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# Effects of pulse strength and pulse duration on in vitro DNA electromobility

David A. Zaharoff, Fan Yuan*

*Department of Biomedical Engineering, Duke University, 136 Hudson Hall, Durham, NC 27708, USA*

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**Abstract**

Interstitial transport of DNA is a rate-limiting step in electric field-mediated gene delivery in vivo. Interstitial transport of macromolecules, such as plasmid DNA, over a distance of several cell layers, is inefficient due to small diffusion coefficient and inadequate convection. Therefore, we explored electric field as a novel driving force for interstitial transport of plasmid DNA. In this study, agarose gels were used to mimic the interstitium in tissues as they had been well characterized and could be prepared reproducibly. We measured the electrophoretic movements of fluorescently labeled plasmid DNA in agarose gels with three different concentrations (1.0%, 2.0% and 3.0%) subjected to electric pulses at three different field strengths (100, 200 and 400 V/cm) and four different pulse durations (10, 50, 75, 99 ms). We observed that: (1) shorter pulses (10 ms) were not as efficient as longer pulses in facilitating plasmid transport through agarose gels; (2) plasmid electromobility reached a plateau at longer pulse durations; and (3) plasmid electromobility increased with applied electric energy, up to a threshold, in all three gels. These data suggested that both pulse strength and duration needed to be adequately high for efficient plasmid transport through extracellular matrix. We also found that electric field was better than concentration gradient of DNA as a driving force for interstitial transport of plasmid DNA.
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*Keywords:* Interstitial transport; Plasmid electromobility; Electric field-mediated gene delivery; Non-viral gene delivery

## 1. Introduction

Non-viral gene therapy has yet to reach its enormous potential as the treatment of choice for the majority of inherited and acquired diseases. Although non-viral gene therapy promises to revolutionize modern medicine, poor transfection efficiencies and inadequate transgene expression have characterized the growing pains of this emerging field. In particular, inadequate development of efficient gene delivery systems has severely limited transfection, and thus, the progress of non-viral gene therapy.

Non-viral gene delivery from the location of administration to the cell nucleus can be broken down into a series of individual transport processes in which physiological barriers to the passage of plasmid DNA must be overcome. However, the vast majority of research conducted in non-viral gene therapy has focused primarily on modifying input parameters of gene delivery technologies while observing quantifiable outputs such as transfection efficiency or therapeutic responses. In this type of research, physiological barriers are either misrepresented or consolidated into a poorly understood, poorly characterized black box. If these barriers remain uncharacterized, progress in enhancing gene delivery will be achieved only in small increments by slight modification of gene delivery input parameters via a trial-and-error approach.

Therefore, there is a definite need to characterize the transport of non-viral vectors through physiological barriers without necessarily measuring expression or therapy. Based on these studies, novel systems of gene delivery can be designed to facilitate DNA transport through various barriers, thereby helping gene therapy realize its full potential.

In this study, we focused on barriers to plasmid transport posed by the interstitium and how they might be overcome by the application of electric field. Interstitial transport of macromolecules, such as plasmid DNA, in solid tumors is limited by small diffusion coefficient, large diffusion distance and inadequate convection [1–3]. In addition to poor transport, naked DNA can be rapidly degraded by nucleases

* Corresponding author. Tel.: +1-919-660-5411; fax: +1-919-684-4488.
*E-mail address:* fyuan@duke.edu (F. Yuan).

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in the interstitial compartment. The half-lives of naked plasmid DNA in mice vary from 5 min to 24 h depending on the site of injection [4–9]. These values are likely to be overestimations of the time period in which the plasmid is still biologically active as most measurements probe for either a small portion of the entire plasmid or radioactivity from a DNA label.

Upon injection of a therapeutic plasmid, whether it be intravenous, intratumoral, or intramuscular, only a portion of the injected DNA will be readily accessible to a target on the cell membrane, i.e., within some critical distance of a cell membrane in which transport can occur before DNA degradation or clearance. This critical distance can be affected by tissue geometry, extracellular matrix composition, non-specific DNA binding to extracellular molecules, DNA size, DNA configuration, and/or external forces. Without considering DNA binding, tissue geometries, and external forces, a conservative estimation of this critical distance is on the order of 1–20 μm based on plasmid diffusion coefficient in 3% agarose gel (see below) and plasmid half-life mentioned above. By adding DNA binding and using a more realistic estimate of plasmid diffusion coefficient in tissues, the estimated critical distance could be reduced by one or more orders of magnitude. Therefore, any enabling technology that would expand this critical distance through enhancing transport and/or decreasing the interstitial residence time of plasmid, would improve the traditionally low transfection efficiencies of non-viral vectors.

