

Bacteria collection from static millilitre samples using a DC field

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Summary:

Efficient bacterial collection from millilitre-scale liquid samples remains challenging for preconcentration-based analyses. We present a device that uses a DC electric field to transport and deposit bacteria onto an agarose pad in a static chamber. The system operates at physiological ionic strength while preserving cell viability by limiting electrochemical reactions. Accumulation of live bacteria at low concentrations (10^3 CFU/mL) is demonstrated at 5V. Transport, viability, and collection efficiency are confirmed by microscopy and plate assays. This approach enables rapid bacterial pre-enrichment and detection.

Keywords: Electrokinetic bacterial collection, Milliliter scale sample processing, Low-concentration bacteria detection, Agarose pad collection

Background, Motivation and Objective

Efficient bacterial collection and concentration are critical for diagnostics and analytical sample preparation. Bacterial cells typically possess a negative net surface charge under physiological conditions and hence, they undergo electrophoretic motion when exposed to a direct current (DC) electric field. Electrophoretic deposition and more sophisticated electrokinetic manipulation strategies like dielectrophoresis (DEP) or ion concentration polarization (ICP) have been widely explored at the microscale, particularly in microfluidic systems for bacterial concentration and sensing [1][2]. Despite these advances, most electrokinetic bacterial concentration strategies rely on microfluidic architectures operating at microliter volumes and often require low-conductivity media to avoid excessive current and electrode reactions. One strategy to mitigate these effects involves electrically coupling the electrodes to the sample chamber through salt bridges or hydrogel interfaces [3]. Such configurations allow ionic current to pass through the system while physically separating the electrodes from the biological suspension, thereby suppressing electrode electrochemistry within the sample volume. Agarose-based salt bridges are widely used in electrophysiological and electrochemical measurements for this purpose [4]. Unlike most electrokinetic bacterial concentration approaches that rely on microfluidic geometries and microliter-scale sample volumes, the present work explores electric field-driven bacterial collection in a milliliter-

scale chamber operating under physiologically relevant buffer conditions. This approach seeks to bridge the gap between electrokinetic manipulation techniques developed in microfluidics and practical bulk sample preparation methods required for analytical and diagnostic workflows.

Description of the New Method or System

We developed a novel milliliter-scale electrokinetic device to collect live bacteria on the surface of an agarose pad. The system consists of a 1 mL bacterial chamber electrically connected to Ag electrodes through agarose salt bridges, which allow electrical conduction while isolating the electrodes from the sample volume. This setup is integrated within a control system, as illustrated in Fig 1. By applying a DC bias (5 V), a current of approximately 1 mA is established across the system. Under the DC electric field, negatively charged bacterial cells undergo electrophoretic transport toward the positive electrode where they adhere on the agarose surface. The device configuration, comprising salt bridges, enables electrophoretic transport of live bacteria under physiologically relevant ionic strengths while suppressing electrode electrochemistry. Under these optimized parameters, bacterial collection from 1 mL sample in about 1.5 h can be accomplished. Remarkably, the collected bacteria remain viable and can be recovered by extracting the agarose pad for downstream analysis.

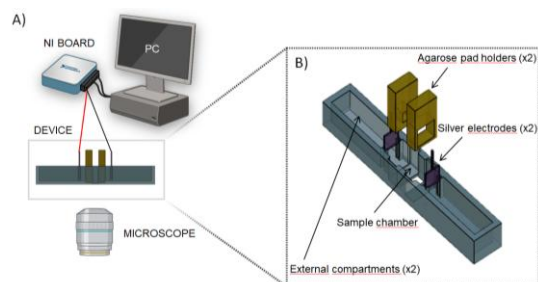


Fig 1. Experimental setup A) Integration of the device with the control system and optical microscopy for real-time observation. B) Zoom on the device showing the silver electrodes, agarose pads, and the 3D-printed PLA chamber compartmentalization

