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DYNAMIC SELF-ASSEMBLY IN E.COLI BACTERIA SUSPENSION

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ABSTRACT
We experimentally investigate self-assembly in bacteria suspension under low frequency alternating electric field. We observe the emergence of electric field-induced bacterial clusters as a function of electric field strength and bacterial concentrations. Above the electric critical field, bacterial cell self-organize into clusters, with further increase in field strength or bacteria concentration, a second critical point is reached, where 3D out of equilibrium structures are formed. Our findings demonstrates that the self-assembly of microswimmers can be controlled via external electric field. The observed cluster size dynamic equilibrium is in contrast with the features of cluster dynamics observed in cancer cells driven by adhesion where the cluster size distribution never reaches dynamic equilibrium. These results can offer a new pathway to self-organize living cells in biomaterials.

Keywords: Electric field, bacteria concentration, self-assembly, dynamic equilibrium, biomaterials

INTRODUCTION
Dynamic self-assembly are widely observed in active matter systems such as bacteria swarms, bird flocks, fish schools, vibrated granular rods, and herds (Zhang et al., 2010; Zhihu, 2018). Generating and controlling autonomous motion within their environment is an essential component for the individual units these system: they can extract energy from their environment and dissipate it for motion. Experiments have demonstrated that in the collective state, active biological systems can exhibit remarkable properties such as enhanced diffusivity (Mino, 2011), formation of sustained whorls and jets (Wu, 2000), and can serve as a good model for non-equilibrium self-organization.

It is well known that high density population of bacterial can develop into a large-scale structures that extend beyond those of their individuals cells. Dombrowski, et al. (2004), observed that concentrated bacteria suspension tend to form large-scale dynamics; the so-called bacterial turbulence. In their experiment, a suspension of swimming bacteria at sufficient density forms a mesoscale, coherent fluid motion. Mendelson et al. (1999), placed population of B. subtilis in a water film above an agar gel and observed mesoscale motions of whorls and jets. The diffusion in such suspensions is considerably enhanced by the mesoscale structures. Another well-known collective motion of bacteria is the band formation observed for magnetotactic bacteria (Thery et al., 2020).

Self-assembly in active matter systems are often controlled by parameters such as temperature, geometry confinement, density, pH and electric and magnetic fields. Electric fields offer an efficient method for manipulating particle motions and have been widely used in directed assembly, particle trapping, nano-manufacturing among others. In particular, self-propelled colloidal particles driven by electric fields typically align into highly ordered coherent structures. Depending on the specific experimental conditions, the outcome of the dynamic self-assembly would be sensitive to the amplitude and frequency of the driving field and properties of the suspending fluids. Although, the application of electric fields display considerable potential in the development of biomaterials with patterned structural features, there are few experiments that focus on its impact on biological systems (Markx, 2008; Gupta et al., 2010).

Recently, Samantaray et al. (2017), experimentally investigated the dependence of self-assembled dynamical phases of living bacterial cells on the electric fields amplitude and frequency. Kang (2014) and Kang & Dhont (2013, 2015) have extensively studied phase transitions of charged fibrous viruses at low frequency range (kilohertz) and observed various phases and dynamical states induced by low frequency electric fields. In these studies, the biological cells are modeled as particles covered with shells, and the interaction among the cells are treated in the dipole approximation. To our knowledge, no studies have been carried out yet on self-assembly of micron-size living bacterial cells under low frequency external alternating electric field.

In this study, we investigate the impact of low frequency external electric fields on the collective behavior of living E.coli bacteria suspension. We observe the development of field-induced bacterial clusters that are electric field strength and bacterial concentrations dependent. Experiments with high bacterial concentrations give rise to large cluster sizes whereas we observe smaller cluster sizes for low bacterial concentrations. Above critical electric field strength, bacterial cells spontaneously form clusters which extends to form 3D structures at higher field strength. Our experiments demonstrate that in bacterial systems, the effect of tuning field amplitude and bacterial concentrations at a fixed low frequency, can enhance the formation of large scale structures and it is possible to design novel biomaterials through such a path.

EXPERIMENTAL DETAILS

We employed *Escherichia coli* bacterial suspension as our physical system. The cell body is typically ~2 µm long, 0.8µm in diameter and swim by rotating several flagella at a frequency of about 100 Hz. The details of bacterial culture preparation are provided in the supporting information. 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 1.1 mm. The ITO coatings are located on the side of the sample. The bacterial 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 500μl. 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 $\mathrm{E(t)} = E_0 \sin(2\pi f)\, t,$ is applied to the electrodes. Where E_0 the amplitude of the field while f is the frequency. We carried out all our experiments at a fixed low frequency, f = 0.3Hz. 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.

