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Note

Electroporation of the skin to deliver antigen by using a piezo ceramic gas igniter

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Abstract

The static electricity generated by triggering a piezo gas igniter is shown to cause electroporation of the skin to deliver antigen. Mice were immunized with chicken albumin by electroporation using a piezo gas igniter and in another experiment, the gas igniter was replaced by a power supply. In both the groups identical immune responses were generated. The change in impedance of the skin of a mice after applying high voltage electrical pulses from a power supply and that with a gas igniter were found to be similar. The piezo gas igniter is an inexpensive and easily available device. It is much more user 'friendly' than a power supply used for electroporation and it may be viewed as a replacement for a syringe with a needle. © 2001 Elsevier Science B.V. All rights reserved.

Keywords: Antigen delivery; Electroporation, Piezo gas igniter; Immune response; Skin impedance

1. Introduction

Application of short, high-voltage pulses provide a way to reversibly disrupt lipid bilayer membranes. Such pulses create pathways to deliver molecules across cell membranes by a mechanism commonly called electroporation. Some of the applications of electroporation are the transdermal drug delivery (Prausnitz, 1999), DNA transfection of cells (Widera et al., 2000) and electrochemotherapy (Heller et al., 1999). It is also known that transdermal drug delivery increases (Misra et al., 1999) by orders of magnitude when high voltage pulses are applied to the skin. Some electrical paraphernalia is always associated with electroporation making it less acceptable at the user level. A syringe with a needle is definitely more handy than a sophisticated power supply used for electroporation. In this paper it is shown that the power supply used for electroporation can be replaced by a domestic piezo gas igniter. Piezo gas igniter has a piezo ceramic disc inside. Application of compressive force on piezo

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ceramic disk produces voltage across its ends. In a typical domestic gas igniter a force of around 2000 N is applied on the piezo ceramic disk at each trigger and a voltage of around 18 KV is generated and a charge of 10^{-6} C is delivered within 10^{-7} s.

Here we describe how the voltage pulses generated by triggering the gas igniter can cause electroporation of the skin. Some of the mice were immunized with chicken albumin by electroporation using a power supply and some by a gas igniter. The change in impedance of the skin of a mice after applying high voltage electrical pulses from a power supply and that with a gas igniter were found to be similar. In both the groups identical immune responses were generated. This new device design has made electroporation as handy as a syringe with a needle.

2. Methods

Inbred, male balb/c ($n = 5$/group) were used for the study. Two days before immunization a portion of the fur on the backs of the mice was removed using a hair-removing cosmetic and on day zero immunization was carried out under ketamine anesthesia. Ten days after immunization, a booster of identical dose was given. A control group, under observation was not given any antigen.

Twenty micro-liter antigen solution (10 μg/ml chicken albumin in PBS) was placed in two hemispherical cups (2 mm inner diameter and 50 μl capacity) stuck to the skin by an adhesive. Platinum wire electrodes were inserted into the antigen solution, but did not touch the skin surface. Fig. 1 shows how the cups containing antigen

