Acanthamoeba Migration in an Electric Field

Jolene Chang Rudell,^{1,2} Jing Gao,^{1,3} Yuxin Sun,¹ Yaohui Sun,¹ James Chodosh,⁴ Ivan Schwab,² and Min Zhao^{1,2}

¹Department of Dermatology, Institute for Regenerative Cures, School of Medicine, University of California at Davis, Davis, California

²Department of Ophthalmology, Institute for Regenerative Cures, School of Medicine, University of California at Davis, Davis, California

³School of Life Science, Yunnan Normal University, Kunming, Yunnan, China

⁴Department of Ophthalmology, Massachusetts Eye and Ear Infirmary, Harvard Medical School, Boston, Massachusetts

Correspondence: Min Zhao, Institute for Regenerative Cures, School of Medicine, University of California at Davis, Davis, CA 95817; minzhao@ucdavis.edu.

JCR and JG are joint first authors.

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PURPOSE. We investigated the in vitro response of *Acanthamoeba* trophozoites to electric fields (EFs).

METHODS. *Acanthamoeba castellanii* were exposed to varying strengths of an EF. During EF exposure, cell migration was monitored using an inverted microscope equipped with a CCD camera and the SimplePCI 5.3 imaging system to capture time-lapse images. The migration of *A. castellanii* trophozoites was analyzed and quantified with ImageJ software. For analysis of cell migration in a three-dimensional culture system, *Acanthamoeba* trophozoites were cultured in agar, exposed to an EF, digitally video recorded, and analyzed at various Z focal planes.

RESULTS. *Acanthamoeba* trophozoites move at random in the absence of an EF, but move directionally in response to an EF. Directedness in the absence of an EF is 0.08 ± 0.01 , while in 1200 mV/mm EF, directedness is significantly higher at -0.65 ± 0.01 ($P < 0.001$). We find that the trophozoite migration response is voltage-dependent, with higher directionality with higher voltage application. *Acanthamoeba* move directionally in a three-dimensional (3D) agar system as well when exposed to an EF.

CONCLUSIONS. *Acanthamoeba* trophozoites move directionally in response to an EF in a two-dimensional and 3D culture system. *Acanthamoeba* trophozoite migration is also voltage-dependent, with increased directionality with increasing voltage. This may provide new treatment modalities for *Acanthamoeba* keratitis.

Keywords: *Acanthamoeba*, directional cell migration, electric fields

Acanthamoeba keratitis is a serious and debilitating, sight-threatening infection of the cornea.^{1,2} Risk factors of *Acanthamoeba* keratitis include contact lens wear, swimming especially while wearing contact lenses, poor hygiene while handling contact lenses, corneal trauma, and chronic ocular surface diseases.^{3–5} However, *Acanthamoeba* keratitis also can occur without any of these risk factors.⁶ *Acanthamoeba* organisms have been found widely in our environment, including contaminated contact lens solutions, bottled water, public water supplies, freshwater lakes, and air.^{7–10} Originally thought to be a rare infection, the prevalence of *Acanthamoeba* infections has risen rapidly in the past few decades.³

Acanthamoeba keratitis is very difficult to treat due to the deep penetration of the cells into the corneal stroma, the drug-resistant cyst life stage, and the lack of commercially available amebicidal agents, in addition to the recurrence of disease being a common complication in many patients. The life cycle of the *Acanthamoeba* consists of two stages, a trophozoite stage and a cyst stage. The trophozoite life stage is the active infectious form, which penetrate the host's cornea.¹¹ The cyst stage is the dormant form, which the organism favors under harsh conditions. Trophozoites and cysts are resistant to treatment in the stroma.^{12,13} Infection can cause radial neuritis in the cornea and can trigger an infiltration of epithelial

dendritic cells in the central cornea, with stromal infiltration and anterior uveitis later in disease.^{14,15} Severe disease can lead to blindness and may even lead to enucleation.¹⁶

To lure the trophozoites out of the corneal stroma and to induce the excystment process would facilitate drug treatment. However, to our knowledge, there is no reported method to guide migration of the trophozoites. The objective of our study was to develop a method to induce the direct migration of the trophozoites in vitro. This first step is necessary before application to animal models of *Acanthamoeba* keratitis and trials in the clinic. In our study, we evaluated the application of electrical fields (EFs) to guide migration of *Acanthamoeba* trophozoites and cysts.

