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Electro-gene transfer to skin using a noninvasive multielectrode array☆

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ABSTRACT

Because of its large surface area and easy access for both delivery and monitoring, the skin is an attractive target for gene therapy for cutaneous diseases, vaccinations and several metabolic disorders. The critical factors for DNA delivery to the skin by electroporation (EP) are effective expression levels and minimal or no tissue damage. Here, we evaluated the non-invasive multielectrode array (MEA) for gene electrotransfer. For these studies we utilized a guinea pig model, which has been shown to have a similar thickness and structure to human skin. Our results demonstrate significantly increased gene expression 2 to 3 logs above injection of plasmid DNA alone over 15 days. Furthermore, gene expression could be enhanced by increasing the size of the treatment area. Transgene-expressing cells were observed exclusively in the epidermal layer of the skin. In contrast to caliper or plate electrodes, skin EP with the MEA greatly reduced muscle twitching and resulted in minimal and completely recoverable skin damage. These results suggest that EP with MEA can be an efficient and non-invasive skin delivery method with less adverse side effects than other EP delivery systems and promising clinical applications.

1. Introduction

In the past two decades electroporation (EP) has received increased attention for its advantages compared to viral vectors for use in gene delivery. EP has been demonstrated to be an efficient non-viral *in vivo* gene delivery method by several independent research groups [1–5]. Diverse electrodes such as calipers, tweezers, needle arrays and microneedle arrays have been designed and tested in different species [6–10]. Various electrical parameters have been studied for their expression efficiency and adverse effects [6,11]. *In vivo* gene delivery by EP has been reported to achieve effective gene expression in various tissues and organs [12], such as liver [1], skin [13], muscle [14], brain [15], eye [16], lung [17], spleen [18], kidney [19], bladder [20], testis [21], artery [22], and tumors [2].

The skin contains large numbers of potent antigen-presenting cells, Langerhans cells and dermal dendritic cells, as well as an abundant blood supply in the dermal layer of the skin [23], which may help transgenic products distribute into distant organs through circulation [24]. These advantages make delivery of therapeutic genes to the skin very attractive, particularly, for i) the treatment of local diseases including skin cancer, chronic ulcer, burn, psoriasis; ii) vaccination against infectious diseases such as HIV, anthrax, malaria, as well as non-infectious diseases like cancer; iii) the correction of systemic or metabolic disorders like anemia in chronic kidney disease. Previous studies have shown that EP efficiently delivers plasmid DNA to the skin resulting in a 10–1000 fold increase of local and serum expression [24–27]. Skin EP delivery was successfully performed in rodent, porcine and non-human primate model systems [13,24,25]. Intradermal delivery of plasmid VEGF (165), FGF-2 or TGF-β by EP has been observed to promote wound healing in rat or mouse models [28–30]. Significant serum levels were achieved by EP delivery of both EPO and IL-12 plasmid DNA to the skin [24,31–33]. A number of studies demonstrated that significant tumor regression could be achieved by electrically mediated delivery of plasmids expressing IFN-α, IL-12, IL-2, IL-15, IL-18, GM-CSF and other transgenes to cutaneous tumors (melanoma, squamous cell carcinoma) [6]. In our mouse melanoma model [32,34], intratumoral EP of IL-12 plasmid resulted in complete tumor regression rates of 80%. Those mice were also resistant to subsequent tumor challenge. Moreover, our phase I human trial of IL-12 EP treatment of metastatic melanoma showed that distant untreated lesions could also regress, suggesting that not only had a local response been mounted against treated tumors but also a systemic memory response had been generated [35].

Current skin EP systems, utilize, for example, invasive needle electrodes as well as plate electrodes (calipers, forceps, etc.) and typically induce significant muscle twitching and discomfort and treatment can result in skin damage [25]. To overcome the pitfalls of these electrode designs, we developed a new non-invasive electrode known as multielectrode array (MEA). In previous studies [27], we reported that skin EP with the MEA could achieve comparable (in rat)

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or higher expression (in guinea pig) as compared to plate electrodes, while the applied voltage and muscle stimulation was greatly reduced. In the current study, we further modified the MEA to include flexible spring electrodes in the substrate to assure a full contact between all of the electrodes and the skin. We then characterized several critical aspects relevant to therapeutic applications. DNA delivery was tested in a guinea pig model, which has similar skin thickness and structure to human skin [36,37]. Localized transgene expression and kinetics were assessed by the measurement of luciferase activity with an *in vivo* bioluminescence scan. The evaluation of the MEA has also included the correlation between expression and the size of the treated area, potential tissue damage, DNA distribution and localization of gene-expressing cells.