To this end, we investigated the influences of pulsed electric fields on plasmid electromobility in agarose gels and compared the electromobility with plasmid diffusion in the same gels. This work follows a recent study from our laboratory, which determined the interstitial electromobility of plasmid DNA in two types of tumor tissues subjected to two different pulsing conditions [10]. One limitation in our previous study was that we could not control the composition and density of extracellular matrix. In addition, we could not separate the retardation effects of cells and extracellular matrix on the electromobility of plasmid DNA. Therefore, we developed an in vitro assay, based on agarose gels, and used it to study the effects of pulse strength and pulse duration on plasmid electrophoresis in the interstitial space subjected to pulsed electric fields.

Gel models have been used widely in studying mechanisms of interstitial transport in tissues [11–16]. The advantage of using gel models is three-fold. First, structures of gels are more reproducible and better characterized than the interstitial space. Second, there exist common mechanisms, such as diffusion, convection, reptation, trapping, and electrophoresis, which govern DNA transport in both gels and the interstitial space. Third, gel preparation is much easier than tissue preparation. Thus, gel models allow more thorough investigation of molecular transport in fiber matrices, as well as testing a variety of pulsing conditions to assess how plasmid DNA responds to different pulsing parameters. Results from our in vitro experiments demonstrated specific effects of electric pulses and pore size in agarose gels on the electrophoretic transport of plasmid DNA. This information would be useful for improving interstitial plasmid transport in vivo.

## 2. Experimental methods

### 2.1. DNA loading into agarose gel

Agarose gels (0.5%, 1.0%, 2.0% or 3.0%) were formed by dissolving the appropriate amount of agarose (0.125, 0.25, 0.50 or 0.75 g, respectively; Fisher, Fairlawn, NJ) in 25 ml of 1 × TAE buffer. The mixture was heated in a microwave oven and stirred until the agarose was completely dissolved. Solutions were weighed before and after heating to confirm that evaporation was negligible. 2.0 μl of 1.0 μm Fluoresbrite™ YG latex microspheres (Polysciences, Warrington, PA) were stirred into to the agarose. About 10 ml of the agarose/microsphere solution was placed in a 10 × 6.5 cm electrophoresis submarine gel tray (E-C Apparatus, Holbrook, NY) with an 8-well comb and allowed to solidify at room temperature. Upon solidification, the comb was removed and the tray was placed in a horizontal electrophoresis chamber (E-C Apparatus, Holbrook, NY) filled with 1 × TAE buffer. 1.5 μl of 0.5 μg/ml rhodamine-labeled, GFP-encoding plasmid DNA (Gene Therapy Systems, San Diego, CA) were loaded into a well. DNA was loaded into the gel with an electric field applied via an electrophoresis power supply (EC250-90; E-C Apparatus, Holbrook, NY). A small square of agarose containing the plasmid was cut out of the tray and placed on a microscope slide. An electrode lid was fixed to the slide with super glue such that the electrodes penetrated the agarose gel and flanked, in parallel, the band of DNA. The electrode lid was composed of a 35-mm petri dish lid with two halves of a stainless steel razor blade fixed, in parallel, 5.0 mm apart, and perpendicular to the lid surface. The razors, or electrodes, were connected to a T820 square wave electroporator (BTX, San Diego, CA). The assembly was fixed to the stage of an LSM 510 confocal microscope (Zeiss, Thornwood, NY) with tape.

### 2.2. DNA electromobility experiments

Fluorescence images of the rhodamine-labeled plasmid and the microspheres in the agarose gels were acquired at 10 × magnification before and after each of 10, 50, 100 or 200 V pulses of 10, 50, 75 or 99 ms at room temperature. One control experiment without electric field was conducted at each gel concentration. The total plasmid or gel movements per pulse were determined by cross-correlation analysis of the fluorescence images of the DNA or microspheres, respectively, between successive pulses. The details of the analysis are as follows. The successive images can be represented as $p(x,y)$ and $q(x,y)$, where $x$ and $y$ are the indices of pixels in

each image. If the image $p$ is fixed and image $q$ is shifted by $i$ and $j$ pixels in $x$ and $y$ directions, respectively, the normalized cross-correlation coefficient of two images is given by

$$r_{ij} = \frac{\sum_x \sum_y [p(x,y) - p_{\text{mean}}][q(x+i, y+j) - q_{\text{mean}}]}{\sigma_p \sigma_q} \quad (1)$$

where $p_{\text{mean}}$ and $q_{\text{mean}}$ are the mean intensities in the images of $p(x,y)$ and $q(x+i, y+j)$, respectively, and

$$\sigma_p = \sqrt{\sum_x \sum_y [p(x,y) - p_{\text{mean}}]^2} \quad (2)$$

$$\sigma_q = \sqrt{\sum_x \sum_y [q(x+i, y+j) - q_{\text{mean}}]^2}$$

The summation above includes only overlapping pixels between $p$ and $q$. We determined the vector of DNA movement by searching $i$ and $j$ at which the correlation coefficient, $r_{ij}$, reached a maximal value. The maximal $r_{ij}$ in our experiments ranged from 0.7 to 0.99. The net plasmid movements in gel were found by subtracting the gel movement from the total plasmid movement during each pulse and averaged over each 10-pulse experiment.