Electrotaxis is the ability of a cell to migrate directionally in response to an EF gradient and the application of this physical procedure has been demonstrated in many different organisms, including a social amoeba *Dictyostelium*.^{17–21} However, to our knowledge, electrotaxis for *Acanthamoeba* spp. has not been reported previously. The ability to induce the excystment process followed by the locomotion of *Acanthamoeba* trophozoites from the stroma to the superficial layers of the cornea could facilitate drug treatment. However, there is no reported method to guide migration of the trophozoites. The objective of the study was to develop a method to induce the

direct migration of the trophozoites in vitro. Thus, we evaluate the application of EFs to guide migration of *Acanthamoeba* trophozoites and cysts. This first step is necessary before application to animal models of *Acanthamoeba* keratitis and trials in the clinic.

MATERIALS AND METHODS

Preparation of *A. castellanii* Trophozoites

The organism used in all experiments is a human ocular isolate of *A. castellanii* from the American Type Culture Collection (ATCC 50514). We used an axenic strain that has been used by many previous studies.^{22–24} *A. castellanii* trophozoites were cultured at 25°C in a peptone-yeast extract-glucose (PYG) medium (ATCC medium 712: proteose peptone 20 g and yeast extract 1 g in 868 mL distilled water, plus 2 mol/L D-(+) glucose 50 mL, 0.4 mol/L MgSO₄·7H₂O 10 mL, 0.05 mol/L CaCl₂ 8 mL, 0.1 mol sodium citrate-2H₂O 34 mL, 0.005 mol/L Fe(NH₄)₂(SO₄)₂·6H₂O 10 mL, 0.25 mol/L NaHPO₄·7H₂O 10 mL, 0.25 mol/L KH₂PO₄ 10 mL) in a tissue culture flask. The trophozoites were resuspended in fresh medium before experiments.

EF Stimulation and Time Lapse Image Recording

EFs were applied as described previously.²⁵ *A. castellanii* trophozoites in two- and three-dimensional (3D) cultures were seeded in electrotaxis chambers, and maintained in PYG medium during EF exposure. The trophozoites were exposed to an EF with field strength ranging from 600 to 1500 mV/mm for 30 minutes at room temperature (25°C). EF strengths were measured at the beginning and the end of the experiment to ensure consistent EF application. An inverted microscope (Carl Zeiss, Oberkochen, Germany) equipped with a CCD camera and the SimplePCI 5.3 imaging system (Hamamatsu Photonics, Hamamatsu City, Japan) were used to capture the time lapse images.

Quantitative Analysis of *Acanthamoeba* Cell Migration

The migration of *A. castellanii* trophozoites was analyzed and quantified as previously reported.²⁶ ImageJ software from the National Institutes of Health (available in the public domain at <http://rsbweb.nih.gov/ij/>) was used to quantify migration directedness and speed by tracing the cell at 30-second frame intervals. Directedness ($\cos \theta$) was used to quantify the direction of cell migration, where θ is the angle between the field vector and the cell migration direction. Directedness is -1 if a cell migrates directly to the left (negative cathode), 0 if a cell migrates perpendicular to the field direction, and $+1$ if a cell migrates directly to the right (positive anode). The equation $\sum i \cos \theta / N$ was used to calculate the average directedness of a cell population, where $\sum i$ is the summation of cosine values obtained from individual cells from the 2 to 3 experiments at a given EF strength, θ is the angle between the field axis and the net cellular translocation direction, and N is the total number of cells contained in all of the experiments at that given field strength.