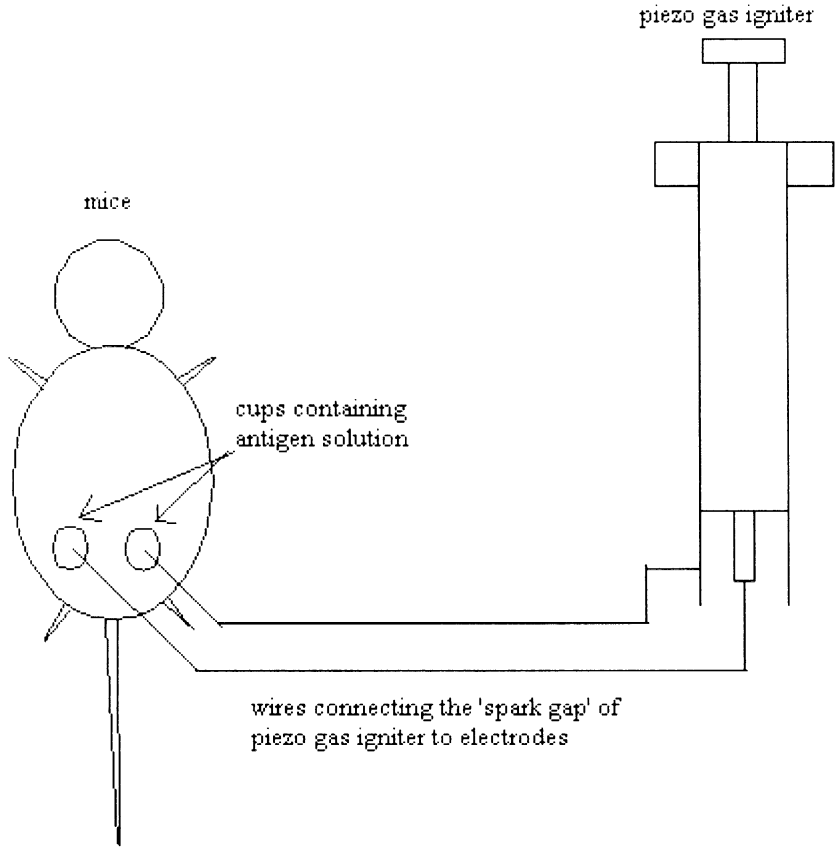

Fig. 1. A schematic diagram showing the connections of a piezo gas igniter to the electrodes placed in the antigen solution.

solution were fixed over the skin and electrodes were connected to a piezo gas igniter.

A domestic piezo gas igniter was modified to deliver charge pulses. An insulated wire was joined by silver loaded conducting epoxy to the central rod of the gas igniter and the other connection was taken from the outer jacket. Ten triggers, each separated by a second, were applied from the gas igniter for immunization. An in-house designed power supply was used to deliver 100 V square wave pulses of 0.1 ms duration. Ten such pulses each separated by a second were applied. The cups containing the antigen solution were left in place for one hour. The amount of antigen diffused into the skin was not estimated.

Impedance was measured before and five seconds after applying voltage pulses by an LCR meter at 1 KHz (Hewlett Packard 4284A). To begin with electrodes were connected to a LCR meter. Electrodes were then removed from LCR meter and electrical pulses were applied. After 5 seconds impedance was again read by connecting LCR meter to the electrodes.

Mice were bleed three times during the course of experiment by retro-orbital plexus using glass capillaries. First, on day zero (before the electroporation), then, ten days after electroporation. A booster by electroporation of identical does was given the same day and finally, 15 days after the booster (25th day) another sampling was performed.

Direct ELISA for antigen specific antibodies was employed to estimate the magnitude of serum immune response. Micro plates (Nunc) were coated with 1μg per well antigen and blocked with 1% BSA solution. They were exposed to a serum dilution of 1:300. The bound antibodies were revealed with goat anti — (mice IgG) (National Institute of Immunology, Reagent Bank) conjugated to horseradish peroxidase.

3. Results

Before applying electrical pulses the resistance of the mice's skin was 25 ± 1.0 KΩ. After applying ten triggers from a gas igniter and ten pulses from a power supply the resistance dropped to 28 ± 7.5 Ω and 17 ± 8.9 Ω respectively at 1 KHz. For all the reported resistance values $n = 5$ and $P = 0.05$. In both the groups the decrease in the resistance was three orders of magnitude. Skin impedance is attributed largely to stratum corneum lipids. Thus changes in impedance suggest changes in lipid structure. A three order of magnitude change in impedance after applying electrical pulses is an indication of changes in skin microstructure (Pliquett et al., 1995) leading to the formation of pores.

Fig. 2 shows the immune response generated after immunization with chicken albumin using a piezo ceramic gas igniter and by the conventional power pulses. Plotted absorbance values are averages of absorbances observed on five animals. The bars on each column indicate standard deviation. The important feature of Fig. 2 is that the two fold change in absorbance observed by using a power supply and a gas igniter are similar on both the samplings, i.e. 10 days after the immunization and fifteen days after the booster.

