ELECTRIC FIELD INDUCED CLUSTERING IN SUSPENSION OF E.COLI BACTERIA

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ABSTRACT

Living microswimmers such as bacteria tend to collective organize into communities in response to external field. As this microswimmers swims, it stirs the fluid and creates flows which generally lead to hydrodynamic interactions with its neighbors. The flows can sometimes affect the collective dynamics of the suspension leading to complex cell interactions. To further understand the role of fluid flow in the dynamics of bacterial suspension, we applied electric field to a suspension of Escherichia coli, which are known for their run and tumble motion. As a consequence, we find that the fluid flow generated by electric field, induces attractive hydrodynamic interaction between the swimming bacterial which in turn leads to bacterial clusters. Indicating that the applied electric force completely disrupted and drowns the self-generated hydrodynamics of swimming bacterial cells. Our results will allow us to examine the relative importance of fluid flow bacteria cell transport and other bacterial process

Keywords: Hydrodynamic interactions, Bacteria, Self-propelled, fluid flow

INTRODUCTION

Microorganisms mostly live in dynamic fluid communities, which is required for most biological processes ranging from mobility, reproduction, infections and survival. At high concentrations, suspensions of swimming bacterial are known to exhibit complex dynamics as a result of long-range hydrodynamic interaction between the cells. Dombrowski et al. (Dombrowski, Cisneros, Chatkaew, Goldstein, & Kessler, 2004) observed large-scale spatiotemporal correlated patterns in suspension of Bacillus subtillis. The cells tended to swim in the same direction as its neighbor, creating flow patterns larger than the scale of an individual cell (Tuval et al., 2005). These studies suggest that the formation of large-scale structures resulted from hydrodynamics interactions between the cells. Since swimmers propel themselves autonomously through a fluid, they are constantly out of equilibrium; understanding their collective properties has become a paradigm of statistical mechanics.

Bacterial hydrodynamics has been studied in great detail over the past three decades. Bacteria such as E.coli swim in the run and tumble motion (C, 2004 ; Macnab, 1977; Turner, Ryu, & Berg, 2000), typically, propulsion is achieved by the rotary action of flagellar motor proteins: concerted rotation in the counterclockwise direction causes flagella to bundle together and propel the cell forward (run motion), but clockwise rotation of some motors causes the flagella to unbundle and the cell to vacillate (tumble motion). Both motions are influenced by chemical stimuli in the environment, or chemotaxis. In the absence of chemical stimuli, bacteria swim in a random fashion with straight runs followed by randomizing tumbles.

The flow generated by swimming microorganisms play an important role in ecological traits such as biogenic mixing, energy expenditure, foraging and transport (Trivedi, Maeda, Abbott, Spagnolie, & Weibel, 2015). Theories of hydrodynamic interaction between cells in suspensions typically characterize the flow fields generated by the microswimmers as the moments of force exerted by the body on a fluid (Elgeti, Winkler, & Gompper, 2015; Lauga & Powers, 2009). A microswimmer that exerts an effective force dipole on a fluid produces a stresslet or rotlet flow. Stresslet is a typical flow profile for swimming microorganisms, such as *Escherichia coli* (Drescher, Dunkel, Cisneros, Ganguly, & Goldstein, 2011). However, if the force is a point force and has a velocity field decaying as $1/r$, it is called Stokeslet flow. Such flows have been observed in the case of *Volvox carteri* (Drescher, Goldstein, Michel, Polin, & Tuval, 2010). The hydrodynamic interactions between swimming bacteria can additionally be affected by confinement, as for example(Wioland, Woodhouse, Dunkel, Kessler, & Goldstein, 2013), where confining surfaces redirect the dynamics of swimming cells. Simulations studies have shown an accumulation of cells near the walls due to confinement. This paper will focus on the effect of external electric field on the dynamics of swimming bacteria. A key challenge in this area is the complexities of designing experiments that couple hydrodynamic interactions and external flows.

