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Short communication

A single molecule detection method for understanding mechanisms of electric field-mediated interstitial transport of genes

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Abstract

The interstitial space is a rate limiting physiological barrier to non-viral gene delivery. External pulsed electric fields have been proposed to increase DNA transport in the interstitium, thereby improving non-viral gene delivery. In order to characterize and improve the interstitial transport, we developed a reproducible single molecule detection method to observe the electromobility of DNA in a range of pulsed, high field strength electric fields typically used during electric field-mediated gene delivery. Using agarose gel as an interstitium phantom, we investigated the dependence of DNA electromobility on field magnitude, pulse duration, pulse interval, and pore size in the interstitial space. We observed that the characteristic electromobility behavior, exhibited under most pulsing conditions, consisted of three distinct phases: stretching, reptation, and relaxation. Electromobility depended strongly on the field magnitude, pulse duration, and pulse interval of the applied pulse sequences, as well as the pore size of the fibrous matrix through which the DNA migrated. Our data also suggest the existence of a minimum pulse amplitude required to initiate electrophoretic transport. These results are useful for understanding the mechanisms of DNA electromobility and improving interstitial transport of genes during electric field-mediated gene delivery.

Keywords: Single molecule detection; DNA electromobility; Interstitial transport; Electric field-mediated gene delivery; Non-viral gene delivery

1. Introduction

The efficacy of non-viral gene therapy in the treatment of solid tumor is limited by poor gene delivery from the extracellular domain into the nucleus of cells since the delivery has to overcome various physiological barriers, including interstitial structures, cell membranes, cytoskeleton, and nuclear envelope, which are tissue- and cell-dependent [1–3]. DNA diffusion in tumor interstitium is negligible as indicated by the small diffusion coefficient ($<10^{-9}$ cm^2 s^{-1}) [2,4]. Convective transport, due to a uniformly elevated interstitial pressure, is also negligible everywhere in solid tumors except at the tumor periphery [1]. Furthermore, the plasma membrane and nuclear envelope are impermeable to naked DNA during passive diffusion. Therefore, non-viral gene transfer is inefficient without development of novel strategies [5–9].

Local application of pulsed electric fields is one the strategies that has been shown to improve delivery of exogenous genes into cells both in vitro [10–12] and in vivo [7–9,13–19]. The improvement occurs through two potential mechanisms: electroporation and electrophoresis [20,21]. These mechanisms, especially electroporation, have been studied extensively in vitro [22–28], in which electric pulses create transient pores in the plasma membrane that allow transport of traditionally non-permeant molecules into cells via both diffusion and electrophoresis [29,30]. However, DNA administered into tissues can be successfully delivered into cells only if these molecules are located within a critical distance from the plasma membrane during electroporation. Beyond this distance, the DNA molecules cannot reach the plasma membrane before the pores created by electroporation are closed. This critical distance depends on the rate of interstitial and transmembrane

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transport. Therefore, any improvement in the transport may significantly increase the extracellular domain within which DNA molecules are eligible for cellular uptake during electroporation.

Both interstitial and transmembrane transport can be improved through electrophoresis [2,31]. A recent report by Satkauskas et al. has demonstrated that following a prerequisite cell permeabilizing pulse, electrophoretic pulses play an important role in determining the efficiency of in vivo gene transfer [32]. The ability of an applied electric field to enhance interstitial transport of DNA has also been investigated in excised tumor tissue [2] and agarose gel acting as a tissue phantom [31]. These studies have uncovered surprising relationships between applied pulsing parameters and resulting gene transport behaviors, and led to questions concerning the underlying mechanisms of transport.

The objective of this study was to develop a reproducible single molecule method for understanding mechanisms of electric field-mediated extracellular transport of DNA. The rationale of the study was that DNA–DNA interactions were negligible during interstitial transport since DNA concentration in tissues was low in most gene delivery studies. As a result, the experimental results were determined mainly by transport behaviors of single DNA molecules. Using this method, we investigated the dependence of DNA transport on pulse magnitude, pulse duration, pulse interval, and pore size in agarose gels exposed to pulsed electric fields.