2. Materials and methods

2.1. Animals

Female Hartley guinea pigs used in this study were 4 to 6 weeks old from Elm Hill Labs (Chelmsford, MA, USA). All experimental procedures were approved by the Institutional Animal Care and Use Committee of the Old Dominion University.

2.2. Plasmids

The reporter plasmids encoded luciferase (gWiz-Luc) and green fluorescent protein (gWiz-GFP) were both from Aldevron (Fargo, ND, USA). Fluorescein-labeled plasmid MIR 7907 and Cy^{TM}3-labeled plasmid MIR7905 (Mirus Bio LLC, Madison, WI, USA) were used to observe DNA distribution.

2.3. DNA injection and in vivo electroporation

Prior to delivery, animals were anesthetized in an induction chamber charged with 3% isoflurane in O_2 then fitted with a standard rodent mask and kept under general anesthesia during the procedure. Guinea pigs received intradermal (i.d.) injections of 50 μL or 200 μL plasmid DNA (2 μg/μL dissolved in saline) on the left and right flanks. Immediately after DNA administration, a MEA electrode with 4×4 2-mm-apart pins was placed over the injection site(s). Voltage was applied (each pair of electrodes was programmed to administer four pulses with total 72 pulses [27], electric field was 250 V/cm, pulse duration 150 ms and 150 ms delay). Electroporation was performed using the UltraVolt Model: Rack-2-500-00230 (UltraVolt, Inc. Ronkonkomo, NY, USA). The electroporation parameters we chose here were based on our recently published study [38] in which we evaluated the effect of different electrotransfer parameters on transgene expression and skin damage using a similar designed MEA electrode in the guinea pig model. The pulse parameters of 250 V/cm and 150 ms were found to give the highest expression with minimal damage to the skin. Increasing the field strength did not result in increased expression. For a single 200 μL injection or four 50 μL adjacent injections, four individual pulse applications were applied without change of pulse parameters.

2.4. Living imaging of luciferase expression

At different selected time points after delivery, animals were anesthetized then administrated intradermally with the same DNA volume of D-luciferin with 7.5 mg/mL in PBS buffer (Goldbio, St. Louis, MO, USA). Assessment of photonic emissions using the IVIS Spectrum system (Caliper Life Sciences, Hopkinton, MA, USA)) was performed 1.5 min after injection of D-luciferin. Background luminescence was determined by measuring luminescence from area without DNA injection.

2.5. GFP expression

Each excised sample was immediately frozen on dry ice. After visualization of GFP expression was observed and obtained by a fluorescence stereoscope (Leica Model MZFL III, Leica, Heerbrugg, Switzerland), the specimens were embedded in tissue freeze media OCT compound (Electron Microscopy Sciences, Hatfield, PA) and frozen at −80 °C freezer. Several frozen sections (8 μm thickness) were cut from each sample. Each section was fixed in 25% Acetone + 75% Ethanol 20 min and then washed twice in PBS. It was dried under dark and mounted into a coverslip with VECTASHIELD® mounting medium with DAPI (Vector Laboratories, Burlingame, CA). Sections were examined by Olympus BX51 fluorescent microscopy (Olympus, Tokyo, Japan) for the presence of GFP.

2.6. Histological analysis

Each specimen was embedded, sectioned and fixed as mentioned above. Sections were dehydrated in 95% ethanol for 30 s, stained in hematoxylin solution for 5 min, rinsed with tap water for 3 min, classified in 1% acid alcohol for 10 s, washed with running tap water for 1 min, blued in 0.2% ammonia solution for 30 s, washed in running tap water for 3 min, rinsed in 95% alcohol, 10 dips, counterstained in eosin Y solution for 45 s, dehydrated through 95% alcohol, 2 changes of absolute alcohol, 10 dips each, cleared in 2 changes of xylene, 10 dips each, mounted with xylene based mounting medium. Sections were examined by Olympus BX51 microscopy.

2.7. Statistical analysis

All values are reported as the mean ± SD. Analysis of luciferase activity was completed using a 2-tailed Student's t-test when comparing two groups. Statistical significance was assumed at $p<0.05$. All statistical analysis was completed using the SigmaPlot 10.0.