A number of studies have previously examined how fluid flow generated by external electric can alter the trajectories of self-propelled particles. For instance, artificial self-propelled colloidal particles that could be steered by external field have

been extensively studied, electric and hydrodynamic interactions between them may arise and result in the formation of highly aligned coherent structures (Aranson, 2018; Mano, Delfau, Iwasawa, & Sano, 2017; Nishiguchi & Sano, 2015; Park & Saintillan, 2011; Yan et al., 2016; Zhang, Yan, & Granick, 2016). The patterns that result from these interactions can be complex and are yet not well understood. By contrast, in spite of considerable recent interest in external flow, its impact on the dynamics of biological active matter is less well explored.

To this end, we experimentally investigate the effect of spontaneous flow field generated by electric field on swimming bacterial suspension. The physical system we studied is aqueous suspension of *E.coli* bacteria. We found an emergent of bacterial clusters that is dependent of electric field magnitude. In our system, flow field is largely controlled by the applied electric force. This strong flow gradient induces cell-cell attractive interaction which in turn leads to formation of large scale bacteria clusters that are transient and reverses when the electric force strength is decreased. This fluid flow can result in long-range interactions between the cells and can drive significant advective transport of cells in far field. We infer that the attractive interaction is from a shear-induced cell migration like we have in many-body hydrodynamic interactions. The problem is biologically relevant in that it could potentially allow us to gain understanding at what drives organ shape formation either *in vivo* (embryogenesis) or in artificial systems (organoids). Our findings can help in the designing strategies and emerging applications of electric force driven microsystems.

MATERIALS AND METHODS

We employed *Escherichia coli* bacterial which have been studied extensively as our physical system. The cell body is typically ~2 µm long, 0.8 µm in diameter and swim by rotating several flagella at a frequency of about 100 Hz. The bacteria samples were washed repeatedly three times using centrifugation and intermittently sonicated to obtain single cells before being suspended in different volumes of clean deionized water according to the required concentrations. In deionized water medium, bacteria do not reproduce and growth is restricted. We used two parallel horizontal conducting glass plates coated with indium tin oxide (ITO) (GULUO Glass), separated by square boundary, carefully constructed and fixated to avoid evaporation. The dimensions of our ITO glasses are 25.4 x 25.4 mm^2 with a thickness of 1.1mm. The ITO coatings are located on the side of the sample. The suspension was introduced into the chamber through a small opening created at the edge of the square boundary. Typically, the amount of sample loaded for each experiment is around $500\mu l$. The sample chamber is then connected to a function generator (GW INSTEK AFG-2105, arbitrary Function Generator). As bacteria cells swim in bulk, a sinusoidal varying alternating electric field of the form $E(t) = E_0 \sin(2\pi f)\, t$, is applied to the electrodes. Where E_0 is the amplitude of the field, f is the frequency. Videos were acquired with a high-speed camera (Leica microscope, 10 frames/sec) under a bright field illumination, using a 40x objective. Experiments were repeated for different bacteria concentrations and various electric field amplitudes.

