Received: 26 August 2022 | Revised: 28 October 2022 | Accepted: 6 November 2022

DOI: 10.1002/elps.202200214

ELECTROPHORESIS

RESEARCH ARTICLE

On-chip dielectrophoretic recovery and detection of a lactate sensing probiotic from model human saliva

Sean Hamilton[1,2] | **Delaney Shea**[1,2] | **Stuart Ibsen**[1,2] | **Michael Brasino**[1,2]

[1]Cancer Early Detection Advanced Research Center, Knight Cancer Institute, Oregon Health and Science University, Portland, Oregon, USA

[2]Department of Biomedical Engineering, School of Medicine, Oregon Health and Science University, Portland, Oregon, USA

Correspondence
Michael Brasino, OHSU Knight Cancer Institute Cancer Early Detection Advanced Research Center (CEDAR), 2720 S Moody Ave, KR-CEDR, Portland, OR 97201, USA.
Email: brasino@ohsu.edu

Stuart Ibsen, Department of Biomedical Engineering, School of Medicine, Oregon Health and Science University, Portland, OR 97201, USA.
Email: ibsen@ohsu.edu

Funding information
National Institutes of Health, Grant/Award Number: R01CA258787; CEDAR center, Grant/Award Number: Exploratory7420320

Abstract

Early detection has led to increased survival for multiple cancers; however, the 5-year survival rate of oral carcinoma (OC) has remained at 40% for the last several decades. Screening for OC is routinely done via visual examinations, followed by tissue biopsy and laboratory testing. Point-of-care testing would be a more convenient and widely available alternative for at-risk individuals. Increased lactate production is a hallmark of many head-and-neck tumors, due to the Warburg Effect, where tumor cells favor glycolysis in the place of oxidative phosphorylation. To detect excess lactate, we have modified the commensal bacterium *Escherichia coli* Nissle 1917 to express fluorescent reporter genes in response to extracellular lactate. Administering this commensal as a mouth wash and subsequently collecting saliva for the detection of the reporter may allow for noninvasive, early detection of cancerous lesions in at-risk individuals. Furthermore, we demonstrate a new on-chip electrokinetic technique to recover these probiotic probes from model saliva fluid to improve the detection of reporter gene activation.

KEYWORDS
commensal, dielectrophoresis, lactate, saliva

1 | INTRODUCTION

Oral cancer (OC) is a major health concern within the United States. In 2022, it is estimated that 54 000 people will be diagnosed with this disease, and that 11 230 will die from it [1]. The prevalence of OC in the United States has decreased over the last several decades primarily due to declining tobacco usage, although currently the majority of patients with OC have never used tobacco. The current gold standard for OC diagnosis is visual inspection followed by tissue biopsy and histopathology of concerning lesions [2]. Patients with early-stage lesions are often asymptomatic, delaying them from initiating this process. Furthermore, biopsies are prone to producing false negatives due to incomplete sampling and tumor heterogeneity. Due to a lack of more convenient screening methods, only 18% of OC is diagnosed before spreading to surrounding tissues or lymph nodes, leading to poor prognosis and increased mortality [1]. The current 5-year survival rate of OC is only 67%, though it is 85% when detected at a localized stage [1]. Thus, there is a clinical need to develop a convenient point-of-care diagnostic test to detect these early-stage lesions and serve as a way to identify patients who would benefit from further diagnostic evaluation. This would greatly decrease oral cancer burden and

Abbreviations: EcN, *Escherichia coli* strain Nissle; OC, oral cancer/carcinoma.

improve patient survival. However, no point-of-care tests are currently approved in the United States to screen for OC.