Cell migration speed was quantified as trajectory speed and displacement speed; trajectory speed is the total migration distance divided by the time taken (in $\mu\text{m}/\text{min}$), while displacement speed represents the straight-line distance from the starting point to endpoint, divided by the time taken (also in $\mu\text{m}/\text{min}$). For each experiment, 30 to 200 cells were counted and quantified, and each experiment was repeated

two or three times as indicated. *P* values that are listed in the results and figure legends refer to comparison at $0 \text{ mV}/\text{mm}$ unless indicated specifically in the text.

Encystment

A. castellanii trophozoites were inoculated into 10 mL encystment medium (PYG medium containing 50 mM MgCl₂) in 50 mL conical flasks at a final concentration of approximately $2 \times 10^5 \text{ cells mL}^{-1}$, and incubated in a rocking incubator for 7 days at room temperature. This protocol has been shown previously to induce effectively 98.5% of trophozoites into cysts.^{13,27}

3D Culture of *A. castellanii* Trophozoites

Low melting point agarose (Catalog # A9414, Sigma-Aldrich, St. Louis, MO) was mixed in PYG medium (0.2% wt/vol). The mixture was heated to 65°C to dissolve the agarose. *Acanthamoeba* trophozoites were harvested from axenic culture in PYG medium as described above (Preparation of *A. castellanii* trophozoites). Trophozoites (0.5 to 1×10^5) were mixed into 1000 μL liquid phase of the agarose-PYG medium mixture, which then was transferred to the electrotaxis chamber to allow cooling and solidifying at room temperature for approximately 10 minutes. A coverslip lid then was placed on top of the chamber before an EF was applied as described above (EF stimulation and time lapse image recording). A focal plane with trophozoites was chosen by varying focus to make sure that the recording plane was not on top or at the bottom of the gel. Time-lapse recording was made and cell migration analyzed using ImageJ software (National Institutes of Health).

RESULTS

Acanthamoeba Trophozoites Migrate Directionally in Response to an EF

In the absence of an EF, no obvious directional migration was observed (Fig. 1A). *Acanthamoeba* trophozoites moved rapidly in a stochastic pattern with a trajectory speed of $12.1 \pm 0.05 \mu\text{m}/\text{min}$ and a directedness of 0.08 ± 0.01 (Figs. 2A–C, $n = 73$). The random movement of the *Acanthamoeba* trophozoites in the absence of an EF is shown by the migration trajectories in time lapse images (Fig. 1A) and in a plot graph normalized to the starting point of the individual trophozoites (Fig. 2A). In an EF of $1200 \text{ mV}/\text{mm}$, *Acanthamoeba* trophozoites migrated directionally toward the cathode with a significantly higher directedness of -0.65 ± 0.01 (Figs. 1B, 2A, 2B; $n = 124$, $P < 0.001$). We then confirmed the directionality of EF-induced cell migration by reversing the EF polarity to see if cell migration also reversed. After reversing EF polarity, trophozoites also reversed direction to migrate toward the new cathode, with a directedness of 0.55 ± 0.03 (Figs. 1C, 2A, 2B; $n = 168$, $P < 0.001$, see Supplementary Movie S1).

Application of an EF significantly increased displacement speed (Fig. 2C). Displacement speed indicates the efficiency of cell migration. In other words, cells made fewer turns during migration and collectively were able to cover a larger area, which was confirmed with electric polarity reversal (Figs. 2A, 2C). In the absence of an EF, the displacement speed of *Acanthamoeba* trophozoites was $4.42 \pm 0.03 \mu\text{m}/\text{min}$ (Fig. 2C, $n = 73$), while with an application of an EF at $1200 \text{ mV}/\text{mm}$ and $1200 \text{ mV}/\text{mm}$ in the reverse direction, displacement speeds were $6.43 \pm 0.05 \mu\text{m}/\text{min}$ (Fig. 2C, $n = 124$, $P < 0.001$) and $5.76 \pm 0.01 \mu\text{m}/\text{min}$ (Fig. 2C, $n = 168$, $P < 0.001$), respectively.

