeting *in vivo*.

Prospects for Safety and Tolerability

For the vaccine developer, a consistent story emerging from over 15+ years of clinical development with plasmid based DNA vaccines collected across 100s of human clinical

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trials covering 1000s of healthy and diseased subjects spanning studies in infectious diseases, cancer, and gene therapy delivery is that of an excellent and consistently unremarkable safety profile. These safety trends have continued to be observed in studies where the DNA has been delivered via EP. Indeed published toxicology studies in animal models have largely yielded no adverse findings and published human clinical studies have not noted vaccine associated serious adverse events when DNA was administered as the drug substance either with or without EP [29,55,60,61]. Tissue biodistribution studies in animals have noted that when found at all, DNA is present only at the injection site (usually skin and muscle). There is a rapid decay in plasmid copy numbers over time [60,61] and early concerns surrounding plasmid integration[62] into the host genome remain unsubstantiated.

EP studies in cancer and HIV amongst others have had good patient recruitment and trial retention, suggesting that this platform can be associated with good patient compliance in diverse clinical protocols including cancer immune therapy [29], HCV therapy [63], HIV prophylaxis [55], and influenza prophylaxis (trials ongoing). The predominant adverse findings (grade 1/2) associated with the IMEP procedure is transient pain that rapidly decays to background within 25 – 30 minutes [64,65]. The transient pain associated with the IMEP procedure was noted to be further decreased substantially in the case of ID EP applicators [60,66], and thus the rationale for the increasing trend towards IDEP. The decreased invasiveness of DNA delivery, shallower depth of penetration and lower current (Amps) parameters to effect optimal delivery all translate into a more tolerable procedure for the subject supporting the observation that IDEP vaccination procedures appear comparable to routine ID/IM vaccination with a needle and syringe.

EP Enhanced Clinical Immunogenicity and Efficacy of DNA Vaccines

The last two years have also seen the completion of the first DNA EP clinical trials and publication of Phase I/II immunogenicity and efficacy data. The exciting findings are that similar to the pre-clinical animal model data, EP appears to enhance the immunogenicity of DNA vaccines relative to DNA alone in the human clinical setting as well. Specifially, Ottensmeier et al reported results from a prostate cancer DNA vaccine study where they detected IFN-γ producing CD8+ T-cells against the target PSMA peptide in approximately 60% of the cases and that EP delivery of DNA stimulated T-cell responses more quickly and with a greater magnitude compared to the cohort that received the DNA vaccine without EP[67]. This group also reported induction of strong humoral responses to the fragment C domain (DOM) of tetanus toxin that was conjugated to the PSMA peptide to facilitate CD4+ T-cell help. The patients receiving the DNA vaccine via EP induced stronger antibody titers (over 14 fold) to DOM relative to those receiving the vaccine without EP[29]. Similarly, Sällberg and colleagues recently reported data from their HCV therapy trial with an NS3/4a based DNA vaccine delivered via EP. The authors noted significant induction of antigen specific IFN-γ producing T-cells in the HCV infected subjects receiving the vaccine via EP. The authors also noted transient reduction in viral load (0.6 $\log_{10}$ to 2.4 $\log_{10}$) in 5/12 vaccinated subjects [63].

Beyond the therapeutic vaccination regimens, EP delivery of DNA has made inroads into prophylactic vaccine regimens as well. Vasan and colleagues reported the induction of antigen specific IFN-γ producing T-cells (response rates and magnitude) when a DNA based HIV-1 candidate vaccine expressing Clade C/B env, gag, pol, nef, and tat genes (ADVAX) was delivered using EP [68] compared to an earlier study where ADVAX was administered via IM injection alone [69]. While overall response rates were only 13–33% in the IM DNA study [68,69] and consistent with the poor immunogenicity reported in other DNA vaccine

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studies, the application of EP increased the response rates to over 75% in the mid and high dose cohorts and magnitude by upto 70 fold over the same dose delivered IM [68].

We recently reported preliminary safety and immunogenicity data from a HPV-16/18 E6 and E7 DNA based candidate vaccine delivered via EP [70]. The study was conducted as a dose escalation study in three cohorts (0.3, 1, and 3 mg of each of two DNA plasmids in the vaccine formulation) and data from the first two dose cohorts was presented at the 50th ICAAC meeting in Boston. No SAEs or vaccine-related Grade 3 or 4 AEs were reported. The antigen specific antibodies and T-Cell ELISpots observed were higher than previous reports from prior studies of HPV poxviral, peptide or DNA vaccines [71,72] and these were observed even at low DNA doses.

Other currently ongoing prophylactic vaccine clinical trials with the DNA EP platform include HIV (two trials sponsored by NIH/DAIDS and the HVTN (HVTN-080) or the USMHRP (RV-262)), avian influenza (Inovio), malaria (NIAID/Ichor), and HIV (Karolinska institute/Cytopulse).