3. Results

3.1. The level and duration of gene expression were significantly increased by intradermal DNA injection and non-invasive skin EP

The correlation between the level and duration of gene expression to the size of the treated area when delivering by EP with the MEA was evaluated by *in vivo* bioimaging. As shown in Fig. 1A, the maximum level of luciferase expression was achieved one day after delivery. While expression in the non-electroporated sites decreased dramatically by day 2 the expression of EP-treated sites was stable until day 15. The average levels of gene expression in the EP-treated groups were 2 to 3 logs higher than in the non-EP-treated groups from days 2 to 15. Among the different EP-treated groups, luciferase expression increased 3.7 to 6.3 fold in 200 μL DNA with one EP application compared to 50 μL DNA with one EP application from days 1 to 8 after delivery. However, the skin receiving 200 μL DNA and four EP applications expressed the highest level of protein with a 4.5 to 15.8 fold increase in expression compared to 50 μL DNA with one EP application from day 1 to day 12 ($P<0.05$ for the most time points). (Table S1). At day 22 after delivery, the luciferase expression of EP-treated skin decreased to the level of DNA injection only, both of which were still slightly increased as compared to background.

Given these findings, we wanted to address whether we could achieve long-term gene expression by repeated deliveries with MEA EP delivery. Based on the previously stated results, a one-time delivery would result in maximum gene expression within 24 h and would remain relatively constant through day 15. Therefore, we aimed to attempt three deliveries at the same site and to produce longer-term expression. The delivery time points were selected to be

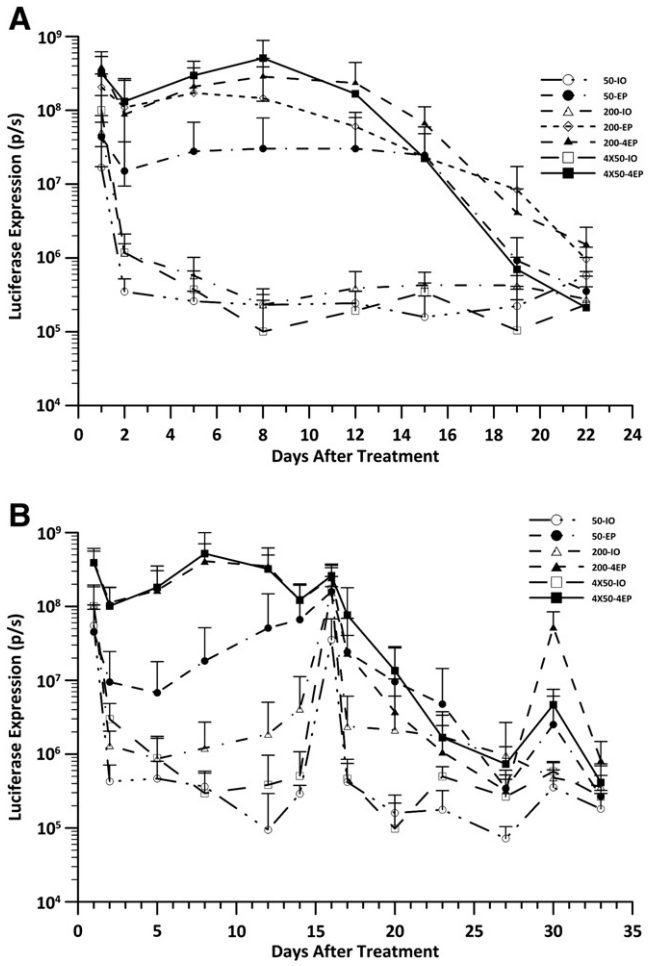


Fig. 1. Kinetic of gene expression in skin after i.d. DNA (gWiz-Luciferase) injection and non-invasive EP. Delivery groups, 50 μL-IO: 50 μL DNA without EP; 50 μL-1EP: 50 μL DNA with 1 EP on the injection site; 200 μL-IO: 200 μL DNA without EP; 200 μL-1EP: 200 μL DNA and 1 EP; 200 μL-4EP: 200 μL DNA and 4 EPs; 50 μL × 4-IO: 4 injections with 50 μL DNA without EP; 50 μL × 4-4EP: 4 injections with 50 μL DNA and each EP on the injection site. A, Time course of luciferase expression in guinea pig skin after 1 delivery at d0. Bars represent mean ± SD. 4–5 sites were analyzed for each delivery. B, Time course of luciferase expression in guinea pig skin with 3 deliveries, separately at d0, d15 and d29. Bars represent mean ± SD. 5–6 sites were analyzed for each delivery. p/s = photons/second.

day 0, day 15 and day 29. Our results from these experiments indicated that subsequent deliveries could not increase or even match gene expression of initial levels nor could it enhance the duration of the expression beyond the initial delivery time frame (Fig. 1B). While in all samples both EP and the plasmid injection only control had similar luciferase expression at one day post second delivery, the expression rapidly decreased and reached background levels by day 12 after the second delivery (day 27). For the third delivery, both non-EP and EP-treated sites could not reach high expression. The gene expression of all sites very rapidly dropped to the background level by day 4 after the third delivery (Day 33). The study was performed twice and reached the same conclusion.