2. Materials and methods

A small-scale electrophoresis chamber was constructed on a glass microslide (VWR, West Chester, PA, USA) to allow continuous observation of single fluorescently labeled DNA molecules undergoing electrophoretic transport in agarose gel (Fig. 1). Platinum wire electrodes (A-M Systems, Inc., Carlsborg, WA, USA), inlaid along opposite walls of the electrophoresis chamber, were used to deliver the electric field. The electrodes were of sufficient diameter (0.127 mm) to span nearly the entire depth of the chamber.

All experiments were performed using bacteriophage T2 DNA (Sigma, St. Louis, MO, USA) stained with YOYO-1 intercalating dye (Molecular Probes, Eugene, OR, USA) at a 1:10 dye to base pair ratio. Solutions of 1.0, 2.0, 3.0, or 4.0% w/v low gelling temperature agarose (Sigma) were prepared in the slightly heated 1× Tris–Acetate–EDTA (TAE) buffer, allowed to cool for 2 min, then mixed with an equal volume of YOYO-1 labeled T2 DNA in 1× TAE containing 6% v/v β-mercaptoethanol. Approximately 50 μl of the mixture was pipetted into the electrophoresis chamber mentioned above. The chamber was then sealed with a glass microslide and the solution formed the gel in 15 min. The final sample medium contained 0.5, 1.0, 1.5, or 2.0% w/v agarose, 3% v/v β-mercaptoethanol, and approximately 3×10^{-6} μg/μl YOYO-1 labeled T2 DNA.

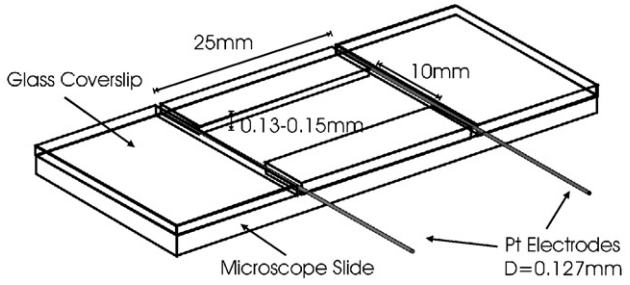

Fig. 1. Schematic of the small-scale electrophoresis device developed for the observation of single DNA molecules undergoing electrophoretic transport under pulsed electric fields.

Images of YOYO-1 labeled DNA during pulsed electric field application were acquired using a 100× oil immersion objective on an inverted microscope (Axiovert 100 TV, Zeiss, Thornwood, NY, USA), and captured with an intensified CCD camera (DAGE-MTI, Inc., Michigan City, IN, USA) connected to a videocassette recorder. The electric field was supplied by an ECM 830 electro square porator (BTX, San Diego, CA, USA). The resulting videos were digitized and analyzed using image analysis software (Image-Pro Plus®, Media Cybernetics, Inc., Silver Spring, MD, USA). Agarose gel deformation due to pulse delivery was negligible as determined by preliminary experiments using 1.0-μm-diameter yellow-green latex microspheres (Polysciences, Inc., Warrington, PA, USA) as a gel marker (data not shown).

DNA movement was quantified and reported in terms of the net DNA displacement per pulse, and the DNA electromobility. Total DNA displacement, d, was defined as the distance between the location of the midpoint of a DNA molecule before application of a 10-pulse train and the location of the midpoint of the same molecule 10 s following the completion of the 10-pulse train. The DNA displacement per pulse, d_p, was calculated by,

$$d_p = \frac{d}{N} \tag{1}$$

where N is the number of pulses in the applied pulse train. The DNA electromobility, μ, is given by,

$$\mu = \frac{v}{E} \tag{2}$$

where v is the magnitude of the velocity vector of the observed molecule, and E is the magnitude of the applied electric field. In this report, v was obtained by,

$$v = \frac{d_p}{t_p} \tag{3}$$

where t_p is the duration of the pulse.

The average pore size in agarose gel has been characterized previously by the radius, r_p, of the largest latex spheres that can enter the gel [33]. The value of r_p is empirically related to the concentration of agarose, ϕ, in the gel through Eq. (4),

$$r_p = 118\cdot\phi^{-0.74} \quad \text{(nm)} \tag{4}$$

in which the unit of r_p is nanometer (nm) and the unit of ϕ is percent weight by volume (% w/v) that is calculated as