Prospects for Clinical Efficacy and Product Development Success

As exemplified above, the early clinical trial data has largely concurred with the larger animal model data in supporting the hypothesis that EP delivery of DNA vaccines enhances immunogenicity of the DNA delivered vaccines. The early clinical trials have focused on development of vaccines for hard to treat targets with a clear unmet need (HIV, HCV and cancer), or for diseases where a cellular immune response was considered important (Immune therapeutic vaccine regimens for HIV, HCV, cancer) – largely as a result of dogma arising from the early days of DNA vaccination.

The key prevailing misconceptions those early studies being:

- **a.** As a platform DNA was good solely at eliciting cellular immune responses but not humoral immune responses
- **b.** DNA vaccination led to a predominantly CD4+ biased response with minimal induction of CD8+ T-cells
- **c.** The cost of DNA vaccine manufacturing (and EP delivery) would make this vaccine platform suitable only for therapeutic vaccination scenarios and out of reach of routine prophylactic vaccination.
- **d.** Due to low potency, DNA is primarily useful as a priming modality in a prime-boost setting.

The application of EP delivery to DNA vaccination has arguably changed our view of all of the conventional wisdom and the weight of recent evidence suggests a much broader potential of DNA vaccination. Indeed, the approach has proved promising in eliciting both cellular and humoral immune responses in animal models and humans. Induction of strong CD8+ T-cells in addition to CD4+ T-cells in primates and humans has been another hallmark of EP delivery and different from what has been observed with DNA alone [34,36,53]. Importantly, although the current costs of cGMP DNA manufacturing at small scale remain relatively high (on the order of $50–100/mg at a 1–10g scale of manufacturing using 500L fermenter process trains), the relative ease of plasmid DNA manufacturing and scale up makes it likely that in the future manufacturers will find ways to lower manufacturing costs by 2–3 $\log_{10}$ at commercial scale (upwards of 10Kg using 3,000 – 30,000L fermenter process trains) [73]. Regardless, while commercial scale up with DNA has not been attempted largely due to the lack of relevant late stage vaccine products, the projected costs of manufacturing DNA at scale appear to be competitive with other licensed

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or other evaluated vaccine platforms – live/attenuated virus, inactivated virus, VLP, or viral vectored (Ad5, MVA). Furthermore, unlike the other platforms that often require mammalian cell culture DNA does not face the same risks with carryover of adventitious viruses [74,75], or large variability in lot-to-lot potency, or the safety of a live vialed final product.

As the ultimate goal of DNA vaccine research should be to develop life saving products in the face of other alternative vaccine development choices and not just find ways to improve immunogenicity of DNA at any cost, a fair question to ask is: *How does the DNA EP technology stack up to other licensed vaccines or developmental vaccine platforms in terms of immunogenicity and efficacy?*

Several recent publications have attempted to answer this question directly in relevant nonhuman primate (NHP) models. Hirao et al evaluated the Merck Ad5 SIV vaccine – an important benchmark for new T-cell based vaccine development – against an optimized SIV DNA vaccine delivered via the CELLECTRA® EP device and noted significant differences in the quantity of IFN-γ responses by ELISpot, greater proliferative capacity of CD8+ T-cells, and increased polyfunctionality of both CD4+ and CD8+ T-cells in the DNA vaccinated group compared to the Ad5 group [39]. Importantly Ad5 immunizations failed to boost following the first vaccination, while the DNA induced responses were continually boosted with three subsequent immunizations (Figure 3a).

In another NHP study, Livingston and coworkers compared the efficacy of an Anthrax DNA vaccine delivered with or without EP and compared the efficacy in a challenge model to that achieved with a licensed anthrax vaccine [50]. The authors report a 100 fold enhanced immune response when the DNA vaccine was delivered via EP compared to standard IM injection. The DNA EP vaccine conferred protection to the animals in a subsequent lethal Bacillus anthracis spore challenge comparable to that achieved with the licensed attenuated anthrax vaccine.

We recently reported on a NHP challenge study with a multicomponent DNA vaccine for smallpox [7]. This vaccine consisted of an 8-plasmid formulation and was delivered in microvolumes via IDEP delivery. We observed high titer antibody responses against all 8 DNA encoded antigens and the vaccinated animals were protected against a lethal monkey pox challenge. The neutralizing antibody titers measured in the vaccinated animals were comparable to those seen with the FDA licensed live attenuated Dryvax® vaccine (Figure 3b). The study also underscored a powerful feature of DNA vaccination – that of being able to develop multi-component vaccine formulations to mimic immune responses from live viral infections and added a new dimension to our ability to design vaccines against complex human and animal pathogens.

In summary, while the lead DNA-EP vaccine programs are still only in the Phase I/II stage, the weight of the available data suggests that many of the desired goals for this platform are within reach and that the approach is likely to have a very bright future.