3.2. Gene expression by skin EP delivery with the MEA was exclusively in the epidermal layer of the skin

Fluorescence stereoscopy and microscopy were used to observe the distribution of the gene transfected cells in the guinea pig skin after i.d. DNA injection and EP. Using fluorescence stereoscopy, no expression was observed in either the non-EP or EP-treated sites at 1 h post-delivery. However, green florescence protein (GFP) expression of non-EP skin was present at day 1, decreased rapidly to scattered dots by day 2, and no expression was observed by day 7 or 9 (Fig. 2A, 50 μL-IO). In the EP-treated skin, GFP-expressing areas were larger than those of non-EP controls and the fluorescence intensity was maintained at similar levels till day 7 (Fig. 2A, 50 μL-1EP or 200 μL-4EP). At day 9, very few fluorescence-bright dots were observed in EP-treated skin. No fluorescence was observed in non-treated controls.

To visualize the localization of gene-expressing cells after non-invasive surface EP, cross-sections of the skin were labeled with DAPI and PI for fluorescence microscopy observation. Surprisingly, almost all GFP-expressing cells from EP-treated skin were located in the epidermal layer at day 2 or day 7 (Fig. 2B). Gene-expressing cells at day 2 were cells with nuclei beneath the stratum corneal layer of the epidermis but by day 7 those GFP-expressing cells had lost their nuclei and moved into the stratum corneum. For DNA injection alone, no expression was observed in the epidermal layer of skin at either day 2 or day 7 (Fig. 2C,D). Skin receiving plasmid injection only expressed the luciferase and GFP transgenes one day after delivery (Figs. 1 and 2A). GFP-expressing cells were observed in the dermis for both DNA injection only and EP delivery groups after one day (Fig. S1). These transgene-expressing cells were scattered in the areas surrounding the DNA injection site and occasionally were seen close to the epidermal layer. However, no expression was found in the epidermis for the DNA injection alone while GFP expression was observed there for the skin treated with EP after delivery day 1 (Fig. S1).

3.3. Skin damage caused by noninvasive electroporation using MEA was limited and completely recoverable

For potential clinical applications, any skin damage including significant infiltration, necrosis and scar formation would limit the therapeutic applications of the MEA. Under our parameters for EP, no severe tissue damage, such as skin burning, ulceration or scar formation, was found from gross observation (Fig. 3A). Skin redness and prints of the MEA array did occur after EP delivery but were not present by day 5. Some hair loss was noted in the area of EP application. However, the hair loss was transient and hair grew back within one week after the delivery. Damage was also assessed histologically by hematoxylin and eosin (H&E) staining. In contrast to DNA injection alone, which did not present with any damage, focal cell vacuolization or degeneration in the epidermal layer was observed for all EP-treated skin (Fig. 3B). By day 7, this cell vacuolization was no longer present. Notably, most epidermal cells were morphologically normal after EP delivery. The statistically significant infiltration and necrosis, which were seen in the epidermal or dermal layer in our previous study with the 4 plate electrode [25], was not observed in this study.

3.4. Skin EP with the MEA facilitated intradermal DNA diffusion into the epidermal direction

Although DNA was administered intradermally before EP, the transfected cells were exclusively indentified within the epidermis, not the dermis (Fig. 2). To elucidate the association between DNA distribution and gene expression, Fluorescein or Cy™3-labeled plasmid was administered either by i.d. injection alone or with EP using the MEA. The skin samples were harvested and analyzed by fluorescence stereoscopy 1 h after delivery. While dense DNA-fluorescence with sharp margins was shown in injection alone samples (Fig. 4B, 50 μL-IO), larger, dimmer peripheral DNA distributions were observed in the skin with EP delivery (Fig. 4C, 50 μL-EP). Under fluorescence microscopy, DNA was distributed symmetrically from high concentration in the injection site to low concentration at both peripheral areas in the dermis (Fig. 4D). There was no labeled DNA which appeared close to the epidermis after DNA i.d. injection

