MICRONEEDLE ROLLER ELECTRODE ARRAY (M-REA): A NEW TOOL FOR *IN VIVO* LOW-VOLTAGE ELECTRIC GENE DELIVERY

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ABSTRACT

This paper reports a novel device, so-called microneedle roller electrode array (M-REA), for minimally invasive *in vivo* electric gene delivery, which utilizes parallel circular blades with microneedle arrays on the edge as electrodes. The microneedle arrays are fabricated by planar laser micromachining. Owing to reduced resistance by penetrating stratum corneum and small gaps between electrodes, electric gene delivery by electroporation can be achieved under low voltage. Additionally, our device provides ability of large area electroporation and causes less damage on the target tissue, benefiting from rolling operation, less invasiveness, good biocompatibility and low voltage. Furthermore, low cost fabrication and convenient operation offer vast potential for practical clinical applications.

INTRODUCTION

As a promising method to deliver molecules into animal tissues, *in vivo* electric gene delivery by electroporation has received attention for clinical applications including DNA vaccination [1] and electrochemotherapy [2]. The first demonstration of *in vivo* electroporation was by dint of the hand-assembled dual-needle electrode and high voltage stimulation. [3] Because the dual-needle device was convenient and general, it has been developed into a widely used equipment. However, large geometric dimension of such needle electrode may cause severe trauma of penetration, and high voltage electric stimulation may bring about serious electric burn. [4] In addition, the electric field generated by the dual-needle is nonuniform in the target tissue that will negatively influence the performance of gene delivery. These problems limit the clinic application of the conventionally manufactured needle-based electroporation equipment. In order to shrink the dimensions of needle into the range of a few hundred microns, microfabrication technique has been utilized. [5] Benefiting from the reduced feature size, microneedle arrays [6-8] have been developed for drug delivery [9], *in vitro* electroporation [10,11] and *in vivo* gene delivery [12,13] with remarkably less damage and lower voltage, and the associated pain can be avoided as well. However, these microneedle devices can only achieve a successful drug delivery on a confined area, which may limit therapeutic effect of the vaccination and/or electrochemotherapy. Furthermore, the use of these microneedle device has received limited practical attention primarily due to the complexity of device design and high cost of the fabrication.[5]

In this paper, we proposed a new tool, M-REA (Microneedle Roller Electrode Array), for *in vivo* efficient gene delivery of nucleic acid molecules. The M-REA can disturb the high-resistance stratum corneum layer, thus the sufficient hypodermic electric field for efficient electroporation can be established under low voltage condition and constrained within the stratum corneum on a large area. The damage on the tissue can be dramatically reduced by using low-voltage electric pulse. Its advantages involving simple operation, high efficiency and safety express vast potential in clinic application.

EXPERIMENTAL

Design and Fabrication

As shown in Figure 1, we integrated stainless steel parallel circular blades with microneedle arrays on the edge, PVC handle and axles to form the M-REA. The stainless steel circular blades were fabricated by laser cutting. The thickness of these blades was around 50 μm to 100 μm. Each microneedle was approximately 1mm long, but only the half is exposed as electrode, which allowed the injection needle to be inserted to a controlled depth. This was critical in delivering the target molecules into the

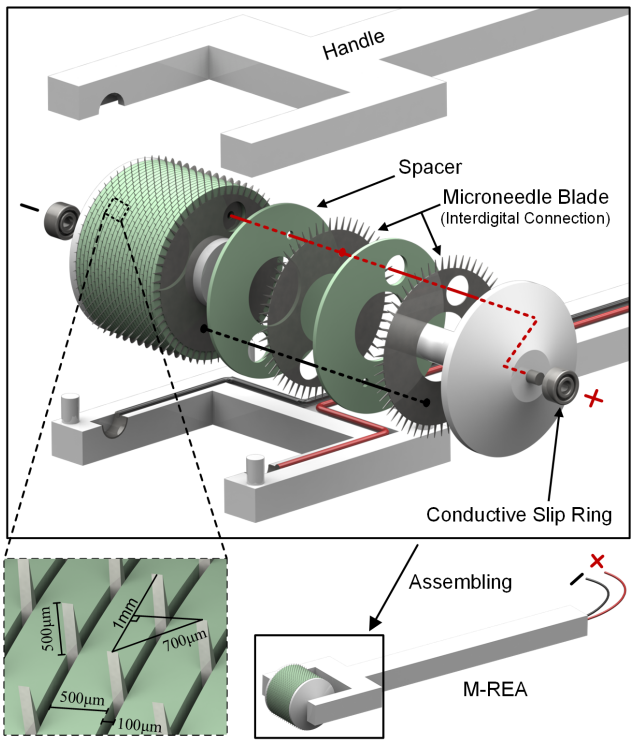

Figure 1: Prototype development of the microneedle roller electrode array (M-REA).

tissue. These blade electrodes were separated by 500-μm-thickness PMMA spacers and interdigitally connected to anode or cathode through the slip rings and hidden wires as shown in Figure1. The specific connection method was that the hidden wire linked to the anode (red line) connected to the smaller holes on these blades, and the bigger holes kept it isolated from other blades. While the ground wire (black wire) took the opposite way of connection.

