Design, modelling and preliminary characterisation of microneedle-based electrodes for tissue electroporation *in vivo*

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Design, modelling and preliminary characterisation of microneedle-based electrodes for tissue electroporation *in vivo*

Conor O'Mahony[1], Ruth Houlihan[1], Konstantin Grygoryev[1], Zhenfei Ning[2], John Williams[2] and Tom Moore[2]

[1]Tyndall National Institute, University College Cork, Cork, Ireland.

[2] School of Biochemistry and Cell Biology, University College Cork, Ireland.

conor.omahony@tyndall.ie

Abstract. We analysed the use of microneedle-based electrodes to enhance electroporation of mouse testis with DNA vectors for production of transgenic mice. Different microneedle formats were developed and tested, and we ultimately used electrodes based on arrays of 500 μm tall microneedles. In a series of experiments involving injection of a DNA vector expressing Green Fluorescent Protein (GFP) and electroporation using microneedle electrodes and a commercially available voltage supply, we compared the performance of flat and microneedle electrodes by measuring GFP expression at various timepoints after electroporation. Our main finding, supported by both experimental and simulated data, is that needles significantly enhanced electroporation of testis.

1. Introduction

Transgenic rodents, whose genomes have been altered through the use of genetic engineering techniques, are used to study normal development and for producing models of human disease to understand pathogenesis and to underpin development of therapies [1, 2]. Transgenic rodents are generally produced by delivery of DNA constructs to fertilised oocytes using microinjection, followed by embryo transfer to pseudopregnant mice. These are laborious, expensive and inefficient techniques that have not changed significantly in thirty years.

Recently, efforts have been made to use electroporation techniques as an alternative to this procedure. Electroporation involves the application of voltage pulses to create an electric field in a tissue sample. This leads to a temporary increase in permeability of cell membranes, facilitating the passage of DNA to cells. DNA injection into testis, followed by electroporation, has been reported as a novel method of producing transgenic rodent offspring [3, 4]. The ability to produce transgenic rodents by testis electroporation would greatly reduce the costs and skill sets needed to produce this essential resource required for the study of developmental biology and disease pathogenesis.

Electroporation pulses are applied using a pair of flat electrodes, applied to either side of the tissue region of interest. The outermost layer of the skin surrounding the testis, the stratum corneum, is only 10 - 20μm thick and is largely composed of dead cells. It is therefore highly resistive and a large percentage of the applied voltage is dropped across this barrier, reducing the electric field inside the tissue and diminishing the efficacy of the electroporation procedure [5]. Skin and the stratum corneum also exhibit considerable variability in thickness and electrical characteristics, leading to uncertainly regarding optimum procedures.

Microneedle devices were originally intended for use in transdermal drug and vaccine delivery [6, 7], but have also generated significant interest for use in electroporation [5, 8-10]. Electrodes formed from arrays of electrically conductive microneedles can pierce the outermost skin layer and make direct contact with the moist, highly conductive epidermis immediately underneath, thereby bypassing the high-impedance stratum corneum and enhancing the electric field within the tissue. The microneedles also produce an increased electric field at the needle tips, which may contribute to increased efficacy. Furthermore, microneedle lengths can be such that they do not stimulate nerve endings within the skin, and therefore their use is painless [11].

This paper addresses the development of microneedle-based electrodes for the production of transgenic rodents using testicular electroporation. Finite-element modelling software was used to simulate the strength and distribution of electric fields established inside the testis. A double-sided wet etching process was then used to create microneedle arrays, which were affixed to tweezers to create prototype electrodes. Preliminary *in vivo* experiments were carried out and showed a strong increase in efficacy over conventional flat electrodes.

These findings may aid in developing an effective and reproducible protocol for the production of transgenic rodents by testis electroporation.

2. Finite element modelling and electrode design

A finite element model was constructed to compare the electric field across the testes achieved using (a) flat electrodes placed either side of the testes with (b) microneedle electrodes placed either side of the testes. A three-dimensional half-model was generated in COMSOL with the testes modelled as an ellipsoid with a major axis of length 19 mm and minor axes of length 11 mm, Figure 1. These dimensions correspond to measurements taken on rat testes. The stratum corneum was modelled as a thin, low permittivity layer surrounding the testes. The relative permittivity of the testes tissue was assumed in this case to be two orders of magnitude greater than that of the outer membrane. The actual permittivity is difficult to measure and in our case an exact value was not necessary since the desired output of the modelling exercise was to establish the relative improvement in the magnitude of the field by incorporating microneedles onto the electrodes. In the case of the electrodes with microneedles, a 2 x 2 array of 300 μm long needles was assumed.