RESULTS AND DISCUSSIONS

E. coli bacteria are known to swim with very fast speed of about 30 µm/s in suspension. At the beginning of our experiment, before electric force was applied, we observed the bacterial performing their usual run and tumble motion with steric repulsion dominating their interactions. When the external electric field is introduced along x and y axis of the containing micro-chamber, the field generates fluid flow which in turn induces attractive hydrodynamic interaction between the swimming bacterial cells. Basically, there will be bacteria rotation and reorientation due to this velocity gradients. The external flow becomes the leading order hydrodynamic interaction between the cells as long as the electric field is turned-on. Snapshot of bacteria clusters dependence is shown in Fig 1(A-D).
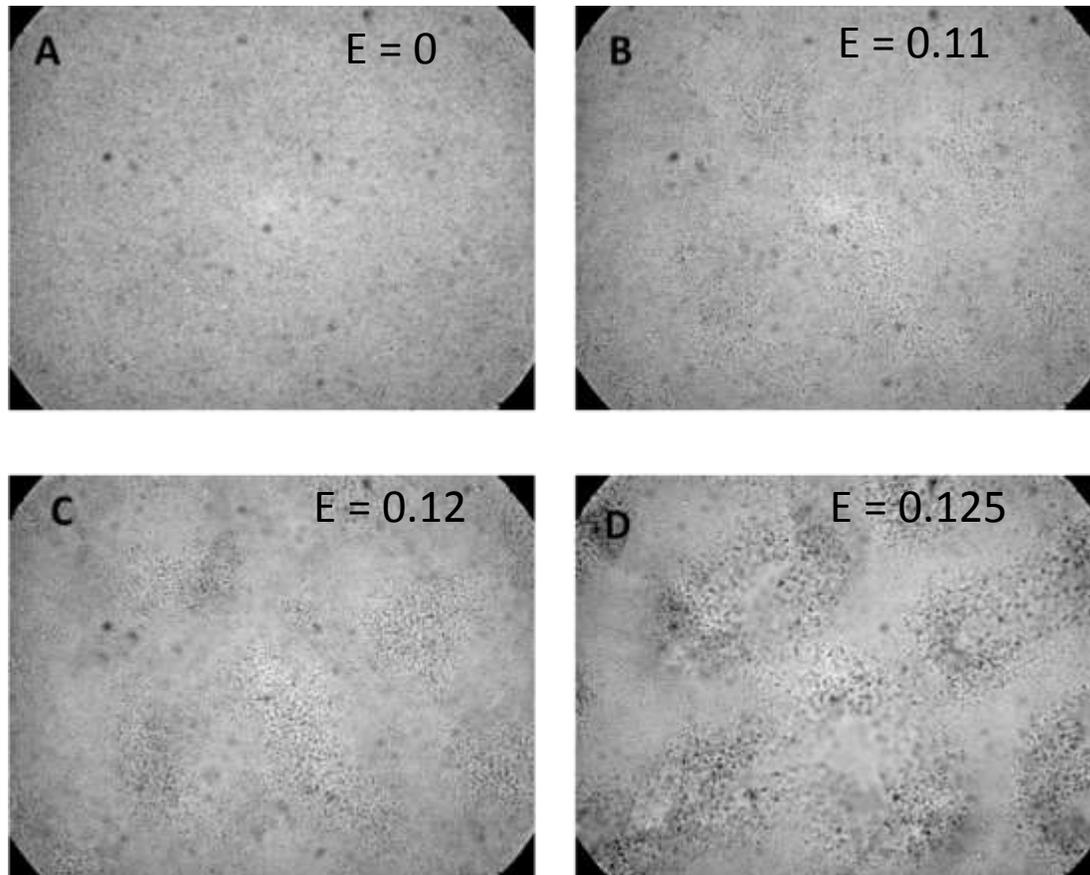
Figure 1: Snapshot bacterial suspension response to external ac electric field E (a) E=0Vμm^{-1} (b) E = 0.11 Vμm^{-1} (c) E = 0.12 Vμm^{-1} (d) E = 0.125 Vμm^{-1}

Above the critical field value 0.125Vμm^{-1}, bacterial clusters of aggregated cells are observed indicating strong attraction between them. A clustering process entails a competition between the pressure induced by the external flow and the swim steric repulsion of bacteria. The external force overcomes. Soon enough, the attractive force overwhelms swimming steric interaction between the cells, resulting in formation of large-scale structures. The structures are transient, they disperse when the field strength is gradually decreased or the electric field is turned-off.