Figure 2(a) represents the schematic view of the electroporation. The delicate microneedles gently and painlessly pierce the skin to generate electric field to convey drugs, which are injected in advance, into the tissue cells when pulses applied. Instead of noninvasive electroporation method, the M-REA can penetrate the high-resistance stratum corneum, thus the successful gene delivery can be carried out with low-voltage electric pulse using X-Porator® EBXP-H1 (Suzhou Etta Biotech Co., Ltd.). Because of the diminutive size of the microneedle and weak applied electric stimulation, the injury on the tissue is slight. The homogeneous electric field generated by the microneedle matrix can also boost the effect of delivery as well.

Electric Field Simulation

The result of electric field simulation (COMSOL 5.2) is shown in Figure 2(b). We set up simplified models to analyze the electric field contribution with the microneedle array penetrating into the skin. The conductivity of the metal microneedle and tissue was set as 1.46 MS/m and 0.1 S/m, respectively. From the result, we can know that M-REA can generate relatively homogeneous electric field beneath the skin. The electric field strength in the majority of the area between the adjacent microneedles is in the range of 400 V/cm to 800 V/cm, which can achieve highly efficient gene delivery. Compared with non-invasive electrode, the effective coverage by using M-REA is much larger

M-REA prototype

The photo of the M-REA prototype is shown in Figure 3. The whole device contained a roller electrode array and a handle. The roller head with diameter of 2 cm had a series of microneedle blades and PMMA spacers. These microneedle blades were connected to the anode and cathode through the hidden wires in the roller head and the handle, described in Figure 1. When doing the gene delivery treatment, the red wire and black wire (Figure 3(a)) were connected to the output and ground of the electric pulse generator (X-Porator® EBXP-H1, Suzhou Etta Biotech Co., Ltd.) respectively. Compared with previously

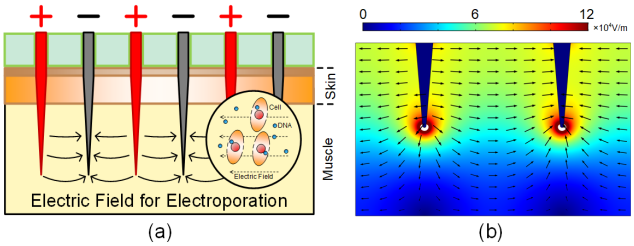

Figure 2: (a) Schematic view of electroporation. (b) Simulation of electric field by the M-REA (50V).

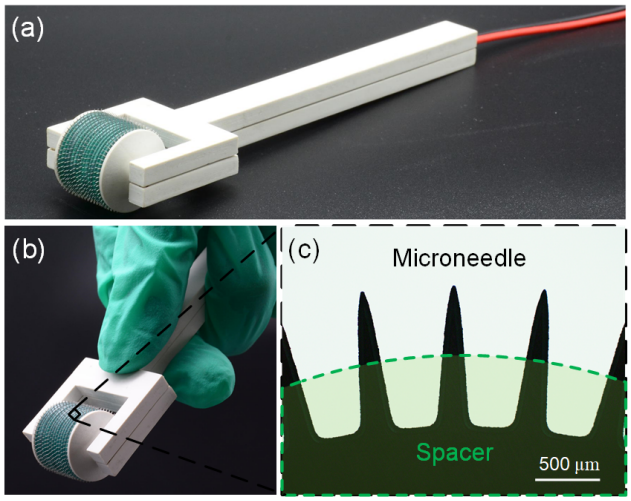

Figure 3: (a) Photograph of the M-REA. (b) The device held in hand. (c) Micrograph of microneedle cut by laser.

reported microneedle-based *in vivo* electric gene delivery equipment, the cost of the M-REA is much lower. Because no complex microfabrication was utilized in the manufacture process, and the whole prototype can be assembled manually. Upon its microneedle array penetrating the high-resistance stratum corneum, the sufficient electric field under skin can be generated under low-voltage stimulation to carry out efficient gene delivery.