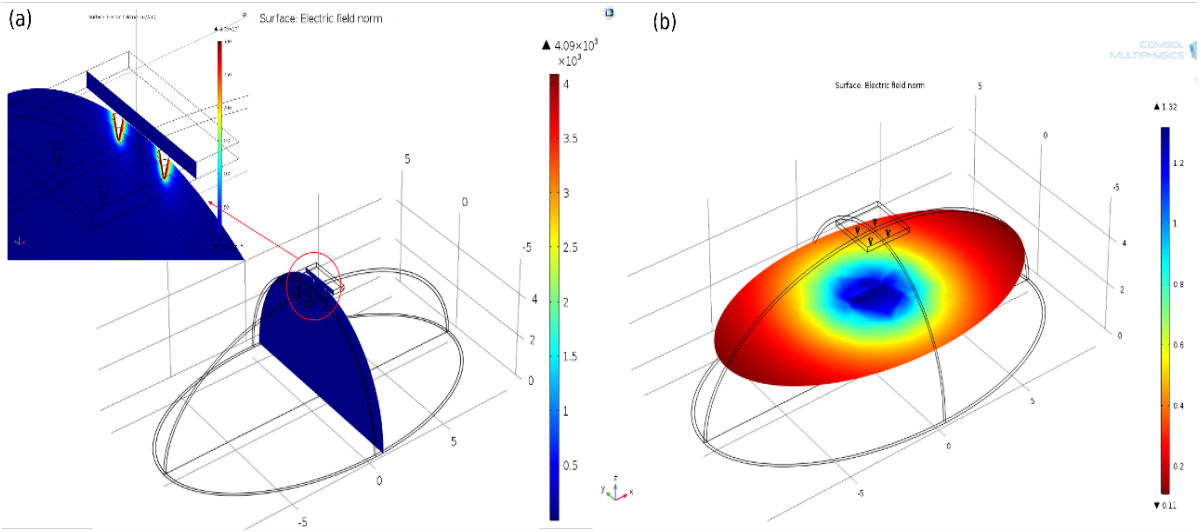

Figure 1. (a) Electric field in a plane perpendicular to the electrode and through the centre of two needles and (b) electric field in the plane mid-way between the electrodes and the centre of the testis ellipsoid.

The applied bias to the electrode was 40 V and the symmetry plane was held at ground. The results of the model with the microneedles show a maximum field three orders of magnitude higher (1.3 V/mm) than that of the flat electrodes (1 x 10^{-3} V/mm, data not shown), in which case the electric field is largely dropped across the layer of skin surrounding the testis.

3. Fabrication and assembly

3.1. Microneedle fabrication

Microneedle fabrication is based on potassium hydroxide (KOH) wet etching of single crystal silicon, where double-sided wafer patterning and an anisotropic etch process is used to simultaneously create a microneedle on the front side of the wafer, and a through-silicon via (TSV) from the back side. Metal deposition on both the front and back of the wafer then establishes electrical contact through this via. This rapid, single-step process eliminates the need for additional processing in order to establish electrical contact between the front and rear sides of the array, and full details are available in [12]. The wafer was then coated (front and back) with a layer of 20nm Ti/200nm Ag before dicing, and electrical contact was made between front and rear surfaces through the KOH-etched TSV openings.

3.2. Assembly

Based on testes measurement as outlined above, arrays of 5 x 4 needles were used. Needle height was 500 mm, pitch was 1725 μm, and the array base measured 8.62 mm x 6.90 mm. To generate prototype electrodes, arrays were affixed to the flat tips of plastic tweezers (Lerloy 6, Farnell, Ireland) using double-sided tape, and interfaced to a voltage source with ribbon cable and wire-to-board connectors. A stopper screw was added to the assembly to limit electrode travel and provide a consistent application force, Figure 2.

Figure 2. Completed device showing electrical connections and stopper screw.

4. Electroporation of adult mouse testis *in vivo*

4.1. Animal model.

Procedures were performed on eight C57Bl/6 male mice aged 35 days with body weight of approximate 20 g. Mice were anaesthetised, scrotal region hair was removed and each testis was injected with 20 μl (0.5 μg/μl) of circular plasmid pCX-EGFP DNA vector. 50 μl of electrogel was coated onto each electrode prior to electroporation to enhance electrical conductivity. Four mild square wave electric pulses (130 V, 5 ms, 50 ms interval) followed by 8 mild square wave electric pulses (30 V, 50 ms, 50 ms interval) were generated by an electroporator (NEPA21, Sonidel, Ireland) and delivered to each testis using flat or needle electrodes, with inter-electrode distance of 4 mm. A new electrode was first applied to the left testis, and reused once on the right testis of the same mouse. After electroporation, the testis area was cleaned with 70% ethanol.

Seventy-two hours post-procedure, the mice were euthanised and testes were harvested and imaged using a Leica DMI3000 fluorescence microscope. The testes were then homogenised and lysed using the M-PER mammalian protein extraction reagent (Thermo Fisher Scientific, Ireland). Protein sample concentration was determined by Bradford reagent (Sigma, UK), as per manufacturer's instructions. Anti-EGFP antibody (Takara Bio, USA) was used at 1:1000 dilution in western blotting. Anti-Gapdh antibody (Sigma, UK) was used at 1:2000 dilution in western blotting as an internal control. Western blotting method was performed as described in [13].