FIGURE 1. *Acanthamoeba* trophozoites migrate directionally in an electric field. Time-lapse images show cell migration without EF (A) and with EF (B, C). When the EF polarity is reversed, the cells switch their direction of migration to move toward the cathode. The bottom panels show the cell positions at time zero (light blue), 10 minutes (yellow), and 20 minutes (blue). Please see Supplementary Movie S1.

In an 1200 mV/mm EF application, trajectory speed increased significantly (Fig. 2C). Trajectory speed of *Acanthamoeba* trophozoites without an EF was 12.1 ± 0.05 $\mu\text{m}/\text{min}$ (Fig. 2C, $n = 73$), while with the application of an EF at 1200 mV/mm and 1200 mV/mm in the reverse direction, trajectory speeds were 13.5 ± 0.11 $\mu\text{m}/\text{min}$ (Fig. 2B, $n = 124$, $P < 0.01$) and 13.2 ± 0.01 $\mu\text{m}/\text{min}$ (Fig. 2B, $n = 168$, $P < 0.01$), respectively.

The Directional Migration of *Acanthamoeba* Trophozoites Is Voltage-Dependent

Different EF strengths were used to induce trophozoite migration to see if directedness is proportional to voltage. Trophozoites migrate randomly without an EF with a trajectory speed of 12.1 ± 0.05 $\mu\text{m}/\text{min}$ (Figs. 2B, 3A; $n = 73$). A small but significant directional migration was observed with an EF of 600 mV/mm with directedness of -0.15 ± 0.05 (Fig. 3A, $n = 238$, $P = 0.012$). In increasing EF voltage application, we found that the *Acanthamoeba* trophozoites migrate toward the cathode with increased directedness. At 900 mV/mm, trophozoites migrate with a higher directedness of -0.23 ± 0.01 (Fig. 3A, $n = 78$, $P = 0.006$). At 1200 mV/mm, trophozoites migrate with a directedness of -0.65 ± 0.04 (Fig. 3A, $n = 124$, $P < 0.001$), which was a 4-fold increase in directedness compared

to 600 mV/mm. Increasing the field strength to 1500 mV/mm did not induce further increase in directedness of trophozoite migration (-0.64 ± 0.03 , Fig. 3A, $n = 217$, see Table). Therefore, the directedness values plateaued at 1200 to 1500 mV/mm (Fig. 3A, see Table). Using ANOVA statistical analysis, values of directedness and speed of migration of *Acanthamoeba* trophozoites differed significantly across the different voltages with a $P < 0.001$ (see Table).

We analyzed the displacement speed of *Acanthamoeba* trophozoites in the presence of an EF. In the absence of an EF, displacement speed is 4.42 ± 0.28 $\mu\text{m}/\text{min}$ (Fig. 3B, $n = 73$). Displacement speed decreased at 600 mV/mm, but subsequent increases in voltage resulted in increased displacement speeds. At 900 mV/mm, trophozoites migrate with a displacement speed of 4.36 ± 0.25 $\mu\text{m}/\text{min}$ similar to no EF (Fig. 3B, $n = 78$, $P = 0.88$). At 1200 mV/mm and 1500 mV/mm, the cells migrated with a significantly higher displacement speed of 6.44 ± 0.27 $\mu\text{m}/\text{min}$ (Fig. 3B, $n = 124$, $P < 0.001$) and 7.04 ± 0.17 $\mu\text{m}/\text{min}$ (Fig. 3B, $n = 217$, $P < 0.001$), respectively (Fig. 3B, $P < 0.001$ see Table).

These results indicated that the directional migration of *Acanthamoeba* trophozoites in an EF is voltage-dependent, with higher voltages correlating with increased directedness and speed, which plateaued at 1200 mV/mm or above (see Table).

FIGURE 2. Quantification of the directional migration of *Acanthamoeba* cells in an electric field. **(A)** Plot graphs of individual cell migration trajectories without EF, and EF to left and to right at 1200 mV/mm. All cell positions at time zero were normalized to the center of each graph. **(B)** Cell migration directedness in EF of 1200 mV/mm with EF direction first to left (*top*) and then reversed to right (*bottom*). Compared to no EF, *** $P < 0.001$. **(C)** Cell migration speeds in EF of 1200 mV/mm in opposite directions as indicated. $n = 73$ to 168 from at least two independent experiments. Compared to no EF, ** $P < 0.01$, and *** $P < 0.001$.