### 2.3. DNA diffusion in agarose gels

Rhodamine-labeled, GFP-encoding plasmid DNA (Gene Therapy Systems, CA) was loaded into 0.5%, 1.0%, 2.0% or 3.0% agarose gels, without latex microspheres, as described above. Six to ten fluorescence images of a DNA band embedded in agarose gels were acquired using a 10× objective for 0.5% and 1.0% gels or a 40× objective for 2.0% and 3.0% gels with an LSM 510 confocal microscope (LSM 510, Zeiss). The time interval between successive images was 10 min. Average intensity line profiles of each image of the DNA band were acquired with image analysis software (Image-Pro Plus, Media Cybernetics, Silver Spring, MD).

We assumed that DNA diffusion was one-dimensional and in the $x$ direction, because the maximum concentration gradient in the $y$ direction was less than 5% of that in the $x$ direction in all cases. To estimate the error, caused by this assumption, in the diffusion coefficient measurement, we performed an order of magnitude analysis on the basis of Fick's second law for diffusion. If $D_1$ and $D_2$ denote the diffusion coefficients obtained by fitting the same experimental data to the solutions of 1-D and 2-D diffusion equations, respectively, then they would be related by the following equation,

$$D_1\left(\frac{1}{L_x^2}\right) = D_2\left(\frac{1}{L_x^2} + \frac{1}{L_y^2}\right) \quad (3)$$

where $L_x$ and $L_y$ are the characteristic lengths of diffusion in the $x$ and $y$ directions, respectively. Because the concentration gradient in the $y$-direction was, at most, 5% of that in the $x$-direction, $L_x$ must be less than 5% of $L_y$. Substituting these values into Eq. (3), we found that the relative difference between $D_1$ and $D_2$ was less than 0.25%, which was much smaller than the error or standard deviation in the diffusion coefficient measurements.

The equation for 1-D diffusion in an infinite medium is as follows:

$$C(x,t) = \frac{1}{\sqrt{4\pi Dt}} \int_{-\infty}^{\infty} F(x') \exp\left[-\frac{(x-x')^2}{4Dt}\right] \mathrm{d}x' \quad (4)$$

where $F(x)$ is the initial concentration profile, $D$ is the diffusion coefficient, $t$ is the time, and $C(x,t)$ is the final concentration profile. The procedure for determining the diffusion coefficient of DNA in agarose gel is as follows. We first substituted an assumed diffusion coefficient and the data of the initial concentration profile, $F(x')$, into the numerical approximation of Eq. (4),

$$C(x,t) = \frac{1}{\sqrt{4\pi Dt}} \sum_{n=0}^{N} F(x_n') \exp\left[-\frac{(x-x_n')^2}{4Dt}\right] \Delta x' \quad (5)$$

where $\Delta x'$ was the pixel dimension, approximately 1.8 and 0.45 μm in images acquired with 10× and 40× objectives, respectively. The calculated concentration profile, $C(x,t)$, was compared with experimental data; and the relative error was calculated. We then changed the value of the diffusion coefficient until the error was minimized. Eq. (5) assumed implicitly that the gel was a uniform porous medium, neglecting the details in the molecular structure of gels. This assumption required that $\Delta x'$ was much smaller than the characteristic length of the concentration profile and much larger than the molecular length scale. Both values of $\Delta x'$ used in this study satisfied the requirements. Any further reduction in $\Delta x'$ by decreasing the pixel dimension would cause more fluctuation in $C(x,t)$ as a function of $x$, but would not significantly improve the estimation of $D$.

## 3. Results

### 3.1. Electrophoretic movement versus applied electric field and pulse duration

The net distances of plasmid DNA movement per electric pulse are shown in Fig. 1. The voltage applied for each pulse has been converted to applied electric field by dividing the former by the interelectrode distance (5.0 mm). As expected, plasmid electrophoretic movement increased with pulse duration and pulse strength at all three