This is despite the prevalence of promising biomarkers for this disease. Specifically, multiple groups have found salivary lactate levels of head-and-neck cancer patients to be significantly higher than that of healthy controls [2, 3, 4]. Lactate is produced as the end product of glycolysis which is metabolically favored by fast growing and aggressive tumors. This metabolic switch, known as the Warburg effect, is one of the most well-studied hallmarks of cancer [5]. High concentrations of lactate in tumor biopsies are correlated with increased metastasis and poor survival in OC patients [3]. Furthermore, OC patients have higher lactate concentrations within their bulk saliva compared to healthy individuals, indicating that lactate produced by a tumor is secreted and accessible within the oral cavity [2, 6]. In the laboratory setting, the detection of lactate in bulk saliva requires specialized equipment that is not suitable for point-of-care testing, such as liquid chromatography–mass spectrometry or biochemical assay. The lactate is also diluted in the bulk saliva reducing overall signal, making detection in that format difficult. However, the local lactate concentrations directly surrounding a tumor (if one exists) will be greater than in the saliva as a whole, so probing localized lactate concentrations should allow for more reliable tumor detection. To do this safely and noninvasively, we describe here an FDA approved commensal bacteria that we have genetically engineered to act as a lactate probe. These bacteria could be applied as a mouthwash so that bacteria are brought in close proximity to the oral tissues where tumors may be present.

Commensal bacteria have long records of safe use in humans and are used in commercial applications. Many of the strains used in these commercial products were originally isolated from the gastrointestinal tracts of healthy individuals and are well tolerated. Recent studies have begun to explore how these bacteria interface with the human immune system to affect disease [7, 8]. Several bacterial species have also been found to preferentially colonize the tumor microenvironment. However, the virulent nature of many of these tumor targeting bacteria has made their application for therapy or diagnosis difficult [9]. Building on this research, there have been many attempts to rationally modify bacteria to enhance their therapeutic properties [10]. Less studied is the use of modified bacteria for early disease detection. The cancer detection application is largely unexplored with a few existing studies focusing mostly on the detection of metastasis or recurrence [11, 12].

In choosing our bacterial probe, we aimed to explore the detection of OC using commensal bacteria that would be well tolerated and could access the tumor location through noninvasive oral delivery. As such, we choose to use the well-studied *Escherichia coli* strain Nissle 1917 (EcN). Commercially available as Mutaflor in the European Union and Canada, EcN is currently prescribed orally for the treatment of ulcerative colitis but has been documented to aid in other ailments such as diarrhea [13]. We sought to modify EcN to probe the human oral cavity for the localized high lactate concentrations associated with OC and to respond with the production of green fluorescent protein (GFP).

The recovery and detection of intact bacteria expressing the lactate activated reporter GFP gene are essential for the future point-of-care screening process. Traditional methods of recovering whole bacteria from body fluids involve the use of physical characteristics of the bacteria themselves. One common method is centrifugation [14, 15] which requires large specialized and expensive equipment and is not a viable option for point-of-care testing. A saliva sample containing individual bacteria can be cultured on agar plates to amplify the dilute population of bacteria for visual detection, [16] but this requires aseptic techniques to perform and does not maintain the desired GFP reporter gene activation signal. Immunocapture techniques are also a possibility that use antibodies or other binders to immobilize the bacteria on a surface, but these techniques suffer from low signal and can require extensive preprocessing of the bacteria, including boiling to break them up into smaller pieces that are easier to capture [17]. None of these existing techniques are designed to recover bacteria from saliva in a way that allows for the detection of individual bacteria expressing GFP.

To address this challenge, we developed an electrokinetic-based bacteria recovery and detection method using a dielectrophoresis (DEP) microfluidic chip [18–22]. DEP uses the differences in dielectric properties between the bacteria and the surrounding undiluted saliva to create a force that draws the bacteria preferentially to predefined regions of a microelectrode array integrated into the chip. With the application of an alternating current (AC), the electrode array generates a nonuniform electric field that induces a DEP force [18]. The DEP force is described in the following equation:

$$F_{DEP} = 2\pi r^3 \varepsilon_0 \varepsilon_m \mathrm{Re}\left([CM]\right) \nabla |E|^2 \quad (1)$$