Cautionary Factors

While the field of DNA vaccines is entering its third decade, *in vivo* EP in the clinical setting is still in its relative infancy. There is much to learn about the clinical effects of transient electric fields on tissues. The results to date with DNA vaccines and EP have created a highly favorable safety profile but, while the safety database of DNA vaccines (with or without EP) is now at several thousand individuals (across 100s of DNA vaccine trials) and growing rapidly, it still has not reached the maturity levels observed with the

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licensed vaccines. Thus potential concerns around long term persistence and potential for integration will have to be addressed on a case by case basis.

Another cautionary aspect of the emerging literature is that while EP delivery may generally improve DNA immunogenicity relative to other methods, there are significant differences in the immune responses elicited depending on the pulse patterns, voltage-current and field strengths, electrode configurations, and impedence of target tissues. Similarly electrode shape, size, and DNA vaccine formulations (optimized sequence, dose, concentration, buffers) also play a critical role in the induction of immune responses and may need to be optimized depending on the particular vaccine target specifications for immunogenicity and efficacy. Based on these considerations, EP delivery of DNA should be viewed in terms of a combination product during its development and that importantly, not all EP device – DNA vaccine combinations are likely to lead to the same outcome based on different design and delivery parameters. This aspect is particularly critical to acknowledge for a newly emergent field in terms of rationalizing some of the variability of immune responses reported in the literature across DNA constructs and across EP devices. From the standpoint of vaccine product development, the apparent lack of standardization may well prove to be challenging, if every vaccine-device combination needed to be ultimately optimized in humans. In that regards the DNA vaccine-EP delivery combination is no different from conventional vaccine approaches (live/attenuated/VLP/recombinant) where considerations such as route of delivery (IM, ID, IV, oral, nasal), dose, and choice of adjuvants/formulations/excipients also need to be optimized in pre-clinical and early Phase I/II studies in response to safety, immunogenicity, stability, and market considerations. However, we remain optimistic that common themes will emerge to further simplify the development paradigms as the first DNA vaccines progress through Phase II – III clinical development and are licensed.

Concluding Remarks

The combination of highly optimized DNA delivered by advanced EP is clearly an important and exciting area of investigation. The numbers of positive outcome studies of DNA-EP in the clinic are steadily increasing and support the notion that this is a vaccine product platform with broad applicability. Excitingly, the immune responses seen to date mimic those seen with viral infections in terms of the induction of both cellular and humoral responses and the magnitude and breadth of the responses. The ability to break tolerance in the cancer setting is also a new and encouraging observation in the few clinical studies reported to date. It is also encouraging that the scalability and the economics of manufacturing at scale allow the DNA vaccine platform to be competitive to other vaccine strategies for either therapeutic or prophylactic vaccine scenarios as well as their deployment in developed or resource poor settings. If these important developments continue to mount and additional successes are reported in research laboratories and in the clinic, we may well look back on this decade as the “DNA vaccine decade”.

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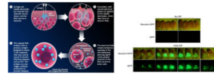

Figure 1.

(a) Schematic depicting the EP process. (b) Enhancement of gene expression following DNA delivery with EP. GFP plasmid was delivered to rabbit muscle via IM injection without EP (top panels) or IM injection with EP (bottom panels). The injected muscle was harvested and then sectioned into 1 mm thick sections to visualize GFP expression either under white light (Muscle + GFP) or under a UV lamp (GFP). The highly fluorescent GFP expression is observed only when the DNA is delivered via EP – representing a 100–1000 fold enhancement in gene delivery to the target tissue (Unpublished GFP images courtesy of Inovio Pharmaceuticals).

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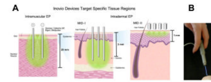

Figure 2.
Electroporation devices developed to target the different depths of the skin/muscle. IM devices include Cellectra®-5P, ELGEN™, Medpulser. The minimally invasive devices (MID) target the dermis/sub-cutaneous layers (MID-I)[53] or the epidermis/stratum corneum (MID-II)[54]. Also shown is a non-contact device where EP is facilitated by piezoelectric discharge (PID)[58].

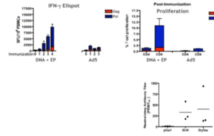

Figure 3.
(a, top) Comparison of cellular immune responses elicited by an optimized SIV DNA vaccine delivered via EP versus an optimized Ad5 SIV vaccine. The DNA EP vaccine yielded stronger and continuously boostable ELISpot responses relative to the Ad5 vaccine. The DNA EP vaccine also led to better proliferative capacity of both CD4+ and CD8+ T-cells and improved polyfunctionality (Figure adapted from [39]). (b, right) Comparison of NAb responses induced by a DNA EP smallpox vaccine to those induced by the licensed Dryvax® live attenuated vaccine in a NHP model (Figure adapted from [7]).

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