Bioelectrochemistry 103 (2015) 120–123

Contents lists available at ScienceDirect

Bioelectrochemistry

journal homepage: www.elsevier.com/locate/bioelechem

Application of increased temperature from an exogenous source to enhance gene electrotransfer

Amy Donate [a], Niculina Burcus [a], Karl Schoenbach [a], Richard Heller [a,b,*]

[a] Old Dominion University, Center for Bioelectrics, 4211 Monarch Way Suite 300, Norfolk, VA 23508, USA
[b] Old Dominion University, College of Health Sciences, School of Medical Laboratory and Radiation Sciences, 5115 Hampton Blvd., Norfolk, VA 23508, USA

ARTICLE INFO

Article history:
Received 10 December 2013
Received in revised form 8 August 2014
Accepted 12 August 2014
Available online 23 August 2014

Keywords:
Gene electrotransfer
Heat
Electroporation

ABSTRACT

The presence of increased temperature for gene electrotransfer has largely been considered negative. Many reports have published on the lack of heat from electrotransfer conditions to demonstrate that their effects are from the electrical pulses and not from a rise in temperature. Our hypothesis was to use low levels of maintained heat from an exogenous source to aid in gene electrotransfer. The goal was to increase gene expression and/or reduce electric field. In our study we evaluated high and low electric field conditions from 90 V to 45 V which had been preheated to 40 °C, 43 °C, or 45 °C. Control groups of non-heated as well as DNA only were included for comparison in all experiments. Luciferase gene expression, viability, and percent cell distribution were measured. Our results indicated a 2–4 fold increase in gene expression that is temperature and field dependent. In addition levels of gene expression can be increased without significant decreases in cell death and in the case of high electric fields no additional cell death. Finally, in all conditions percent cell distribution was increased from the application of heat. From these results, we conclude that various methods may be employed depending on the end user's desired goals. Electric field can be reduced 20–30% while maintaining or slightly increasing gene expression and increasing viability or overall gene expression and percent cell distribution can be increased with low viability.

1. Introduction

Direct injection of DNA is an easy method for delivering genes. However gene expression is relatively low and requires the use of chemical, electrical, or mechanical enhancers to increase expression levels. Electrical enhancers like gene electrotransfer (GET) have been used increasingly for several gene delivery applications including: cancer vaccines and therapeutics, infectious disease DNA vaccines, and gene replacement for metabolic disorders, and have been translated to the clinic with some success in the areas of infectious disease and cancer vaccines and therapeutics [1–6]. Despite the successes, GET still has its drawbacks. A primary one is the pain and potential cellular damage associated with administration of electrical stimulation.

In addition to the pain of electrical stimulation, insertion of needles into tissue can also add to the pain. However, using this approach, levels of gene expression and distribution can be increased and more tightly controlled. Methods have been employed to counteract some of these issues. Gene expression levels and distribution can be adjusted and regulated based on the type of electrode, pulse number, duration, and amount or concentration of DNA [6–8].

Pain can also be reduced by several of these methods. Non-invasive electrodes have been designed for skin delivery in an effort to reduce discomfort from needle insertion [7,9–11]. However, the drawback has been the need for higher applied voltages to penetrate the stratum corneum. An additional hurdle is the depth within the tissue of gene expression. Newer non-invasive electrode designs have incorporated multiple electrodes. The multi-electrode array allows for a lower applied voltage due to the small gap between electrodes [12,13]. This type of electrode design has been successful in generating immune responses against infectious agents but was ineffective for increasing expression systemically [14–16]. Additionally, while adding electrodes and reducing the gap allows for a reduction in applied voltage it also in turn reduces the penetration of the electric field. Other similar electrodes have been designed, like the MID, that minimally penetrate the tissue, these designs have been successful but again require penetration of the tissue [17,18].

To enhance the applicability of GET it is important to address these issues. It will be necessary to determine if non-invasive GET can be enhanced while still maintaining the safety of low voltages. One possibility is to increase membrane fluidity to allow for greater penetration and uptake of the DNA. One possible approach is to increase the temperature prior to electrotransfer. The idea of heat has been considered a negative factor when associated with electrotransfer. Much work has evaluated whether GET conditions used for gene delivery increase temperature and whether this is a contributing factor for the gene delivery.

* Corresponding author at: Director, Center for Bioelectrics, 4211 Monarch Way, Suite 300, Norfolk, VA 23508, USA. Tel.: +1 757 683 2690; fax: +1 757 451 1010.
E-mail address: rheller@odu.edu (R. Heller).

http://dx.doi.org/10.1016/j.bioelechem.2014.08.007
1567-5394/

It has been shown that in fact this is not the case and that the electric field is the cause for gene delivery [19]. However, it is also known that the phospholipid bilayer is designed for 37 °C and when the phospholipid bilayer is heated the phospholipids move apart and the membrane becomes more fluid-like [20]. This effect could be of benefit to non-invasive GET. The application of low to moderate heat may increase the membrane fluidity of the skin allowing the electrical fields to more easily drive gene delivery. Our goal was to evaluate this process under low to moderate heating conditions combined with specific GET conditions. The research was designed to determine if pretreatment with heat could increase gene expression. In addition, the effect on cell viability was evaluated as well as determining if lower voltages could be used when pretreating with heat.