FIGURE 3. Voltage dependence of directional migration of *Acanthamoeba* trophozoites. **(A)** Cell migration directedness is highly voltage-dependent. Increasing the voltage significantly increased cell directedness. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ compared to no EF. Arrows show the EF polarity. **(B)** Migration speed in different EFs. Displacement speed is voltage-dependent at higher EFs. $n = 73$ to 238 from at least two independent experiments. P values are that when compared to 0 mV/mm unless otherwise indicated. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. N.S., no significance ($P > 0.05$).

TABLE. EF-Guided Migration of *Acanthamoeba* Trophozoites

Culture Condition	Voltage, mV/mm	<i>n</i>	Directedness, $\cos \theta$	Trajectory Speed, $\mu\text{m}/\text{min}$	Displacement Speed, $\mu\text{m}/\text{min}$
2D	0	73	0.08 ± 0.08	12.06 ± 0.47	4.42 ± 0.28
	600	238	$-0.15 \pm 0.05^*$	$7.66 \pm 0.14^\dagger$	3.30 ± 0.10
	900	78	$-0.23 \pm 0.08^\dagger$	$9.28 \pm 0.28^\ddagger$	$4.36 \pm 0.25^\ddagger$
	1200	124	$-0.65 \pm 0.04^\ddagger$	$13.48 \pm 0.32^\ddagger$	$6.44 \pm 0.27^\ddagger$
	1500	217	$-0.64 \pm 0.03^\ddagger$	$11.04 \pm 0.19^{\ast\ddagger}$	$7.04 \pm 0.17^\ddagger$
3D	0	117	-0.06 ± 0.07	3.38 ± 0.07	1.16 ± 0.05
	1500	235	$-0.60 \pm 0.03^\dagger$	$6.10 \pm 0.10^\dagger$	$1.79 \pm 0.07^\dagger$

Data are mean $\pm$ SE. Statistics was done with ANOVA and 2-tailed Student's *t*-test.

$*P < 0.05$ when compared to the no EF control (0 mV/mm).

$^\dagger P < 0.01$ when compared to the no EF control (0 mV/mm).

$^\ddagger P < 0.01$ when compared with the values at 600 mV/mm.

$^\S P < 0.01$ when compared to the values at 600 mV/mm and 900 mV/mm.

FIGURE 4. Electrical field stimulated and guided migration of *Acanthamoeba* cells in 3D agar. **(A)** Time lapse images of *Acanthamoeba* cell migration in 0.2% (wt/vol) 3D agar (*marks a cell out of the focal plane). **(B, C)** EFs of 1500 mV/mm significantly increased migration speed (include displacement speed and trajectory speed, **[B]**) and directedness **(C)**; $n = 117$ to 235 from at least three independent experiments. *** $P < 0.001$ compared to the no EF control. **(D)** Collection of individual cell migration trajectories with and without EF. All cell positions at time zero were normalized to the center of each graph. Please see Supplementary Movies S2 and S3.

At 600 mV/mm, trajectory speed decreased to 7.66 ± 0.14 $\mu\text{m}/\text{min}$ compared to no EF (Fig. 3B, $n = 238$, $P < 0.001$), but subsequent increase from 600 to 900 mV/mm increased the trajectory speed significantly to 9.28 ± 0.28 $\mu\text{m}/\text{min}$ (Fig. 3B, $n = 78$, $P < 0.001$). Trajectory speeds were increased

significantly at 1200 mV/mm compared to no EF at a speed of 13.48 ± 0.11 $\mu\text{m}/\text{min}$ (Fig. 3B, $n = 124$, $P < 0.01$). In an EF of 1500 mV/mm, trophozoites migrated toward the cathode with a trajectory speed of 11.04 ± 0.19 $\mu\text{m}/\text{min}$ (Fig. 3B, $n = 217$, $P = 0.016$) which is statistically similar to the trajectory

speed of no EF. At higher voltages of 1800 mV/mm, most of the trophozoites had detached and presumably were dead (data not shown).