4.2. Results

Inspection of whole testis under microscopy indicated extensive fluorescence in both flat and needle electrode-treated testes as shown in Figure 3.

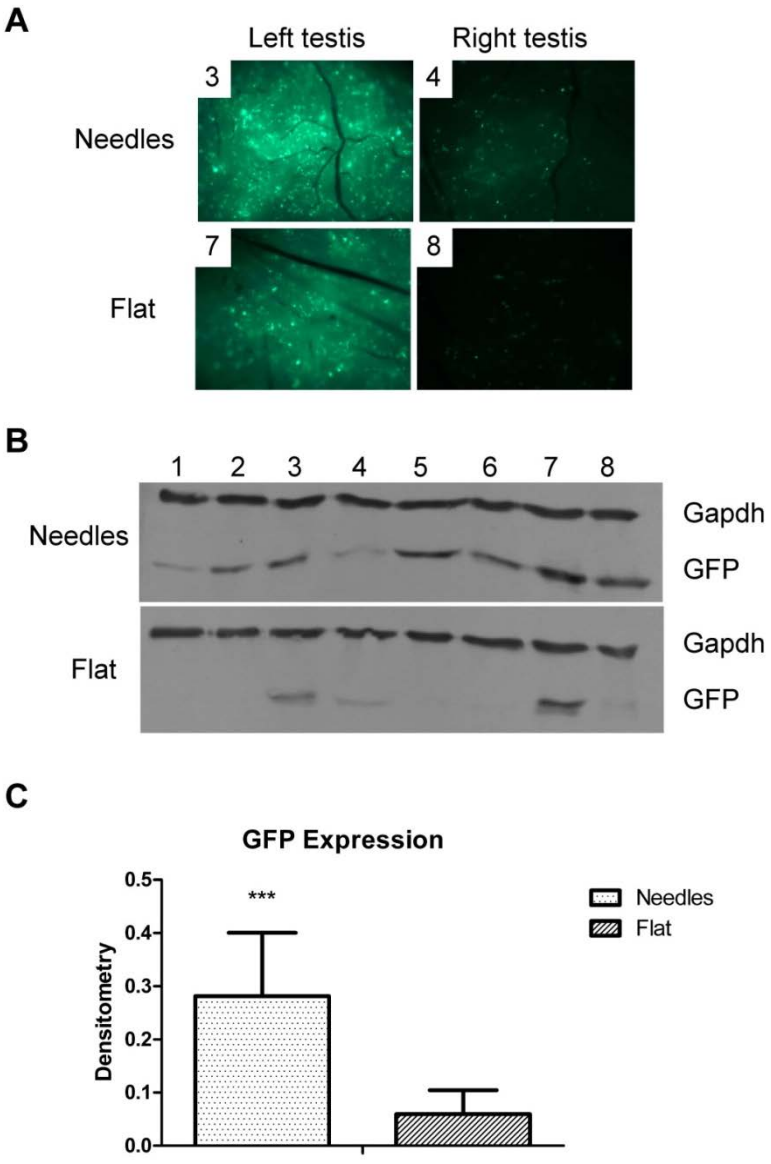

Figure 3. A) Selected examples of fluorescent images of whole testis transfected with GFP from two mice. B) Western blot of all eight testes treated with needle (upper panel) and flat (lower panel) electrodes. C) GFP expression from samples measured by densitometry.

However, there was a striking reduction in fluorescence intensity on second use of electrode on right testis (Figure 3A), which may be due to heat damage after first use on left testis. This difference between first and second use of electrodes was also evident after western blot analysis and densitometry. To provide quantitative data, densitometry was performed following western blotting and probing with antisera raised against GFP and Gapdh (loading control). Results are combined data for eight testes each for flat and needle electrodes and indicated that needle electrodes were significantly superior to flat electrodes as measured using GFP expression normalised to within-sample Gapdh expression (Figure 3C).

5. Discussion and Conclusion

This work has developed microneedle-based electrodes for the production of transgenic rodents using DNA microinjection and electroporation. Preliminary experiments *in vivo* in mouse models suggest that needle electrodes are significantly superior to flat electrodes for electroporation of testis cells. Histological analysis of some testes indicated that both somatic and germ cells express GFP following testis electroporation (data not shown). However, we did not observe GFP transgenics among one hundred offspring from mating testis-electroporated mice. One possible explanation is that we used the C57Bl/6 strain, which may not be optimal for this procedure, and so future work will involve testing the needle electrodes on mice from other genetic backgrounds [4]. Future work will also solve an issue of apparent heat damage to electrodes following a single use, by using different metallic coatings on electrodes.

Acknowledgements

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