where r is the radius of the bacteria particle, ε_0 is the permittivity constant for a vacuum, ε_m is the permittivity constant for the suspending media, such as saliva, and $\nabla|E|^2$ is the gradient of the electric field squared. *CM* is the frequency-dependent Clausius–Mossotti factor, also written as $K_i(\omega)$, which is the contrast factor of the electrical permittivity of the particle and surrounding media [23]. The *CM* factor is dependent on the type of particle, the

simplest case being a homogenous spherical particle as shown in Equation (2), followed by Equation (3) which is the version for a nonspherical bacterium adapted from Fernandez et al. [24]:

$$Spherical\ K_i\ (\omega) = \frac{\left(\varepsilon_p^* - \varepsilon_m^*\right)}{\left(\varepsilon_p^* + 2\varepsilon_m^*\right)},$$
$$\frac{\varepsilon_i^*}{(i\ =\ p,m)} = \varepsilon_i\ - j\frac{\sigma_i}{\varepsilon_0\omega} \quad (2)$$

$$Ellipsoid\ K_i\ (\omega) = \frac{1}{3}\frac{\left(\varepsilon_p^* - \varepsilon_m^*\right)}{\varepsilon_m^* + A_i\left(\varepsilon_p^* - \varepsilon_m^*\right)} \quad (3)$$

As stated above, ε is the permittivity constant for either the particle or the media annotated with a subscript p or m, respectively, the additional term of ε_0 is equal to the permittivity of free space, ω is the angular frequency, j is equal to $\sqrt{-1}$, σ is the electrical conductivity, and A_i is an x, y, z axis-specific depolarization factor. An expanded version of Equations (2) and (3) can be found in Fernandez et al. [17].

Under positive DEP, the collection of bacteria occurs at the electrode edge because this is where the electric field gradient is the highest. This concentrates the bacteria creating a localized signal amplification thereby increasing the signal-to-noise ratio of the GFP fluorescence. This application of DEP is unique because a hydrogel coating over the electrodes allows its successful operation in physiological fluids with comparatively high electrical conductivity, like saliva which has an electrical conductivity of 0.45 S/m, in a barrier free and label free manner that does not rupture the bacteria. This hydrogel coating has been effective in separating the metal electrodes from the high conducting biological media as shown in previous research for exosome and DNA isolation from plasma or whole blood, and we are using it here in undiluted saliva [19, 20, 25]. This maintains the integrity of the bacteria and GFP signal and allows for detection and quantification of the GFP fluorescence signal through automated optical analysis of microscopy images [18]. It only requires 20 µl of saliva simplifying sample collection, preparation, and handling. It has been shown previously that DEP can be used to successfully move and trap bacteria [21, 26]. Dielectrophoresis has also been utilized for the detection of oral cancers through the direct collection and analysis of epithelial cells with the 3DEP system or by a DEP impedance measurement [27–30]. Our approach uses DEP to recover our engineered bacteria from undiluted model saliva fluid and detect GFP fluorescence indicating exposure to lactate as an oral cancer metabolite. This work represents a preliminary study using these engineered bacteria to probe metabolite levels for lactate in combination with DEP isolation and quantification of the resultant bacterial signal.

2 | MATERIALS AND METHODS

2.1 | Cloning and plasmid preparation

The lactate responsive elements of our design, including the LldR coding sequence and Lld promoter, were PCR amplified from the *E. coli* DH5α via primers LldR-fwd, -rev and LldProm-fwd,-rev, respectively (listed in supplemental). These amplicons along with vector pET His6 GFP TEV LIC cloning vector (1GFP) (provided as a gift from Scott Gradia, Addgene plasmid 29663) were used as templates for a second round of PCR in which amplicons were extended with homologous ends followed by assembly via the NEBuilder HiFi DNA Assembly kit (New England Biolabs). This resulted in the replacement of the LacI coding sequence and LacI promoter with the LldR coding sequence and reasonably strong constitutive promoter bba_J23118, and the replacement of the T7 RNA polymerase responsive promoter with the Lld promoter responsive to LldR regulation. The assembly reaction was transformed into NEB5α chemically competent *E. coli* (C2987H) and plated on LB agar plates containing 100 µg/ml carbenicillin (Sigma Aldrich, C1389). Resulting colonies were pricked, grown overnight in LB with carbenicillin (100 µg/ml) and miniprepped with a Qiagen kit (27106). Sanger sequence confirmation was attained via Genewiz sequencing services.