***Acanthamoeba* Trophozoites Migrate Directionally in Response to an EF in a 3D Culture System**

We tested the response of *Acanthamoeba* trophozoites to an EF of 1500 mV/mm in a 3D culture system to mimic the environment of an *Acanthamoeba* infection in the cornea. To confirm that trophozoites were in the agar gel in 3D, we changed the focal plane to make sure that the recording plane is in, not on the surface or at the bottom of the gel (Supplementary Movie S2). In the absence of an EF, *Acanthamoeba* trophozoites migrated randomly with a directedness of -0.06 ± 0.07 (Fig. 4A, $n = 117$). An applied EF of 1500 mV/mm induced a significant migration toward the cathode with a directedness of -0.60 ± 0.03 (Fig. 4A, $n = 235$, $P < 0.001$, Supplementary Movie S3).

The applied EF significantly increased trajectory speed and displacement speed. Cells migrated with increased motility and efficiency. In the absence of an EF, trophozoites migrated with a trajectory speed of 3.38 ± 0.07 $\mu\text{m}/\text{min}$ and displacement speed of 1.16 ± 0.05 $\mu\text{m}/\text{min}$ in 3D cultures (Fig. 4B, $n = 117$). An applied EF significantly increased trajectory speed and displacement speed to 6.10 ± 0.10 $\mu\text{m}/\text{min}$ and 1.79 ± 0.07 $\mu\text{m}/\text{min}$, respectively (Fig. 4B, $n = 235$, $P < 0.001$). This represents over 80% and 50% increase in trajectory speed and displacement speed, respectively. Trophozoite migration is shown by migration trajectories in time lapse images and in a plot graph normalized to the starting point of the individual trophozoites (Figs. 4C, 4D). These results indicated that an EF can induce directional and increased migration of *Acanthamoeba* trophozoites in a 3D environment.

***A. castellanii* Cysts Do Not Migrate in Response to an EF**

We also tested the effect of EF application on the cyst life form of *Acanthamoeba*. *Acanthamoeba* cysts formed after 7 days shaken in encystment medium at room temperature as described previously.¹³ An EF of 1500 mV/mm was applied to the cysts embedded in the gel. We found that cysts did not show obvious migration in response to an EF, but some trophozoites exhibited directional migration toward the cathode (see Supplementary Movie S4).

DISCUSSION

Acanthamoeba spp. reside deep in the corneal stroma and form cysts to evade drug treatments, which makes *Acanthamoeba* keratitis very difficult to treat. An effective technique to drive *Acanthamoeba* out of the corneal stroma might facilitate pharmacologic treatment. Because application of EFs has been shown to induce directional migration of many types of cells, we aimed to investigate the possibility of using EFs to drive migration of *Acanthamoeba* trophozoites. Out of the corneal stroma, the *Acanthamoeba* trophozoites can be exposed to drugs to eradicate the organism. In this initial study, we found that *Acanthamoeba* trophozoites respond significantly to EFs. *Acanthamoeba* trophozoites move directionally toward the cathode. The direction of cell migration was reversed when the polarity of the EF was reversed. Importantly, *Acanthamoeba* trophozoites also responded directionally to the application of an EF in a 3D culture system with increased migration speed.

Patients with *Acanthamoeba* keratitis typically present with photophobia, severe pain out of proportion with the extent of tissue damage, conjunctival hyperemia, and tearing.¹⁴

Severe disease can lead to blindness and even lead to enucleation.¹⁶ *Acanthamoeba* keratitis is notoriously difficult to treat. Currently, no drugs are licensed for treatment of this infection, to our knowledge, and the optimal approach for treatment in a given patient is unknown. Typically, a number of topical chemotherapy agents are used in combination in a rigorous treatment regimen, including chlorhexidine, propamidine, and pentamidine. Topical corticosteroids sometimes are used, although their efficacy in treating *Acanthamoeba* keratitis has been controversial, with some studies showing benefit,^{28,29} and a larger proportion of studies showing worse outcomes or no benefit at all.^{30–32} Patients typically are treated for many months at a time, even up to a year or more. Recurrence is common after stopping treatment, and *Acanthamoeba* keratitis is a difficult infection to eradicate. Often, patients are recalcitrant to medical treatment alone, and many of these patients require therapeutic keratoplasty for definitive treatment.⁵