For a typical piezo gas igniter, in each trigger a force of approximately 2000 N is applied by the spring of the gas igniter to the piezo crystal and a voltage of 18KV is generated. The out put electrical charge can be calculated by knowing the piezo strain d33 constant of the piezo material used inside the gas igniter. The '*d*' constant relates mechanical stress (force per unit area of ceramic) and strain (fractional deformation of the ceramic to electrical voltage or charge. The number '33' written along with *d* (*d*33) specify the direction of stress and polarization. For the gas igniter used in this study the *d*33 constant was 400×10^{-12} C/N. Thus the charge delivered at each trigger can be calculated by the formula (Waanders, 1991):

$$\text{Charge } (C) = d33 \text{ constant} \times \text{Force}$$

In each trigger, a force of approximately 2000 N is applied on the piezo ceramic disk and therefore

$$\text{Charge} = 400 \times 10^{-12}\ C/N \times 2000\ \text{N}$$

$$= 8\text{X}10^{-7}\ C.$$

The amount of charge delivered in subsequent triggers will also be the same as same static electricity is generated everytime force is applied to

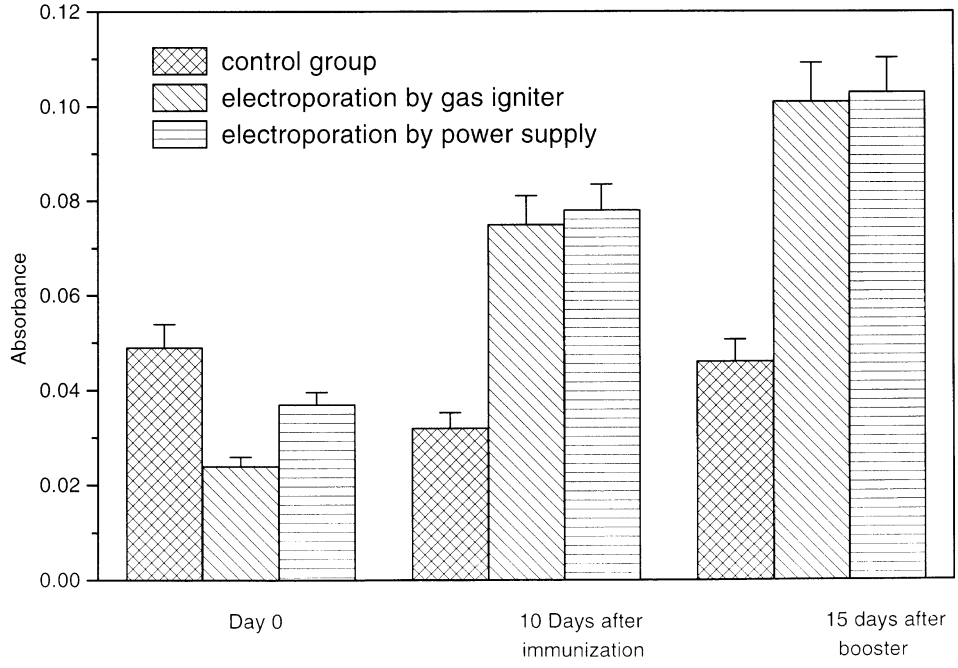

Fig. 2. A comparison of the immune response generated after immunizing mice using electroporation caused by a piezo gas igniter and a power supply. Plotted absorbance values are average of absorbances observed on five animals. The bar on each column indicate standard deviation.

the piezo crystal. This charge is less than the charge delivered with the power supply with ten 100 V pulses of 0.1 ms duration across the skin which has a typical DC resistance of 10 KΩ ($C = V \times t/R = 100 \times 10^{-4}/10^4 = 10^6$) before electroporation and 100 Ω after electroporation ($C = 10^4$) caused by the first pulse.

Comparing the voltage pulses of the gas igniter and the power supply, the pulse from a gas igniter is of high voltage and of very short duration (around 10^{-7} s). Using the gas igniter, less charge is delivered to the skin which results in less discomfort to the recipient. It has been reported by performing experiments in vitro that long, medium voltage pulses result in better mass transport than short, high voltage pulses (Vanbever et al., 1999), the results reported here do not contradict these findings. As marginally higher amount of antigen delivered by long, medium pulses does not necessarily invoke higher immune response. Long pulses also cause discomfort for longer duration of time, which is not desirable.

These results show that charge pulses generated by triggering a typical gas igniter can cause electroporation of the skin to deliver drug of interest. This device design can easily be adapted to perform other electroporation experiments. Further investigations are necessary to study the details of immune response generated after antigen delivery by electroporation using a gas igniter.

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