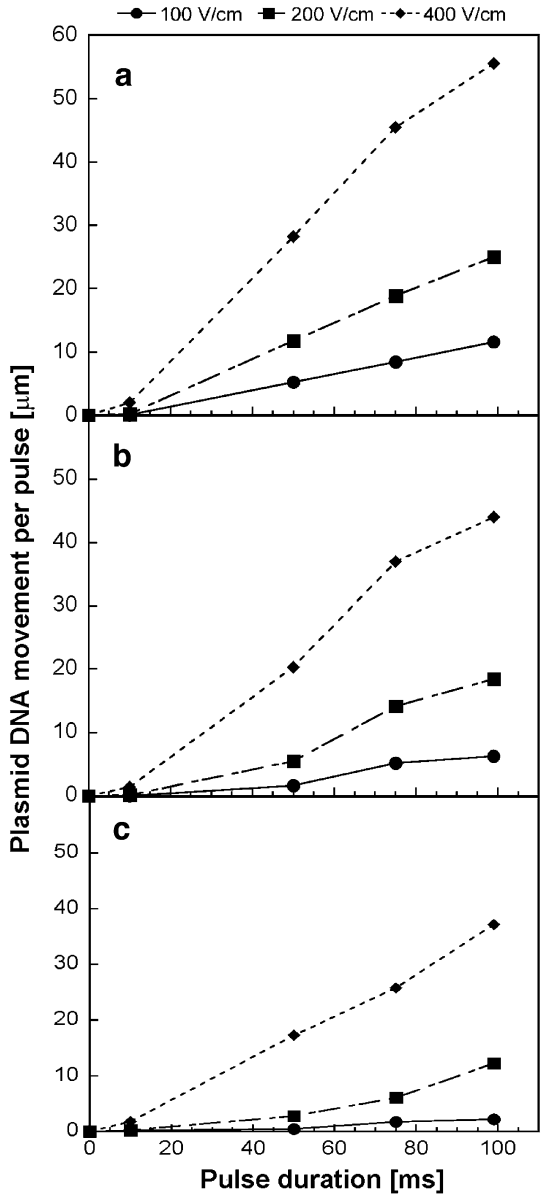


Fig. 1. Plasmid DNA movement versus pulse duration in (a) 1.0%, (b) 2.0% and (c) 3.0% agarose gels. Applied electric fields were 100, 200 and 400 V/cm, respectively. Electrophoretic movement of plasmid increased with both pulse strength and pulse duration. Each symbol represents the mean of 50 measurements taken from five independent experiments. Standard errors of means are comparable to the size of the symbols. The 0 ms data point was obtained from averaging 10 measurements in a control experiment.

gel concentrations. Longer, stronger pulses lead to greater plasmid movement.

Plasmid movement was inversely related to gel concentration. At higher gel concentrations, plasmid transport was more hindered by agarose fibers. There was no measurable plasmid movement during either image acquisition or the time interval between pulses. Plasmid movement did not change significantly over the course of any ten-pulse experiment ($p>0.05$; Spearman rank correlation).

### *3.2. Electromobility versus applied electric field, pulse duration and gel concentration*

To elucidate better the specific effects of pulse strength and duration on plasmid electrophoresis, plasmid movements shown in Fig. 1 were converted to electromobilities. Electromobility is defined as plasmid velocity divided by electric field. Plasmid velocity was calculated by dividing net distance of plasmid movement by pulse duration.