2. Materials and methods

2.1. Cell lines and medium

The human Keratinocyte cell line, HaCaT, was used for all in vitro experimental studies. Cells were cultured in DMEM + pen-Strep and 10% FBS. Cells were harvested from flasks with 0.25% Trypsin-EDTA and re-suspended at 5 million cells/ml in complete media.

Plasmids: 5 μl of either Gwizluc or GwizGFP (Aldevron) at 2 mg/ml were used in this study to evaluate gene expression.

2.2. Experimental setup and groups

For these experiments there were 3 experimental setups.

1. Oil temperature 43–45 °C for 1.5 min for a final media temperature of 43 °C.
2. Oil temperature 45–48 °C for 2 min for a final media temperature of 45 °C.
3. Oil temperature 40–43 °C for 1.25 min for a final media temperature of 40 °C.

Untreated controls were included in each experiment. Initial temperature of cells was room temperature ranging between 22–24 °C. Table 1 describes the experiments performed with each temperature.

2.3. Application of heat

An oil immersion bath was used to heat the HaCaT cells to various temperatures. 150 μl of cell suspension was put into each 1 mm gap electroporation cuvette and placed in the oil immersion bath. Various oil temperatures and times were evaluated to determine the amount of time necessary to heat each sample to our desired temperatures. Oil temperatures ranged from 43–48 °C and time in oil ranged from 1–2 min. The time in heat for each sample was determined by the closest 15 s to the average of all samples. Time in heat was determined to be 1 min 15 s for 40 °C, 1 min 30 s for 43 °C and 2 min for 45 °C.

2.4. Electroporation

150 μl of cells was electroporated at various temperatures and times. Cells were left in the oil immersion bath while pulsing. Electroporation was performed using the BTX ECM 830 at applied voltages of 90, 75, 60, or 45 V using 1 mm electroporation cuvettes. Pulse length (t_p), pulse number (p) and frequency (*f*) remained constant at 5 ms, 1p, and 1 Hz respectively. Untreated cells and non-electroporated cells were used as controls. After treatment, cells were removed from cuvette and 140 μl added to each well of a 12 well cell culture plate.

2.5. In vitro bioluminescent imaging

Luciferase gene expression was measured 24 h after treatment. Complete media was removed from cells and 500 μl of complete media + 150 μg/ml Luciferin was added to each well. Cells were incubated in Luciferin-media mix for 5 mins at 37 °C before imaging. Caliper Life sciences IVIS Spectrum was used to measure luciferase expression. All luciferase data is presented as average total flux in photons/s (p/s).

2.6. Flow cytometry

GFP expression/distribution was measured by flow cytometry 24 h after treatment. Samples were harvested using trypsin and washed and resuspended in 200 μl of DPBS. Propidium Iodide was used to evaluate viability. 10,000 events were collected for each sample and percent GFP events calculated.

2.7. MTT viability

Viability was measured 24 h after treatment using MTT. 10 μl from each treatment was plated for MTT assay in a 96 well cell culture plate. 90 μl of complete media was added to each well. Untreated control and standard curves for cell number were used to measure viability. 25 μl of MTT was added to each well after 24 h and incubated at 37°. After 2 h media + MTT was removed and 100 μl of DMSO (sigma) was added to each well and shaken for 10 min at 100 rpm. Optical density was measured using a plate reader at 540 nm.

3. Results and discussion

3.1. Changes in luciferase gene expression from heat pretreatment GET

The first objective of this study was to determine if increased temperature affected overall gene expression. To test this, we delivered luciferase plasmid by GET at four different applied voltages with each being administered at three elevated temperatures. Luciferase expression was measured after 24 h. Increasing the temperature during the administration of GET can, result in changes in gene expression (Fig. 1). Fig. 1a shows the p/s of luciferase from each electrotransfer condition with and without the addition of heat for each of the three temperatures. Interestingly, different temperature and GET combinations had differing effects on gene expression. This becomes clearer when evaluating the fold changes of heat pretreated GET over GET alone for each condition at each temperature (Fig. 1b). 90 V and 75 V with heat represent the highest fold increases in gene expression; however this is seen at different temperatures (90 V with 40 °C and 75 V with 43 °C). Significant increases in gene expression are not seen between our four voltages at room temperature. However, evaluating each temperature individually shows that with only a slight increase in temperature of 40 °C significant increases in gene expression can be seen. At 40 °C and 90 V there is a significant increase in gene expression over 60 V and 45 V. As voltage is decreased to 75 with the same 40 °C gene expression is significantly

Table 1
Shows the experimental setup for each of the laser heated and electrotransfer conditions. All conditions were evaluated by luciferase gene delivery and expression. GFP expression was evaluated with all voltages but only at 43C and No heat.

	900 V/cm	750 V/cm	600 V/cm	450 V/cm	DNA only
Heat 40 °C	Luciferase	Luciferase	Luciferase	Luciferase	Luciferase
Heat 43 °C	Luciferase/GFP	Luciferase/GFP	Luciferase/GFP	Luciferase/GFP	Luciferase/GFP
Heat 45 °C	Luciferase	Luciferase	Luciferase	Luciferase	Luciferase
No heat	Luciferase/GFP	Luciferase/GFP	Luciferase/GFP	Luciferase/GFP	Luciferase/GFP