2.2 | Bacterial strains and sensing conditions

E. coli Nissle 1917 (EcN) was purchased from Ardeypharm GmbH and made chemically competent through treatment with magnesium and calcium chloride (Sigma) following a previously published protocol [31]. EcN carrying the LldGFP plasmid was grown to mid-log phase in LB media supplemented with 100 µg/ml carbenicillin at 37°C with shaking. EcN control without sensing plasmid was grown in LB without carbenicillin. When the culture had an OD_{600} of 0.55, it was spun down at 4000 g for 5 min and resuspended in the same volume of artificial saliva with mucin (Pickering Laboratories, 1700-0316) with various concentrations of sodium lactate (Sigma 71718). The artificial saliva had a mineral composition and pH similar to human saliva, with mucin added to increase viscosity. Cells were incubated at 37°C with shaking to allow them to sense the lactate.

2.3 | GFP fluorescence measurements

GFP fluorescence measurements were taken using a Tecan Spark 20M (SN: 1605005980) plate reader in opaque sided, clear bottom, 96 well plates. Bacteria in sensing media with various concentrations of lactate were added at 100 µl per well, and readings were taken every half hour. Readings were taken at an optical density of $\lambda = 600$ nm (OD_{600}) and fluorescence expression of eGFP at an excitation wavelength of $\lambda_{ex} = 466$ nm and an emission wavelength of $\lambda_{em} = 510$ nm.

2.4 | DEP isolation and quantification

After Tecan fluorescent quantification, the artificial saliva samples were introduced into three-chamber microfluidic DEP chips (ExoCell EVF1P flow cell, 3-channel, EF-CRT-00002) purchased from Biological Dynamics (San Diego, CA, USA). The microfluidic chips utilized planar electrodes to generate an AC DEP force. An amount of 20 µl of sample was loaded into the inlet ports of the microfluidic chips, and a KD Scientific syringe pump (SN: D104199, CAT: 78-8111) was used to flow in the sample at a rate of 5 µl/min and a total volume of 20 µl. Once the chamber was filled with sample, the electric signal was applied. The electric signal was generated using an Agilent function waveform generator (SN: MY48006111, Model: 33210A) set to High Z Mode and a Newtons4th Ltd amplifier (SN: 510-01201 Model: LPA01). The amplifier was set to AC mode, low bandwidth, and 4× amplification, producing an applied signal of 14 kHz and 12 V_{pp}. After collection, a wash step of 48 µl of 0.5× concentration PBS (6.5 mS/cm conductivity) buffer at a flow rate of 5 µl /min washed away uncollected material. The wash volume was equivalent to four times the channel volume. After the wash step, imaging and fluorescent quantification were performed using an automated MATLAB program as previously described in Gustafson et al. 2021 [4]. Briefly, the fluorescent intensity of the collection area around the electrode perimeter was quantified, followed by local background subtraction, and artifact removal. Fluorescence of bacterial expressed eGFP was detected at an excitation wavelength of $\lambda_{ex} = 466$ nm and an emission wavelength of $\lambda_{em} = 510$ nm using a Zeiss Axio Imager Vario A2 microscope.

3 | RESULTS AND DISCUSSION

Our lactate responsive genetic circuit, shown in Figure 1A, was created from synthetic components, along with several parts from the endogenous *E. coli* LldPRD operon which is involved in lactate metabolism. This includes a lactate binding transcriptional regulator LldR, and the Lld Promoter which it regulates. The LldR protein has been shown to regulate this promoter both positively and negatively by recruiting RNA polymerase when bound to lactate and blocking it when unbound to lactate [32]. In order to drive a high level of reporter transcription, the LldR was expressed under a strong RBS and constitutive promoter from the Anderson library [33]. This expression cassette was placed next to the Lld driven reporter construct but in an opposite orientation as to prevent read through transcription. For our reporter gene, we used monomeric-enhanced GFP for high fluorescence intensity and fast maturation time.