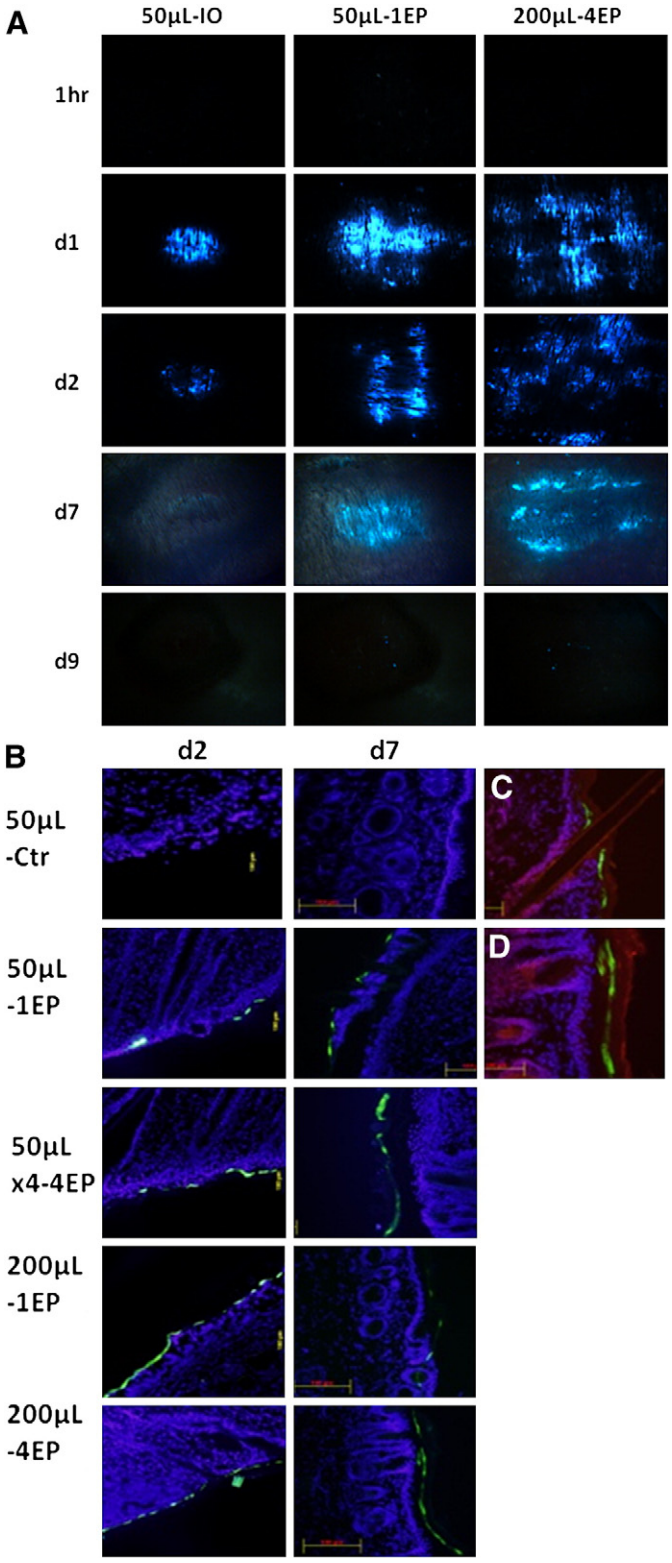


Fig. 2. Distribution of gene-expressing cells after i.d. DNA (gWiz-GFP) injection and non-invasive EP. Skin samples were collected post-delivery, 1 h, day 1, day 2, day 7 or day 9. Samples were analyzed by immunofluorescence microscopy. Delivery group, 50 μL-IO: 50 μL DNA without EP; 50 μL-1EP: 50 μL DNA with 1 EP on the injection site; 50 μL×4-4EP: 4 injection of 50 μL DNA and each EP on the injection site; 200 μL-1EP: 200 μL DNA and 1 EP; 200 μL-4EP: 200 μL DNA and 4 EPs. A, One representative picture of 3 treated sites. (B, C, D) Total 6 cryosections (2 sections per sample) of each delivery were analyzed. Cell nuclei were blue-stained by DAPI. GFP-expressing cells were shown in green. (C, D) Cell nuclei and stratum corneum was shown red-stained by propidium iodide. B, One representative section of each delivery was presented for post-delivery day 2 and day 7 (magnification = 100, scale bar = 100 μm). C, One representative section from post-delivery day 2 (magnification = 200). D, One representative section from post-delivery day 7 (magnification = 200, scale bar = 100 μm).

alone (Fig. 4D). However, EP changed this pattern. The relative scattered and spread distribution was seen from injection site to the epidermal direction. A few labeled DNA spots were observed in the epidermis (Fig. 4E).