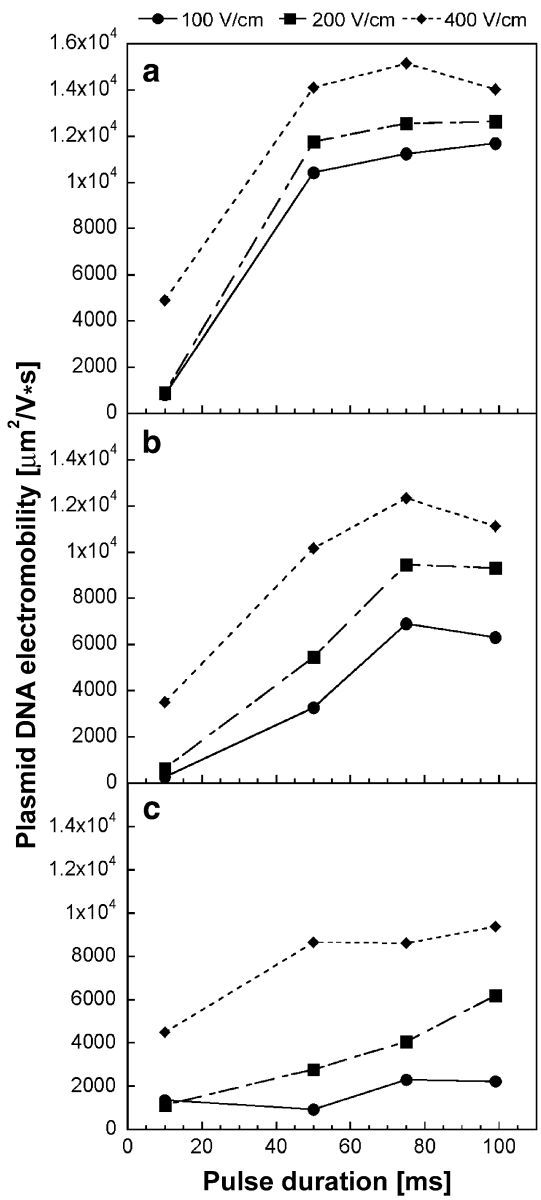


Fig. 2. Electromobility of plasmid DNA as a function of pulse duration in (a) 1.0%, (b) 2.0% and (c) 3.0% agarose gels. Plasmid electromobility in all gels was lower for shorter duration (10 ms) pulses as compared with longer duration (75–99 ms) pulses. Plasmid electromobility reached a plateau somewhere between pulse durations of 10 and 50 ms in 1% gel. The occurrence of the plateau was shifted to a higher pulse duration when the gel concentration was increased. Each symbol represents 50 measurements taken from five independent experiments. Standard errors of means are comparable to the size of the symbols.

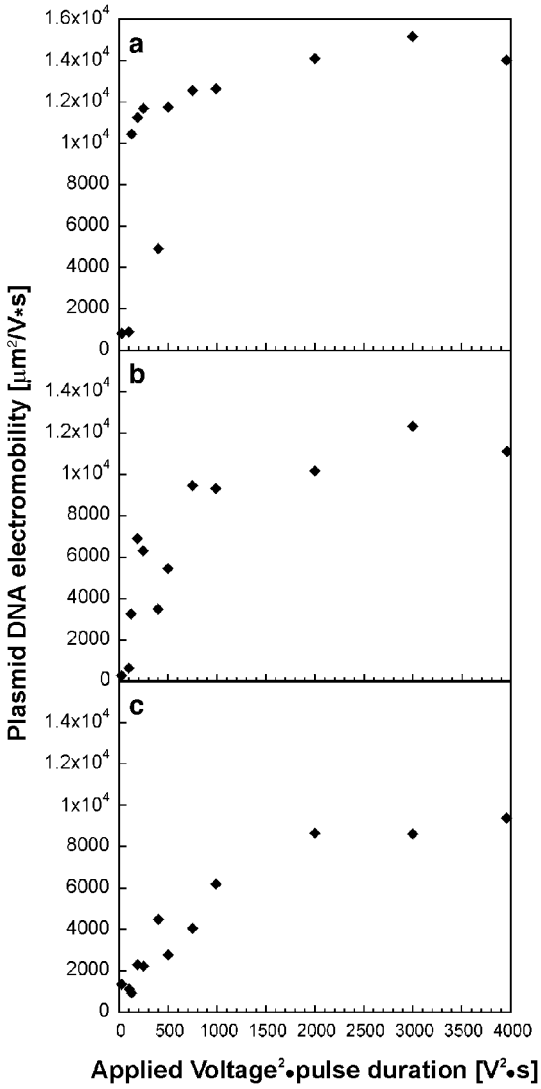


Fig. 3. Electromobility of plasmid DNA as a function of applied voltage squared times pulse duration, which is proportional to the applied energy, in (a) 1.0%, (b) 2.0% and (c) 3.0% agarose gels. Electromobility reached a plateau in all gels. The threshold energy at the plateau onset was shifted in favor of higher energies as gel concentration increased. Each symbol represents 50 measurements taken from five independent experiments. Standard errors of means are comparable to the size of the symbols.

Plasmid electromobility increased with pulse duration and then reached a plateau when pulse duration was between 10 and 50 ms in 1% gel (Fig. 2a). The occurrence of the plateau was shifted in favor of higher pulse duration when the gel concentration was increased (Fig. 2b and c). At all gel concentrations, shorter duration pulses (10 ms) were not as efficient as longer pulses in facilitating plasmid transport (Fig. 2). In the literature, electric pulses with strengths in the 1 kV/cm range and durations of approximately 100 μs have been used for electroporation of cells in vivo [17–21]. We observed that plasmid movement per pulse in the same agarose gels was only 0.05–0.10 μm when the pulse strength and duration were 2.0 kV/cm and 99 μs, respectively (data not shown), which was two to three orders of magnitude less than the data shown in Fig. 1. Thus, electric pulses with 1 kV/cm strength and 100 μs duration are sufficient for electroporation of cells but cannot efficiently facilitate plasmid DNA transport in the interstitium of tissues.

### 3.3. Electrophoretic mobility as a function of applied energy

The electromobility data are re-plotted in Fig. 3 as a function of applied voltage squared times pulse duration, which is proportional to applied energy. Plasmid electromobility increased with applied energy up to a threshold in all gel concentrations. For energies above the threshold, plasmid electromobility was constant or increased minimally with energy. The threshold energy increased with gel concentration.