E. coli Nissle 1917 (EcN), purchased from Ardeypharm GmbH, was transformed with this construct on a high copy plasmid, grown to late log phase in rich media and then spiked into an artificial saliva sample with various concentrations of sodium lactate. As shown in Figure 1B, transformed EcN exposed to lactate showed increased fluorescence indicative of their surrounding lactate concentration within 2 h. Importantly, the fluorescence could clearly detect the millimolar concentrations of lactate that have been documented in oral cancers compared to surrounding tissue [4]. The lactate senstivity of the bacteria in artificial saliva was also similar to that in rich media, indicating that sensing is possible without the bacteria requiring specific growth conditions. This sensitivity level is similar to other lactate sensing circuits built in bacteria [34, 35].

We next attempted to use these bacteria to detect lactate gradients in a two-dimensional hydrogel, analogous to the oral mucosal lining. For this, transformed EcNs were grown to late log phase in rich media and then immobilized in an agarose gel on which a porous puck soaked with various concentrations of sodium lactate was placed. As shown in Figure 1C, modified EcN produced an intense fluorescence response corresponding to the expected lactate gradient diffusing from the puck. This mimics the way these engineered bacteria could be used to detect lactate diffusion from a tumor within the oral cavity.

We next sought to develop a sensitive method of detecting and quantifying the fluorescent output of these bacteria with a method suitable for future translation to a low-cost portable test. Our goal was to detect bacteria at the lowest possible concentration, as they will likely be diluted in bulk saliva during and after exposure to the oral cavity. We show that direct fluorescence measurement in human saliva using a plate reader or similar device is hampered by the high autofluorescence background signal from endogenous saliva components, including mucin and other proteins. Saliva composition also varies widely between individuals and even within the same individual over time, making controlling for this background difficult [36]. This necessitates the development of a method

