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Light induced DEP for immobilizing and orienting *Escherichia coli* bacteria

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ABSTRACT

Manipulating bacteria and understanding their behavior when interacting with different substrates are of fundamental importance for patterning, detection, and any other topics related to health-care, food-enterprise, etc. Here, we adopt an innovative dielectrophoretic (DEP) approach based on electrode-free DEP for investigating smart but simple strategies for immobilization and orientation of bacteria. *Escherichia coli* DH5-alpha strain has been selected as subject of the study. The light induced DEP is achieved through ferroelectric iron-doped lithium niobate crystals used as substrates. Due to the photorefractive (PR) property of such material, suitable light patterns allow writing spatial-charges-distribution inside its volume and the resultant electric fields are able to immobilize *E. coli* on the surface. The experiments showed that, after laser irradiation, about 80% of bacteria is blocked and oriented along a particular direction on the crystals within an area of few square centimeters. The investigation presented here could open the way for detection or patterning applications based on a new driving mechanism. Future perspectives also include the possibility to actively switch by light the DEP forces, through the writing/erasing characteristic of PR fields, to dynamically control biofilm spatial structure and arrangement.

1. Introduction

The growth and invasiveness of many bacterial species rely on their ability to form biofilms, complex matrices of proteins, sugars and signaling molecules secreted by the bacteria to achieve a sort of orchestrated growth. Biofilms are one of the most critical causes of infection in several fields as health-care and food-enterprise. The understanding of the early stage of their formation could help in improving future treatment or avoiding their growth. Biofilms development and structural stability depend on several factors acting both at the beginning stage, as the chemical interaction of each single cell with the host surface, and later on with the particular and organized positioning of the bacteria giving rise to the biofilm. On the other side, cell manipulation is nowadays a key step in several research areas from tissue engineering to biosensors, besides the basic research in terms of biochemical pathways study [1,2]. Some well-established methods exist for single cell and cell population handling. For single cell manipulation a quite new technique is the so-called Optical Tweezers (OT) where a laser beam focused on a small area generates forces able to trap micrometer sized objects (viruses, bacteria and eukaryotic cells) [3]. OT allows accurate three dimensional control with nanometer accuracy by an all-optical approach. In particular, Jordan and coworkers [2] have demonstrated the manipulation of multiple bacteria in 3D volume by holographic OT. However, the number of samples that is possible to control with OT is limited. Therefore, new engineered nanodevices, characterized by functionalized substrates have been developed as alternative methods. Very recently, chips have been coated with components of extracellular matrix (ECM) caged with photo-deprotectable groups. By using focused light it has been possible to create patterns on the chip with de-protected ECM factors in order to guide cell adhesion towards those points [4]. When cell manipulations such as identification and sorting are needed, the gold standard methodologies to identify and separate a certain subpopulation are the Fluorescence Activated Cell Sorting (FACS) [5], magnetic activated cell sorting [6] and chemically functionalized pillar-based microchips [7]. These techniques, although effective and promising, are limited by the necessary knowledge of the properties of the desired cell types, they are time-consuming, and require special training to be executed. An alternative to the mentioned methods is dielectrophoresis (DEP); a technique that eliminates extensive sample preparation (no antibody labeling, one needs only to prepare a single sample) and provides a high selectivity at separating rare cells. DEP is the motion of a polarizable particle in a suspending medium due to the presence of a non-uniform

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electric field [8–10]. With no needs to know the surface properties of the desired cells, DEP can noninvasively sort populations through differences within the interior of cells, as well as their exterior. In conventional DEP techniques, metallic microelectrodes with various geometries are patterned on a microfluidic device using conventional lithography techniques [11–13]. DEP has been used for the isolation of several rare cell types including human cervical, breast and colorectal cancer and leukemia cells [8,14,15]. Cell isolation has been done through both batch separation [16–18], where rare cells population is trapped by positive DEP force while background cells pass through the microdevice, and continuous separation [14], where rare cells continuously separate from background cells. Among the several applications of the DEP technique in biology we focus the attention on the immobilization of microbial organisms where the study of the early stage development of biofilms is desirable [19–21]. In literature, it has been largely demonstrated that DEP through local micro/nano-electrodes allows a time dependent accumulation of bacteriophages; thus resulting an excellent technique not only to immobilize but also to concentrate microorganisms [22–27]. Recently, new DEP principles have been demonstrated based on electrode-free approaches that allow to realize devices with great versatility in terms of liquid/polymer patterning geometries. The electric fields able to manipulate liquids and immobilize particles are generated by exploiting the properties of ferroelectric crystals avoiding external electric circuits and voltage generators. In particular, it has been successfully proved that pyroelectric (PE) and photorefractive (PR) fields can be employed for dispensing liquid droplets down to atto-liter volumes [28], for trapping microparticles [29] and for creating liquid/polymer structures such as biodegradable polymer microneedles [30], tunable micro-lenses [31] or microfluidic channels [32]. The driving stimuli for PE and FR fields generation are thermal and optical, respectively.