### 3.4. Electrophoresis versus diffusion

The diffusion coefficient of a 5.1 kb supercoiled plasmid DNA in agarose gels is shown in Fig. 4. As expected, plasmid diffusion coefficient decreased exponentially with gel concentration. To compare plasmid transport via electrophoresis versus diffusion, we defined a dimensionless parameter, $P_{EE} = vL/D$, where $v$ is the electrophoretic velocity, $L$ is the transport distance, and $D$ is the diffusion coefficient. $P_{EE}$ is similar to the Peclet number, which compares the relative contribution of convection (electrophoresis in our case) versus diffusion to molecular transport. Using the data shown in Figs. 1 and 4, we found that transport via electrophoresis was up to five orders of magnitude faster than diffusion in agarose gels when the transport distance, $L$, was between 1 and 10

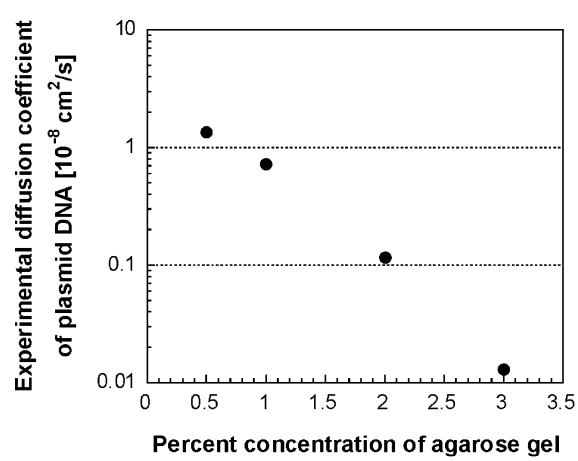


Fig. 4. Diffusion coefficient of a fluorescently labeled, 5.1 kb supercoiled plasmid as a function of agarose gel concentration. The diffusion coefficient decreased with gel concentration. Each symbol represents the mean of 20 measurements taken from three independent experiments. Standard errors of means are smaller than the size of the symbols.

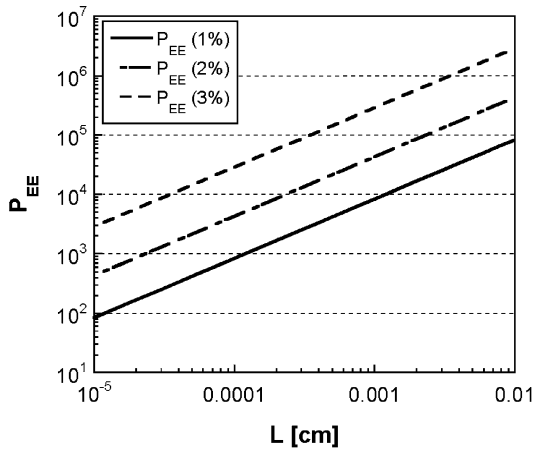


Fig. 5. The ratio, $P_{EE}$, of electrophoretic versus diffusive flux as a function of transport distance $L$. Electrophoresis was three to five orders of magnitude faster than diffusion over the distance of 1–10 μm. The ratio increased with $L$ and the concentration of gel.

μm (Fig. 5). This advantage of electrophoresis over diffusion increases with both transport distance and gel concentration.

## 4. Discussion

We characterized the plasmid electromobility in agarose gels at various pulsing conditions to simulate electrophoretic movement of DNA in the extracellular matrix of tissues. We observed that plasmid movement in agarose gels was greatest for pulses with high amplitude and long duration. This was not surprising as longer, more intense pulses create a larger and stronger driving force for the transport of plasmid through porous media. When the distance of plasmid movement was converted to electromobility and thus normalized by pulse duration and amplitude, we found that electromobility first increased with pulse duration and then reached a plateau (Fig. 2).

In comparing these results to our previous study [10], we found the distance of plasmid movement in agarose gels to be one to two orders of magnitude larger than that in tumor tissues in the same electric fields. We expect that similar results will occur in other tissues, because biological tissues are densely packed with cells and extracellular matrix whereas agarose gel mimics only the latter. Quantitatively, one may predict the electromobility of plasmid DNA in vivo on the basis of its values in agarose gels if the information on local arrangement of cells and the structure of extracellular matrix is known.

Mechanisms of the dependence of plasmid DNA electromobility on pulse duration are still unclear. Here, five possibilities are explored individually: (1) overcoming inertial forces during plasmid acceleration; (2) DNA orientation; (3) DNA–DNA interactions; (4) DNA–ion interactions; and (5) entropic trapping.