Besides, the size of MRE was also suitable and convenient for hand operation (Figure 3(b)). By slightly piercing the skin, the Microneedle array on the head could easily penetrate the skin, and the treated area within one round could reach 1 cm × 1.7 cm that was large enough for small-animal model. For big-animal application, the user can increase the treated region by rolling the device and multiple electroporation.

RESULTS AND DISSCUSSION

Insertion Test

To test the ability of penetration of the M-REA, 5μl (1μg/μl) Cy5-labelled siRNA was applied on the depilated skin, and the skin is impermeable to the Cy5-labelled siRNA solution. Then we used the M-REA to roll on the skin once (2) or five times (3), while the control mouse (1) was treated without microneedle. Ten minutes later, the mouse skin was thoroughly washed and fluorescently imaged.

As shown in Figure 4(a), the treatment with M-REA enhanced the permeation of the siRNA, because the mouse #2 and #3 expressed high doses of fluorescence. And the fluorescence of mouse #3 was obviously stronger than mouse #2, revealing that repeated penetration of the microneedle can make the skin more permeable to small molecules. By contrast, the mouse #1 was still impermeable to siRNA for that the fluorescence was low. Additionally, according to Figure 4(a), the fluorescence of the treated area on the leg showed uniform fluorescence intensity, indicating that the M-REA could offer a uniform penetration within the target region. The fluorescent area was large as well. The image result tells that the micro needle of the M-REA can insert the skin easily and the effect of penetration is uniform. Moreover, this insertion

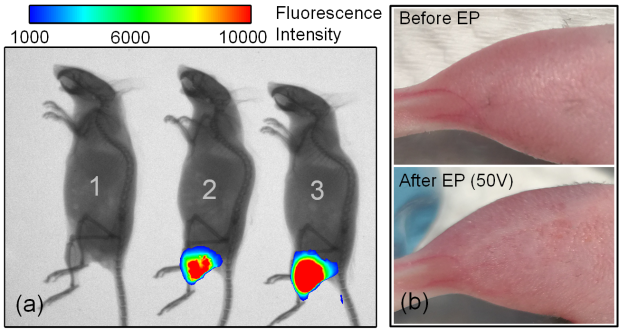

Figure 4: (a)The skin insertion test. After putting a drop of Cy5-labelled siRNA on the depilated skin, the M-REA rolled on the skin once (2) or five times (3), while the control mouse (1) was treated without microneedle. (b) Skin surface before and after electroporation by M-REA.

treatment can help strengthen the permeability of the large area of the skin.

Evaluation of Invasiveness

For some specific biologic applications or clinic therapy, high-voltage gene delivery and forceful needle penetration can cause severe trauma on the target tissue, which directly affects the subsequent operation and curative effect. Therefore the tissue damage by the electric current should be considered as the main concern that prevents the use of traditional needle-based gene transfer devices in many clinical applications. To address these issues, the physical dimension of the microneedle has been shrunk to hundreds of micrometers and the applied voltage has been reduced to a few tens of volts. The M-REA, as a new tool of low-voltage electric gene transfer device, can provide a minimally invasive *in vivo* electroporation with high efficiency and slight tissue damage on the tissue.

To verify the actual injure induced by penetration of the microneedle roller and electric pulse, visual examination of skin appearance before and after 10 electric pulses (50 V amplitude, 10 ms duration, 1s interval) treatment is shown in Figure 4(b). It was clear that the damage was very slight, as only some tiny pores on skin, which could recover in a few days. No any bleeding was discerned and no functional disability of the mouse leg was observed as well. Therefore, our proposed M-REA can offer a safe *in vivo* electric stimulation.

Plasmid DNA Transfection

To verify capacity of the electric gene delivery, *in vivo* DNA electroporation experiments involved with the pulse amplitude on the mouse thigh muscle have been done. In these experiments, RFP (red fluorescence protein) expression was quantitatively analyzed using in vivo imaging system to determine the gene delivery efficacy. The myocytes of the living mouse thigh were set as the target tissue for transfection. In RFP plasmid delivery experiment, 40μl (2μg/μl) hyaluronidase was firstly injected into the muscle of the female C57BL/6 mouse thigh after unhairing. After reaction for 30 minutes, 40μl (1 μg/μl) RFP plasmid were injected. Then the roller head of the M-REA was gently placed on the target surface and lightly pushed into the tissue. Keeping the microneedle array piercing the skin, we applied 10 electric pulses (50V,