Results

We fabricated the device in Fig 1 by 3D printing in PLA. A sample chamber was implemented between two agarose pads which isolated it from the electrode compartments. A pair of electrochemically chlorinated silver (Ag/AgCl) electrodes were placed outside the bacterial sample, in two compartments containing 1.5 mL of 0.1x PBS. The agarose salt bridge (3% agarose salt bridges, 4 mm thick) enables ionic continuity and electrical connectivity while acting as a collecting surface for bacteria. By combining the agarose salt bridges with a low-current DC field and chlorine-free chemistry, we effectively eliminated the formation of toxic electrolysis by-products, thereby preserving the physiological integrity of the sample. The transport dynamics were monitored by using an inverted Olympus microscope (40x objective) interfaced with a National Instruments (NI) card and a power supply, controlled by custom developed software. The velocity, v of a bacteria cell driven by a DC electric field, depends on the bacteria electrophoretic mobility, μ and the intensity of the electric field, E : $v = \mu E$. Electrophoretic mobility depends on the zeta potential of the bacteria surface (about -40 mV), and permittivity and viscosity of the liquid in which the bacteria is immersed. With a potential $V = 5V$, distance $d = 20$ mm, between the two electrodes and ~50% attenuation of the electric field inside the sample chamber, the driving velocity is $v \sim 2.1 \mu\text{m/s}$, which corresponds to the experiments. For a 1 mL cubic chamber with the width $w = 1$ cm, the estimated time required to collect all the bacteria on the agarose pad is $t \sim 80$ min. Since this is quite time-consuming experiment, for the proof of concept we run experiments lasting only 15 min and extrapolated the results. *E. coli* bacteria at 10^3 CFU/mL concentration were used as sample and the collection evaluated by the plate-count method. Agarose pads were extracted, and collected bacteria were recovered by centrifugation and then plated. After 24 h of incubation, significantly more colonies were observed on the anode

pad than on the cathode for DC field, and similar few colonies on both pads for control (no field), confirming transport and collection of viable bacteria as expected (Fig 2a). On average, about 17% of the initial bacterial population was recovered on the positive electrode after 15 min ($n = 5$ experiments). Extrapolating to the theoretically estimated time, a collection efficiency of 90% could be reached in 80 min. The presence of the bacteria on the anode and cathode pads was checked also with stained bacteria (NucRed) by confocal microscopy (Fig 2b). The normalized amounts of collected bacteria on the two pads, in the presence and without electric field are presented in Fig 2c, showing the net accumulation on the anode pad under the application of the DC field. These preliminary results demonstrate that by combining milliliter-scale operation, biocompatible electrolytes, and suppression of electrode electrochemistry, one can obtain a simple electrokinetic system and practical approach for bacterial preconcentration and detection in analytical and diagnostic workflows.

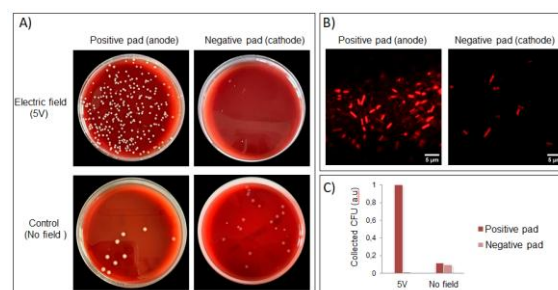


Fig 2. Recovery and viability analysis. A) Representative agarose pads after culture. B) Confocal images of labeled bacteria collected on agarose pads. C) Statistical CFU count comparing transport vs. control

References

- [1] MP. Hughes, Strategies for dielectrophoretic separation in lab-on-a-chip systems, *Electrophoresis* 23, 2569–2582 (2002); doi: 10.1002/1522-2683(200208)23:16%3C2569::aid-elps2569%3E3.0.co;2-m
- [2] S. Park, Continuous dielectrophoretic bacterial separation and concentration from physiological media of high conductivity, *Lab Chip* 17, 2893–900 (2011); doi: 10.1039/c1lc20307j
- [3] PH. Barry, Liquid junction potentials and salt bridges in electrophysiological measurements, *J. Membr. Biol* 121, 101–117 (1991); doi: 10.1007/BF01870526
- [4] A.J. Bard, *Electrochemical Methods: Fundamentals and Applications*, 2nd ed.; Wiley (2001)