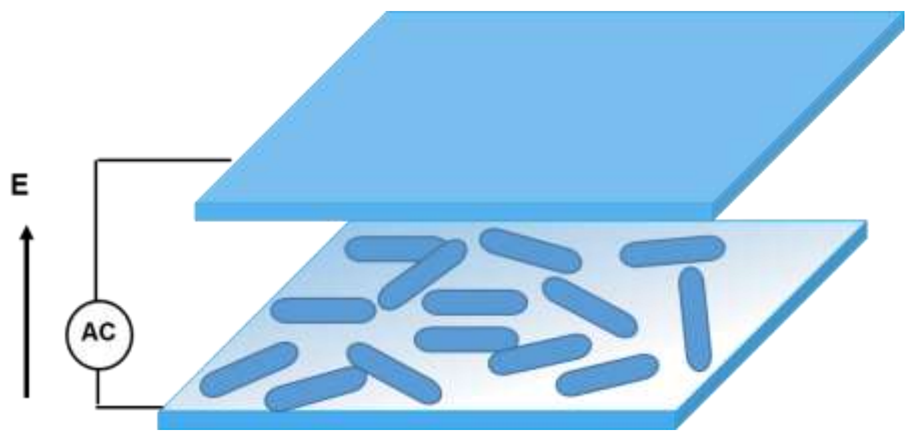

Figure 1: A schematic representation of the experimental setup

RESULTS AND DISCUSSIONS

Without an applied electric field (E = 0), the cells are diffusive and are randomly distributed as can be seen in fig 1a. When a sinusoidal alternating electric field is introduced in the suspension, it triggers collective swimming of the cells and in turn leads to cluster formation. The clustering process depends on the electric field amplitude and bacterial concentrations. The clustering occurs when the field strength is above the critical value E_c = 0.12V/µm. Likewise, high bacterial concentration favors bacteria clustering. If the field value is below the critical value, cells form disordered clusters similar to those observed in active colloids. Snapshots of a typical electric field-induced bacteria clustering process are shown in (fig.1a-c).

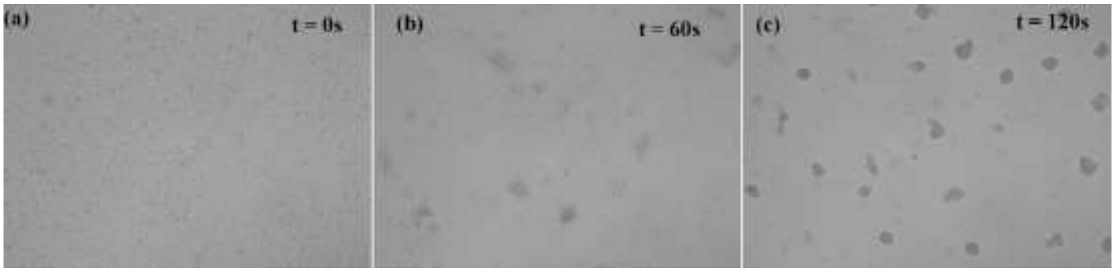

Fig. 2. Snapshot of cluster evolution. (a) t =0s the electric field is turned-off, the cells swim randomly in the microfluidic chamber (b) t = 60s, electric field is turned-on which triggers collective motion in the suspension. (c) three dimensional bacteria clusters are formed.

In order to establish the time evolution of clusters, we study the effects of electric field strength and bacterial concentrations on the cluster sizes. We start with computing the mean cluster size ⟨s⟩ as a function of time for different values of E and ϕ. Fig 3a reveals that the system quickly reaches a steady state characterized with ⟨s⟩ ≈ 25µm. High field values give rise to large mean cluster sizes. This is similar to what was observed in active colloid (Zhang et al., 2016). This quantitative similarity may originate from the fact that when the field value is large they all experience higher activity and when the field value is lower, they all have slower activity. For different values of ϕ at a fixed E (fig. 2b), high concentration ⟨s⟩ reaches steady state at relatively longer time with larger mean cluster size. The large fluctuations observed in plots could be as a result of cluster merging and splitting. Clearly, the cluster size show stronger dependence on ϕ than E.

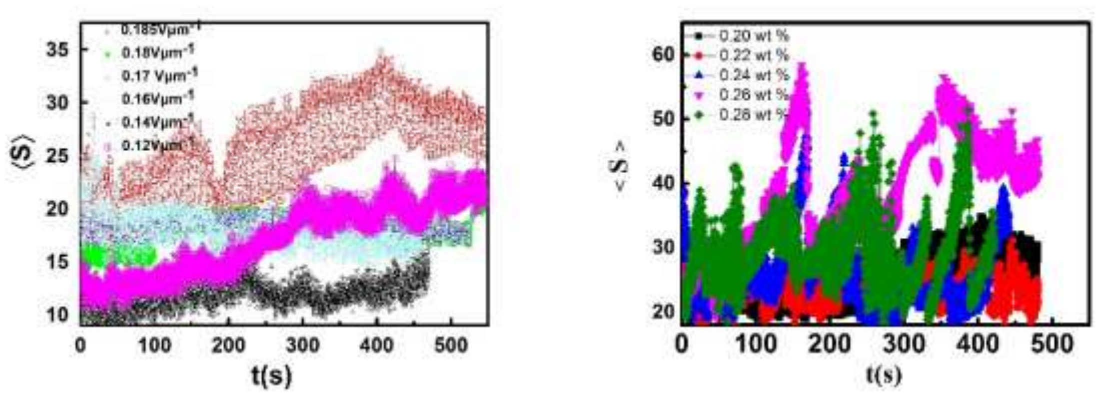

Fig. 3. Mean cluster size distribution. (a) Different values of field-induced steady state cluster sizes (b) Various bacterial concentration