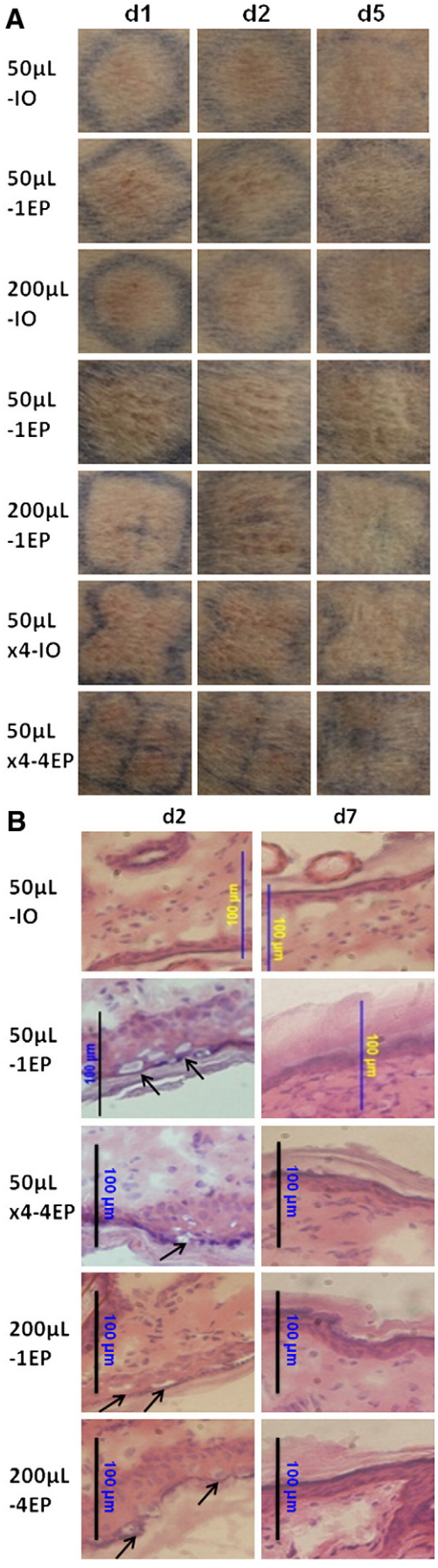
4. Discussion

While many studies focus on the application of skin EP for superficial cancers [6,39], a few studies have demonstrated that significant serum levels of products could be obtained by EP gene transfer to skin [24,31,34]. Considering the easy access and large area of the skin, the expression level could be potentially increased by increasing the area treated to achieve the effective protein concentration in serum. Indeed, luciferase expression could be significantly enhanced by increasing the delivery area. Here we demonstrated that local protein expression levels can be increased by an average 7.8 fold (d1 to d12, $p<0.01$) by quadrupling the size of the treated area (200 μL-4EP compared to 50 μL-1EP). It could, however, be interpreted as marginal electric field effect because four pulse deliveries were applied adjacently. The marginal areas were exposed to repeated electrical field, so more cells could have been transfected and/or more DNA transferred into the same cells. To achieve more protein product locally or systemically, we can simply apply multiple injections and pulse deliveries or expand the MEA without any change of EP parameters, for example the current 4×4 array electrodes could be expanded to a 7×7 array to assure a 4-fold increase of size.

One of the critical aspects for skin EP is the duration of expression after electrogene transfer. The kinetics of luciferase expression in mice has been studied by several groups [24–26,40–42]. A significant increase in gene expression was obtained by skin EP with plate electrodes in two weeks [24–26,40]. Different expression patterns were reported, which may be due to different electrodes and/or parameters of EP chosen by the different groups. EP with needle electrodes showed increased expression for longer than 3 weeks [41,42], most likely because needles can achieve deeper penetration of electrical field or may facilitate DNA diffusion from the injection site into the adjacent dermis or even muscle layers [42,43]. Interestingly, in guinea pig, luciferase expression in the epidermis reached the first peak at day 1, then slightly dropped at day 2 and slowly reached the second peak at day 8. The significant expression after EP can last up to 15 days. If EP delivery method targets to the epidermal layer of the skin as in this study, the duration of transgenic expression very likely depends on the epidermal turn over.