### 4.1. Overcoming inertial forces

Upon application of a constant force, a finite time period is required to accelerate all molecules from rest to a steady state velocity, due to inertial and drag effects. The same is true for plasmid movement in a uniform electric field. Thus, we estimated the time constant for a sphere, of similar size and charge as the plasmid used in our experiments, to reach a steady state velocity in free solution subjected to an applied electric field. The governing equation describing the motion of the sphere is:

$$qE - fv = ma \tag{6}$$

where $q$ is the total charge on the sphere (0.066 $e^-$ per base pair [22] times 5000 bp or $5.3 \times 10^{-17}$ C/plasmid); $E$ is the strength of the electric field (40,000 N/C for a 400 V/cm electric field); $f = 6\pi\mu R_g$ is the Stokes' frictional drag coefficient on a sphere; $R_g = 100$ nm is the radius of gyration for a 5.4 kb supercoiled plasmid [23]; $\mu = 0.01$ g/cm s is the viscosity of water; $m$ is the mass of sphere (approximately $5.1 \times 10^{-18}$ g/plasmid); and $a$ is the acceleration of the sphere. Eq. (6) can be solved analytically for $v$ given the initial condition $v = 0$ at $t = 0$.

$$v(t) = \frac{qE}{f} - \frac{qE}{f} e^{-\frac{ft}{m}} \tag{7}$$

The velocity of the sphere increased exponentially before reaching its steady state velocity of $qE/f$ when $t$ was approximately $3 \times 10^{-11}$ s, based on values of $f$ and $m$ given above. This time constant was much smaller than the time to reach the plateau shown in Fig. 2.

The assumption of the spherical shape of the plasmid is based on the fact that conformations of long DNA molecules are globular in solutions [24]. Furthermore, changing the conformation of the DNA would have a minimal effect on the time constant. A completely elongated 5 kb DNA chain moving parallel or perpendicular to the electric field reached the steady state velocities in $2–3 \times 10^{-11}$ s. Thus, the inertial and drag effects could be neglected as a possible mechanism to explain the pulse duration-dependence of electromobility.

### 4.2. DNA orientation

Another possible mechanism for the pulse duration-dependence of electromobility was the orientation of DNA in electric fields. It is well known that charged macromolecules such as DNA become aligned in electric field [25–30]. The orientation of DNA as a rod perpendicular and parallel to an electric field leads to minimum and maximum DNA velocities, respectively, due to the differences in frictional forces. Therefore, the maximum possible change in velocity attributable to DNA orientation occurs between these two extremes. From Eq. (7), we see that the velocity of

a particle is inversely proportional to its frictional force. The normal, $f_N$, and tangential, $f_T$, coefficients of friction for a needle-like rod (length≫radius) have been solved for low Reynolds number flow [31],

$$f_N = -\frac{4\pi\mu L}{ln(L/r) + 0.5} \tag{8}$$

$$f_T = -\frac{2\pi\mu L}{ln(L/r) - 0.5} \tag{9}$$

where $\mu$ is the viscosity of the solution, $L$ is the length of the rod and $r$ is the radius of the rod. The ratio of $f_T$ to $f_N$ is equivalent to the velocity ratio for a rod moving parallel and perpendicular to an electric field. If $L$ is half of the length of a 5.1-kb circular plasmid, or 867 nm based on 0.34 nm per base pair, and the radius of double stranded DNA, $r$, is 1.2 nm [32], then a DNA rod moving parallel to the electric field is 1.7 times faster than a DNA rod moving perpendicular to the electric field. This maximum difference in velocity due to DNA orientation cannot explain the 3- to 10-fold increase in velocity observed in Fig. 2. While DNA orientation likely contributes to the increase in electromobility, the effect is not sufficient to explain the pulse duration-dependence of electromobility as well.

### 4.3. DNA–DNA interactions

There are three regimes describing the transport of polymers in solution: dilute, semi-dilute and entangled (concentrated). In a dilute regime, the concentration of polymer is low enough that the polymer chains do not interact and can be considered as isolated particles. In an entangled or high concentration polymer solution, all polymer chains influence the transport of one another. Semi-dilute solutions exhibit properties of both dilute and entangled solutions. The maximum concentration of polymer in dilute solutions is

$$c^* \approx \frac{\mathrm{MW}}{N_A\left(\frac{4}{3}\pi R_g^3\right)} \tag{10}$$

where MW is the molecular weight of the polymer, $N_A$ is Avogadro's number and $R_g$ is the radius of gyration of the polymer [33]. For the DNA used in our experiments, $c^*$ is approximately 1.2 mg/ml. The concentration of DNA in our experiments was approximately 0.05 mg/ml. At this concentration, DNA solution is dilute and DNA–DNA interactions can be neglected.

### 4.4. DNA–ion interactions

Next, the interactions between DNA and ions are considered. A charged surface, e.g. DNA, in a static ionic solution will develop a layer of ions of opposite charge, i.e. counterions, near its surface. The counterions balance the charge of the surface and they together form the electric double layer. Upon application of an electric field, DNA will undergo electrophoresis, however, its movement is affected by the counterion atmosphere. The effect of the double layer on the electrophoresis of DNA is threefold.