Thus far we have demonstrated that low frequency external electric field can be used to induce and direct self-assembly in bacterial suspension. The observed field-induced bacterial clustering exemplify the emergence of collective motion often seen in active matter systems. In our experiments, we observe dynamic cluster size distribution similar to previous study on cancer cells driven by adhesion (Khain et al., 2009). However, in contrast to the mentioned experiments, where the cluster size distribution never reaches dynamic equilibrium, our observed CSD evolves towards dynamic equilibrium as shown in fig 4. As the bacteria concentration increases, the exponential width increases.

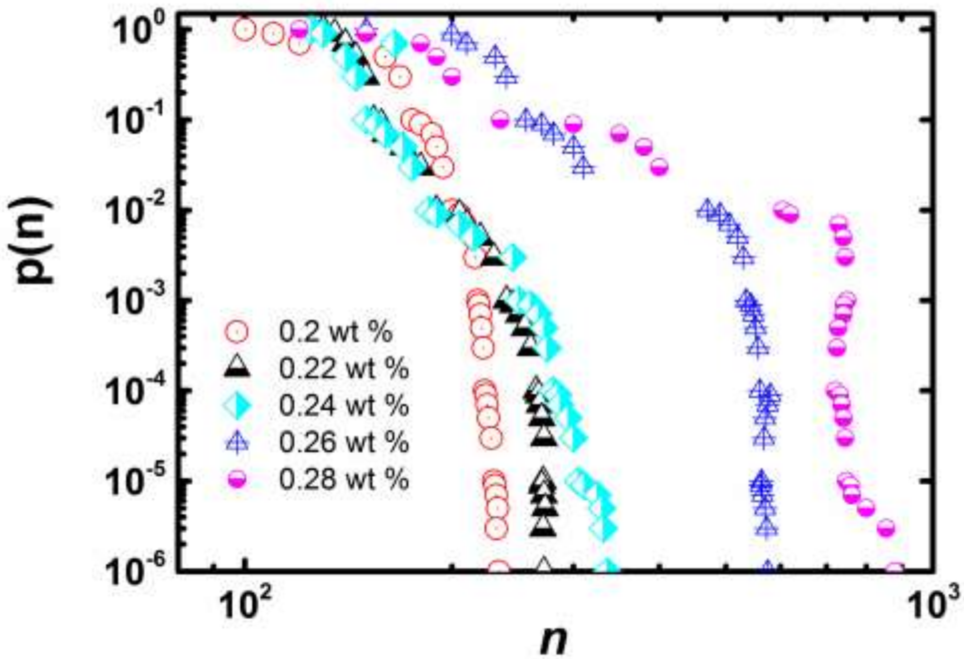

Fig. 4. Cluster statistics: The cluster size distribution of various bacterial concentrations.

In this work, we acknowledge that we do not have the details of bacterial effective interparticle interactions under external electric field. However, we assume that double layer polarization could be the plausible field-induced mechanism in our experiments. The reason is that we carried out all our experiments in low frequency electric field regime. The mechanism governing low frequency- dependent polarization had been previously explained in studies on the field induced phase transition of fd-virus (Dhont & Kang, 2008, 2014). At low frequency alternating electric field, polarization of the electric double layer is dominant such that dielectric polarization is excluded. Since bacterial have surface charges in deionized suspension (Samantaray et al., 2017), and our experiments were carried out at a fixed low frequency and sufficiently low field strength, it is possible that the field-induced clustering and structure formation in our experiments is due to the field-induced deformation of electric double layer surrounding bacterial cells.

In the experimental work of Kang et al. (2014), various field induced phases such as stripes, nematic and dynamical states, were observed. In contrast, we used low frequency regime (mHz), yet there is a close analogy between the clustering and phase transitions of observed in charged colloids. The cluster fusion and splitting in our experiment is similar to the dynamical state of fd-virus, where non-chiral domains melt and reform (Dhont & Kang, 2014). Although, Samantaray et al. (2017), found a phase diagram based on high frequency electric field as a function of field amplitude and concentration. They considered bacterial cells as polarized rods, and predicted a phase diagram which contains oriented, chain and 2D columnar phases. On the other hand, our experiments were based on double layer model, however, the phase diagram observed have are in qualitative agreement with the previous studies.

CONCLUSION

We have reported a new type of self-assembly in living bacterial cells under low frequency external alternating electric field. We found that bacterial cells exhibit a transition at a critical field amplitude and concentration values characterized with the emergence of a field-induced 3D structure with a remarkable dynamic properties. Our experiments demonstrates that by tuning field amplitude and bacterial concentration, one can control and manipulate the responses of bacterial self-assembly. The field induced cluster fission and fusion observed can provide valuable pathway to the designing of new complex functional biomaterials on the micron-scale. The outcome of this studies will have potential application in the design of novel biomaterials for specific biosensor applications.

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