Multiple EP treatment applications were often utilized to treat cancer in animal models or clinical trials [25,32,34,35]. In this study, multiple deliveries were designed to achieve long-term expression and assess the feasibility of skin EP for protein replacement. Unfortunately, luciferase expression patterns after the second and third deliveries were shown to be completely different as compared to the first delivery. No definite interval of high expression was observed after the second and third deliveries. The presence of anti-luciferase IgG antibodies was discovered in the guinea pig serum after three EP deliveries and is most likely the cause of the change in expression patterns (Fig. S1). Vandermeulen et al. also demonstrated that high titers of anti-luciferase IgG antibody were induced by multiple intra-pinna electroporations (one priming and two boosts) in mice [44]. These results indicate that since luciferase is an exogenous protein capable of eliciting an immune response, it is not a good reporter for multiple deliveries or long-term expression studies in guinea pigs. On the other hand, the capability to induce an immune reaction to a weak antigen by skin EP is helpful for researchers to design an effective vaccination against infectious diseases or cancer [10,44–51].

The distribution of transfected cells by EP is dependent on both the skin differences between the animals as well as the electrodes employed. Our results show that uniform epidermal expression in guinea pig skin can be obtained by EP with the MEA. The study of



intradermal DNA EP with the caliper electrode demonstrated that the transfected cells were present at the dermis in mouse while at the epidermis in xenograft human skin [40,44]. Moreover, EP with tweezer electrodes resulted in transgenic expression in the lower dermal region of rabbit skin [52]. However, EP with needle array electrodes could result in transfected cells in the dermis, epidermis, hypodermis even around the muscle layer, but mainly in the panniculus carnosus muscle layer of the mice [42,43] or dermis of the pig [53]. For plate electrodes, the electrical field went through all layers of skin between the two plates [54]. For the needle electrodes, the electrical field was confined between the two (array) needles in the skin [54]. However, the electric field generated by the MEA is designed to decrease the depth of penetration thereby reducing muscle contraction. We observed significantly reduced muscle twitching when using the MEA as compared to the 4 plate electrodes or needle electrodes.

It is necessary to point out that non-invasive electrodes such as plates and the MEA do not directly affect DNA distribution after i.d. administration. On the other hand, the needle electrodes may penetrate the injection site and facilitate DNA diffusion into the surrounding area. This is a potential explanation for the spread of expression usually observed by EP with needle arrays [42,53]. The histological characterization of skin also plays a role in the distribution of transgenic expression. With the same plate electrodes or i.d. DNA injection only, both Zhang's and Hengge's groups demonstrated that gene-expressing cells in the dermis for mouse skin but in the epidermis for xenografted human skin [40,55]. The epidermal expression in guinea pig by the MEA may also be associated with its similarity to human skin structure [36,37].

Consistent with our previous report [27], EP with the MEA could greatly reduce the adverse effects of needle or plate electrodes while comparable or higher expression levels were achieved. Minimal skin damage was observed grossly as well as histologically and complete recovery after EP was observed. Tissue damage such as the dermal necrosis or burning seen in previous studies done by our group [25] and others [56] was not observed in this study. When multiple deliveries with the MEA were applied to the same sites, skin redness and hair loss were slightly increased for both DNA injection alone and EP, but completely healed by day 5 (Fig. S2). These results were consistent with our previous finding in mice where skin damage was increased by repeated gene delivery with plate electrodes [26]. Both studies suggest that repeated application of EP pulses at the same site should be avoided.

Based on the DNA distribution and gene expression we can see there are two types of expression for non-invasive EP skin delivery with the MEA in guinea pigs. One is local expression around DNA injection site with the duration of 1–2 days. Another is epidermal expression distant from DNA injection site with the duration of 15 days. The first pattern is obviously independent of EP because it occurred in both DNA injection alone and EP-treated locations (Figs. 1A,B and 2A). The latter pattern is specifically related to MEA EP because it did not occur with DNA injection alone. The two patterns of transgenic expression may explain why the luciferase expression with EP dropped slightly at day 2. It is possible that day 1 expression with EP included the component related to non-EP dependent expression and that waned rapidly. Further histological analysis of DNA distribution (Fig. 4D,E) and gene expression location (Figs. 2B and S3) demonstrated that MEA EP first facilitates DNA diffusion from