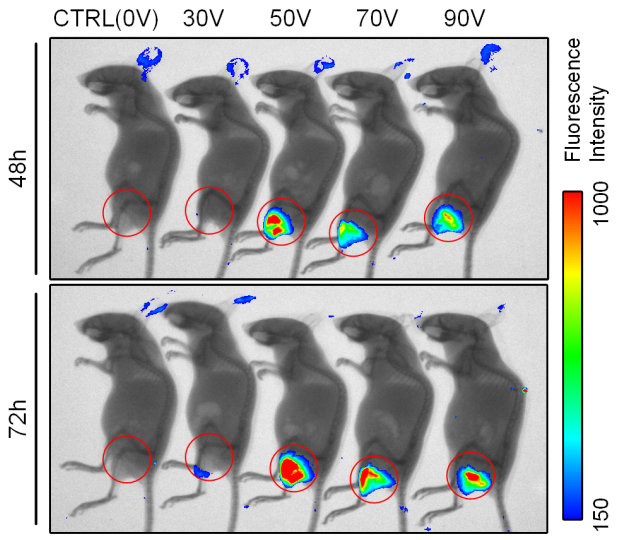

Figure 5: Whole-body fluorescence images of the mice whose thigh received different treatments for RFP plasmid in vivo electroporation 48h and 72h after electroporation. (10 pulses, 10 ms duration, 1 s interval)

10 ms duration and 1 s interval) to convey the molecules into the muscle cells. After that, these treated mice were collected and cultivated. The RFP fluorescence was monitored at 24 hours, 48 hours and 72 hours.

The whole-body fluorescence intensity images (48 and 72 hours after electroporation) are shown in Figure 5. The negative control showed no RFP expression, while other electric pulse treated groups expressed fluorescence protein in different extent. The fluorescent area was largest at 50V, and the intensity was very uniform as well. Using higher voltage pulse, the expression of RFP plasmid could keep maintained at a high level. While too weak electric condition (30V) did not efficiently help enhance gene delivery, which indicated that only if the electric field strength is larger than the certain threshold value, efficient electroporation can be achieved.

The mean fluorescence intensities (MFIs) of the targets were quantitatively analyzed shown in Figure 6. The trend of the data from different groups was similar at 3 time points of observation. With the time increasing, the

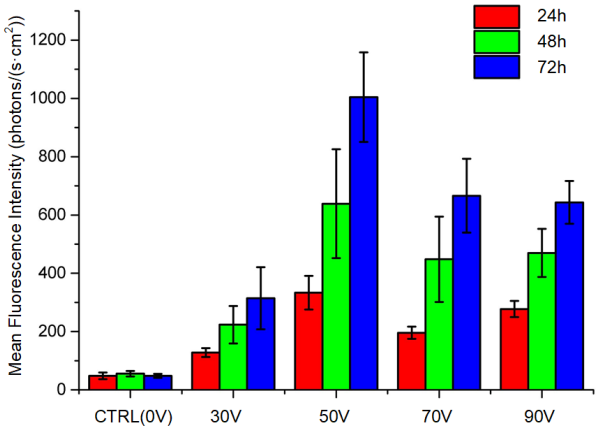

Figure 6: The relationship between mean fluorescence intensities (MFIs) of the electroporated mice thigh and voltage within 3 days. (n=4)

fluorescence intensity enhanced. The MFI increased remarkably from 30V to 50V, and reached its peak at 50V. Higher voltage (70V and 90V) caused a decrease in MFI, which stated that the damage from high field in tissue cells probably negatively affected the gene expression, and their nearly same data revealed that the DNA expression might reach saturation over 70V. These results proved that the M-REA was capable for efficient low-voltage *in vivo* electroporation.

CONCLUSION

In this paper, we have proposed microneedle roller electrode array (M-REA) as a promising new tool for *in vivo* gene delivery. The M-REA can achieve a safe low-voltage electroporation because of the narrow space between the electrode and the small dimension of the microneedle. Its ability of penetration has been examined by evaluating the fluorescence of the penetrated Cy-5 labelled siRNA, proving that this roller device can readily insert into the tissue and help enhance the permeability of the skin. In addition, the safety and efficiency have been verified as well. The surface of the target tissue after electric pulse stimulation showed no apparent trauma and no functional disability. The plasmid DNA delivery experiment can indicate that the M-REA can achieve its best performance at 50 V voltage pulse. Both of higher and lower voltage than that can negatively affect the delivery efficiency. Low cost, high efficiency and safety, the M-REA has huge potential for the biology research and the clinic application.

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