The first is the retardation effect, which reduces the electromobility of a spherical particle in an ionic solution by, at most, 33% depending on the double layer thickness and particle dimension [34]. The second is relaxation, which depends on the ratio of the particle's radius to the double layer thickness and the particle's zeta potential ($\zeta$). The $\zeta$ of plasmid DNA varies from −30 to −80 mV [35]. When $\zeta$ is −80 mV, relaxation and retardation together lead to a 50% reduction in electromobility [34]. The third is the surface conductance effect [34]. The change in surface conductance is only significant when the double layer thickness is much smaller than the radius of the particle [36]. In our case, the double layer thickness is about half of the radius of double stranded DNA. As a result, surface conductance can be neglected.

Taken together, DNA–ion interactions may affect DNA mobility through three mechanisms: retardation, relaxation and surface conductance. However, none of them can adequately account for the dependence of DNA electromobility on pulse duration shown in Fig. 2.

### 4.5. Entropic trapping

The interfiber gaps, commonly referred to as pores, in agarose gels have been measured by size exclusion of latex spheres [37]. The average pore size depends on the concentration of agarose gels. The dependence is described by,

$$P = 118 \cdot A^{-0.74} \tag{11}$$

where $P$ is the pore radius in nm and $A$ is the percent concentration of agarose in a range between 0.2 and 4.0 [37]. Based on Eq. (11), the average radii of pores are 118, 71 and 52 nm in agarose gels with concentrations of 1.0%, 2.0% and 3.0%, respectively. Incidentally, collagen, whose levels have been correlated with transport impairment of macromolecules in tissues [10,38], has interfibrillar distances of 20–40 nm for well-aligned bundles and 75–140 nm for poorly aligned bundles in tumor tissues [38]. The radius of gyration of a 5.4-kb supercoiled plasmid is approximately 100 nm [23], which is either comparable to or larger than the interfibrillar distances in both agarose gels and tumor tissues.

Although agarose gels have a defined mean pore size and are generally considered to be macroscopically homogeneous, the disordered distribution of fibers creates a heterogeneous distribution of pores at the microscopic level. Thus, an agarose gel can be considered as a distribution of larger voids connected by narrower passages or pores. When the radius of a molecule is comparable to the mean pore size,

transport is significantly hindered by frictional and steric interactions between that molecule and the pores [38,39]. Therefore, in order for a DNA random coil to move through the narrow passages of agarose gels, it must be elongated in the direction of motion and shrunken in the perpendicular direction and therefore lose entropy [40]. The narrow passages function as entropic barriers to the transport of DNA.

The application of electric field has been shown to reduce the height of the entropic barrier [41]. Rousseau et al. suggest that the entropy barrier or entropic trapping does not occur if the electric field is beyond a critical level [41]. However, the electric field applied in Rousseau's study was continuous. If the duration of the electric field is finite, then pulse duration must be considered since entropy has units of energy per unit temperature and the applied electric energy is proportional to the product of voltage squared and pulse duration. When plotted as a function of applied energy as shown in Fig. 3, plasmid electromobility increased first and then reached a plateau at all gel concentrations. The threshold energy for the electromobility to reach a plateau increased with increasing gel concentration. Since other mechanisms unlikely explain the pulse duration-dependence of electromobility, and energy is proportional to pulse duration, the data shown in Fig. 2 and Fig. 3 are likely to be explained by the entropic trapping mechanism.

In biological tissues, which are much denser and more transport impairing, one should expect the threshold energy to be outside the physiologically relevant range covered in this study. Therefore, increasing the energy of a pulse will always increase plasmid transport through the interstitium.

### 4.6. Electrophoresis vs. diffusion

To date, there has been no measurement of large nucleic acid diffusion in tissues and very few measurements in agarose gels. Pluen et al. measured the diffusion coefficient of a 4.4-kb linear DNA fragment in 2.0% agarose gel via the fluorescence recovery after photobleaching (FRAP) technique. From their figure, the diffusion coefficient is approximately $5.5 \times 10^{-9}$ $cm^2/s$ [14]. This is about five times greater than the diffusion coefficient measured in this study for a 5.1-kb rhodamine-labeled, supercoiled plasmid in the same gel (Fig. 4). The discrepancy could be caused by the fact that the 4.4-kb fragment was linear and therefore could presumably reptate, or migrate head first, through the gel, whereas the 5.1-kb plasmid was supercoiled, and therefore, less flexible. The mobility of supercoiled plasmid is in general lower than linearized plasmid in gels with pore sizes similar to plasmid radius of gyration [23].

As shown in Fig. 5, electrophoresis is faster than diffusion as a mode of plasmid transport by several orders of magnitude. The advantage increases with transport distance and gel concentration or medium density. In tissues, which are much denser than agarose gels, we expect to observe a greater advantage of electrophoresis. Therefore, employing electric field is a feasible and powerful strategy for enhancing plasmid transport, decreasing plasmid residence time and thus plasmid degradation in the interstitial space [42,43].

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