Fig. 3. Gross observation and histology of skin after i.d. DNA injection and non-invasive EP. A, Skin observation after delivery. Pictures were taken at post-delivery day 1, day 2 and day 5. One representative picture of 4 to 5 sites was shown here. Delivery group, 50 μL-IO: 50 μL DNA without EP; 50 μL-1EP: 50 μL DNA with 1 EP on the injection site; 200 μL-IO: 200 μL DNA without EP; 200 μL-1EP: 200 μL DNA and 1 EP; 200 μL-4EP: 200 μL DNA and 4 EPs; 50 μL × 4-IO: 4 injections with 50 μL DNA without EP; 50 μL × 4-4EP: 4 injections with 50 μL DNA and each EP on the injection site. B, Hematoxylin and eosin-stained skin samples. One representative of 3 treated sites was presented here for post-delivery day 2 or day 7. Arrows indicate the focal cell vacuolization. (magnification = 200, scale bar = 100 μm).

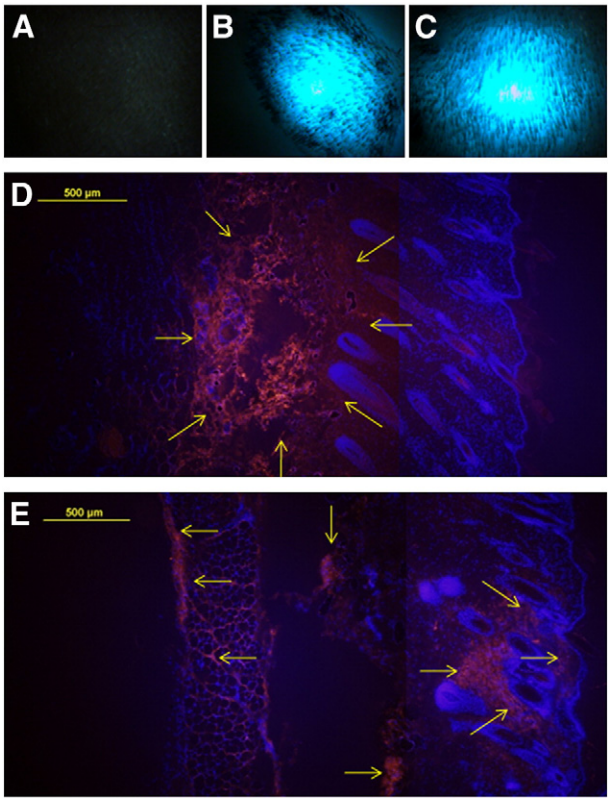


Fig. 4. DNA distribution in the skin after i.d. DNA injection and non-invasive EP. A, B, C, Skin observation by flurescence stereoscope after delivery with fluorescein-labeled plasmid. Pictures were taken at post-delivery 1 h. One representative picture of 2 sites was shown here. Delivery group: A, control; B, 50 μL-IO: 50 μL DNA without EP; C, 50 μL-1EP: 50 μL DNA with 1 EP on the injection site. D, E, total 4 cryosections (2 sections per sample) of each delivery were analyzed. Cell nuclei were blue-stained by DAPI. Cy™3-labeled DNA was shown red as indicated by arrows. D, One representative section of 50μL-IO was presented. E, One representative section of 50 μL-EP was presented (magnification = 100, scale bar = 100 μm).

the dermal layer into the epidermal layer and then electrotransfer of DNA into epidermal cells.

5. Conclusion

Efficient gene delivery can be obtained by skin electroporation with a non-invasive multielectrode array. The high expression can be maintained for up to 15 days after single skin EP with MEA. The gene expression level can be easily multiplied by increasing the delivery area without any change of EP parameters. Skin EP with MEA was found to target the epidermal cells for gene transfer. In contrast to plate electrodes, skin EP with MEA significantly reduced muscle twitching and resulted in minimal and completely recoverable skin damage. However, multiple EPs with MEA are not recommended to apply in the same site because of the potential of skin damage. Further studies will focus on whether we can translate these findings into vaccination, cancer immunogene therapy or long-term endogenous gene expression for protein deficiencies.

Supplementary materials related to this article can be found online at doi:10.1016/j.jconrel.2011.01.014.

Conflict of interest

With respect to duality of interest and financial disclosures, Dr. R. Heller is an inventor on patents which cover the technology that was used in the work reported in this manuscript. In addition, Dr. R. Heller owns stock and stock options in Inovio Pharmaceutical Corporation and has an ownership interest in RMR Technologies.

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