This discovery that *Acanthamoeba* trophozoites respond to EFs is important for potential treatment of *Acanthamoeba* keratitis. It is thought that the topical medical agents may not penetrate into the cornea to reach the trophozoites residing deep in the cornea. However, it may be possible to use an EF to move the trophozoites directionally closer to the corneal surface to make them more accessible to amebicidal drugs, leading to more effective medical treatments and negating the need for invasive surgery. In this way, the application of an EF might be used in combination with drugs for treatment of *Acanthamoeba* keratitis to improve clinical outcomes in these patients. There are several possibilities for applying an EF onto an infected cornea. An EF could be applied directly to the cornea with the use a bioelectric contact lens that can directly generate an EF.³³ An alternative way might be to use chemicals that can change membrane potentials across the cornea to generate indirectly an electric membrane potential difference, which also can trigger migration of *Acanthamoeba* trophozoites closer to the corneal surface.

Other possible options for treatment of *Acanthamoeba* keratitis exist, although not much research has been tested directly in a clinical setting. It already is known that the innate immune response is critical for the resolution of disease, particularly macrophages and neutrophils, which are critical elements of the innate immune response to protozoan pathogens, including *Acanthamoeba*.^{34–36} Application of EFs may offer an additional possibility to combine with decystment, amebicidal agents, immune therapy, and perhaps photodynamic therapy for effective treatment.^{32,37–40} Application of an EF might stress trophozoites and induce cyst formation; however, we did not see any sign of encystment during our experiments. The opposite outcome also theoretically is possible, and we intend in future experiments to determine if electrical stimulation combined with other methods can induce decystment, which, if successful and combined with electrotaxis of trophozoites, may offer even more powerful approaches to treat *Acanthamoeba* keratitis.

Although the ability of an EF to provide a directional movement of *Acanthamoeba* trophozoites in a 3D culture system has been demonstrated (Fig. 4, Supplementary Movie S3), the effect of EFs on *Acanthamoeba* spp. in the corneal environment and on an infected cornea with *Acanthamoeba* keratitis remains unknown. This stresses the importance of future studies using animal models of *Acanthamoeba* keratitis, in which model migration of *Acanthamoeba* trophozoites can be tested.

It is important to consider how the electrotaxis of *Acanthamoeba* trophozoites might induce unwanted effects on corneal cells. We and others have demonstrated that corneal cells, including epithelial cells, keratocytes, and

endothelial cells, have a strong electrotaxis response. The corneal epithelium maintains a transepithelial potential difference. A compromised epithelial barrier, as after wounding, produces small wound EFs measuring approximately 150 mV/mm.^{41–43} Bovine and human corneal epithelial cells migrate robustly to the cathode in an EF. Corneal keratocytes and endothelial cells migrated to the anode,^{26,43–47} but these responses plateaued around 150 to 200 mV/mm,^{25,48} and our previous data showed that EFs over 200 mV/mm induced less directional migration.⁴⁴ Therefore, our results suggested that a field strength of 150 mV/mm is likely to be too weak to induce migration of *Acanthamoeba* trophozoites, and that higher field strengths effective for inducement of trophozoite migration (600–1500 mV/mm) will have minimal effects on corneal cell migration. *Acanthamoeba* trophozoites and corneal cells, therefore, appear to have different optimal voltage response ranges.

Our research addressed new possibilities for treating *Acanthamoeba* keratitis with the potential for improved success rates for medical treatment without resorting to invasive surgery. This project also elucidated a novel mechanism for migration of *Acanthamoeba* trophozoites, with increased insight into the migratory behavior of this opportunistic organism.

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