Here, we demonstrate that is possible to apply such electrode-free DEP cues for immobilization and orientation of biological objects. The PR effect is the best choice as the high temperature reached in the PE devices could alter the normal conditions and damage the samples. In the recent years DEP trapping of nanoparticles by PR induced electric fields has been largely studied [33] and supported by experimental validations [34,35]. In literature, the interaction of PR field with biological sample has been investigated to induce death in tumor cell cultures [36]. In the present paper such fields are used to manipulate bacteria cells maintaining them alive. In order to test the applicability of this innovative technique we tested the ability of *Escherichia coli*, Gram negative bacteria, to respond to the external electric field on the surface of ferroelectric iron-doped lithium niobate crystals (Fe:$LiNbO_3$) [37]. The experimental results and discussion on advantages and drawbacks will be described in the following section.

2. Research methodology

2.1. Bacteria culture

E. coli DH5-alpha was plated and incubated on agar plates. The day before the beginning of experiment, a single bacterial colony was picked up and cultured in Luria-Bertani (LB) broth medium (10 g/l NaCl, 10 g/l tryptone, 5 g/l yeast extract) at 37 °C in a shaker incubator for 16–18 h to achieve saturation conditions. A 1:5 volumetric dilution of cell culture was then grown in LB until reaching the log phase corresponding to a cell concentration of

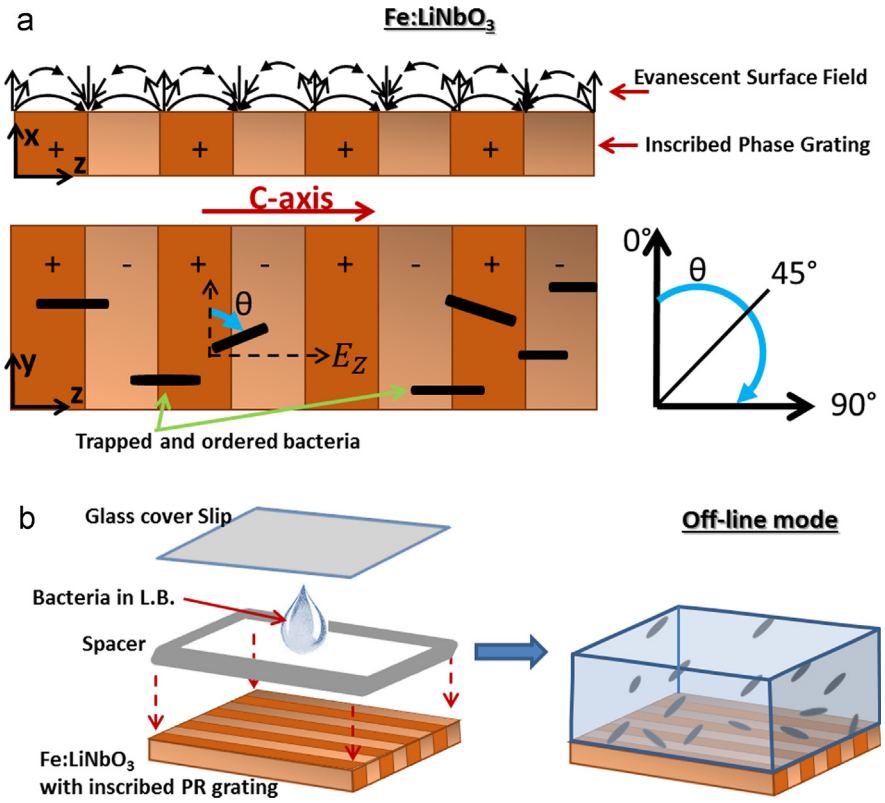

Fig. 1. (a) Schematic representation of evanescent field and consequent *E. coli* orientation. The angle, θ, between each bacterium and the direction of grating planes, is measured. (b) Drawing of the sample: a closed chamber containing about 30 μl of bacteria suspended in LB, the bottom surface of the chamber is Fe:$LiNbO_3$.