To quantify the impact of the external flow on the orientations of the swimmers, we used PIV analysis to measure the flow field of the bacteria under the external electric field.
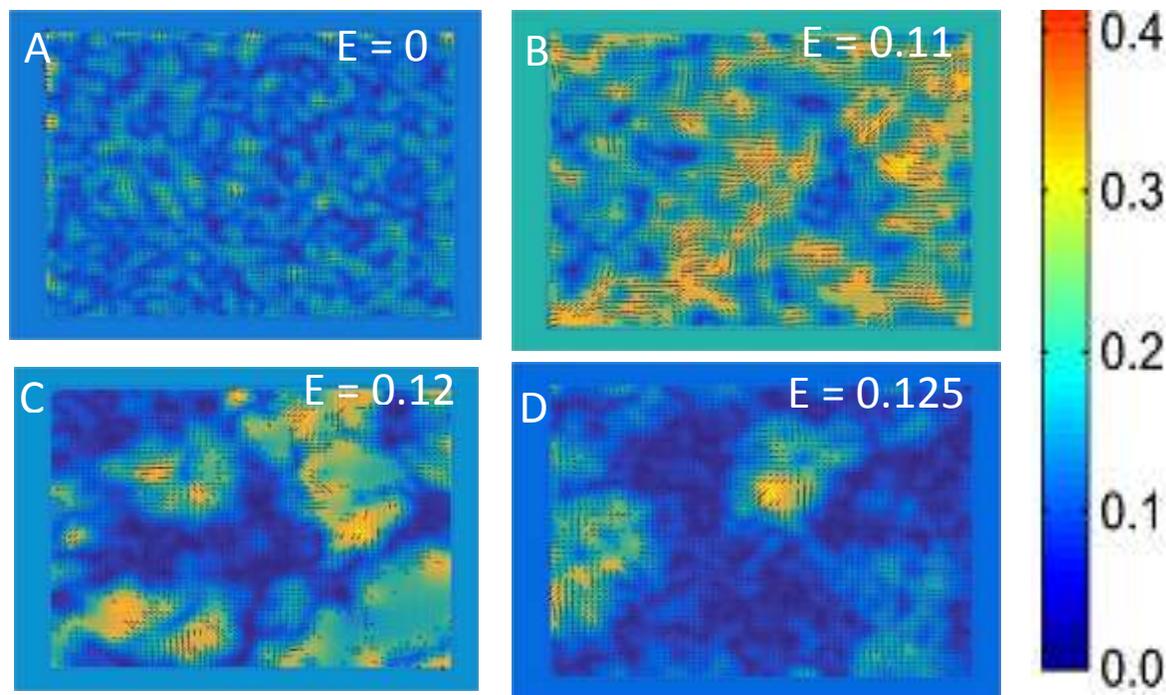
Figure 2: The flow velocity created by electric field around swimming bacterial. Arrows depict the direction, and colors show the velocity magnitude of bacterial flow (a) before electric field was applied (b-d) increased electric field amplitude.

Fig. 2 illustrates the bacteria flow for different field values. The colour bar indicates the level of the flow pattern. For samples with electric field (fig. 2a), significant flow is observed. As the effect of electric field decreases and the flow decays as compared to Figs. 2(b)–2(d). This is apparently due to change in bacteria behavior under the influence of electric field. Since the Reynolds number of an individual bacterium is exceedingly small, the flow is described by the linear Stokes equation (Lauga & Powers, 2009), coupled with an additional force. For a bacterium swimming in x direction, the flow can be approximated by a hydrodynamic dipole, $u(r) = pr(3x^2/r^2 - 1)/8\pi r^3$ where $r = /r/$ is the distance vector relative to the center of dipole, $p = aVl^2$ is the dipole strength, and a is the constant determined by the shape–aspect ratio.

These flow field measurements around freely swimming microorganisms provide the basis for a deeper understanding of a number of issues in biological fluid dynamics, including the interactions of microorganisms with surfaces, with each other, and the rheology of suspensions.

CONCLUSION

We have examined the effects of fluid flow generated by external alternating electric field on the suspensions of swimming microorganisms in confined microchamber. Specifically, we find that there exist a critical field value which induces dominant external flow which enhances bacteria aggregation. The external flow could significantly change the trajectories of swimming organisms. Therefore, it is possible to predict beforehand how the properties of self-propelled particles (such as microorganisms) will be altered by an external flow, which is one of the goals of this study.

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