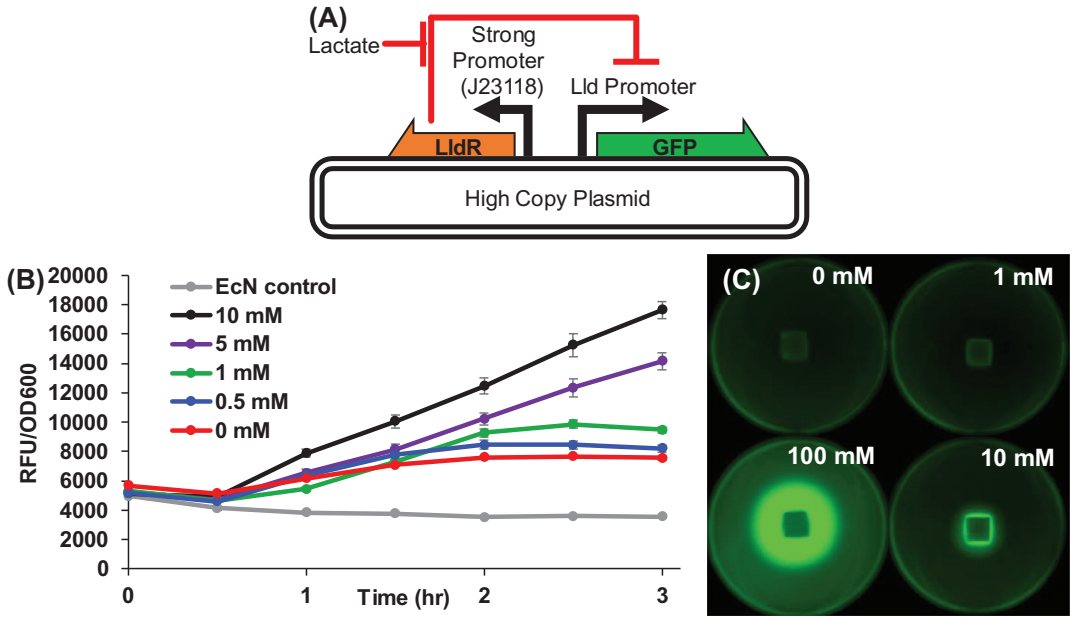

FIGURE 1 Demonstration of a genetic sensor for lactate detection in *Escherichia coli* Nissle 1917. (A) Diagram of the lactate sensing genetic circuit using the responsive transcription factor LldR and its cognate Lld promoter, both from *E. coli*. LldR has been placed under the strong constitutive Anderson promoter J23118 and in the opposite direction from the LLd promoter to prevent read through transcription. (B) *E. coli* strain Nissle (EcN) was transformed with the lactate sensing circuit and grown in LB broth until mid-log phase. Then diluted 1:100 in artificial saliva with the listed concentration of sodium lactate and incubated at 37°C with shaking. Green fluorescent protein (GFP) fluorescence and cell density were read periodically in a Tecan plate reader. Data represents average and standard error of five replicates. (C) EcN transformed with the lactate sensing circuit was grown to mid-log phase in liquid media followed by mixing one to one with 1% agarose in fresh media and plating in 10 cm diameter petri dishes. Filter paper pucks soaked with various lactate concentrations were placed on the center of each gel, and bacteria were allowed to grow at 37°C for 16 h., after which gel fluorescence was imaged. Lactate concentrations clockwise from top left: 0, 1, 10, and 100 mM

that separates the bacteria from the bulk saliva in a quick and easy manner suitable for future clinical translation. To both concentrate and purify the bacteria for detection, we used a newly developed biomarker quantification strategy based on a DEP microfluidic chip [18–20, 25, 37].

Our transformed bacteria were used to detect lactate concentrations in artificial saliva as before then diluted in additional artificial saliva before measuring fluorescence using either our in-lab plate reader or using DEP as shown in Figure 2A. DEP chips use a relatively small volume (20 µl) of sample and were run with an applied signal of 14 kHz and 12 V_{pp}. Bacteria within each DEP chip responded to a positive DEP force causing collection around the perimeter of planar circular electrodes on the bottom surface of the sample chamber. The bacteria were held with enough force to allow a wash to remove non-collected material and fluid. As shown in Figure 2B, the collected bacteria were directly imaged using fluorescent microscopy, and the fluorescence of these rings was quantified using a previously developed optical quantification software to determine the sample fluorescence intensity [18]. Signal and background levels measured by the plate reader and DEP chips are compared in Figure 2C. The ratios between the 1 mM lactate signal and the background 0 mM lactate signal are shown for each bacteria dilution factor in Figure 2D. At the 1× bacteria dilution level (approximately 8×10^8 CFU/ml), the DEP and the plate reader methods showed similar average ratios between signal and background (1.63 and 1.75, respectively). Here, the high bacteria concentrations created a fluorescent signal that was well above the auto fluorescent background of the artificial saliva. However, when sensing bacteria were diluted in additional artificial saliva to mimic expulsion from the oral cavity, the average signal-to-background ratio for the plate reader dropped significantly due to the increased proportion of the fluorescent signal coming from the autofluorescence of artificial saliva (1.13 and 1.05 for 20× and 100× dilutions, respectively) (*t*-test, two tailed, equal variances not assumed, $p < 0.01$ for 20× and $p < 0.01$ for 100× compared to 1×).

As expected, bacterial isolation with the DEP chips allowed for the removal of the artificial saliva which lowered the background. The DEP average signal-to-background ratio did not drop from 1× when diluted (2.07 and 1.88 for 20× and 100× dilutions, respectively), likely due to the purification of the bacteria from the artificial saliva. The observed increase in the ratio with increasing dilution was not statistically significant (*t*-test, two

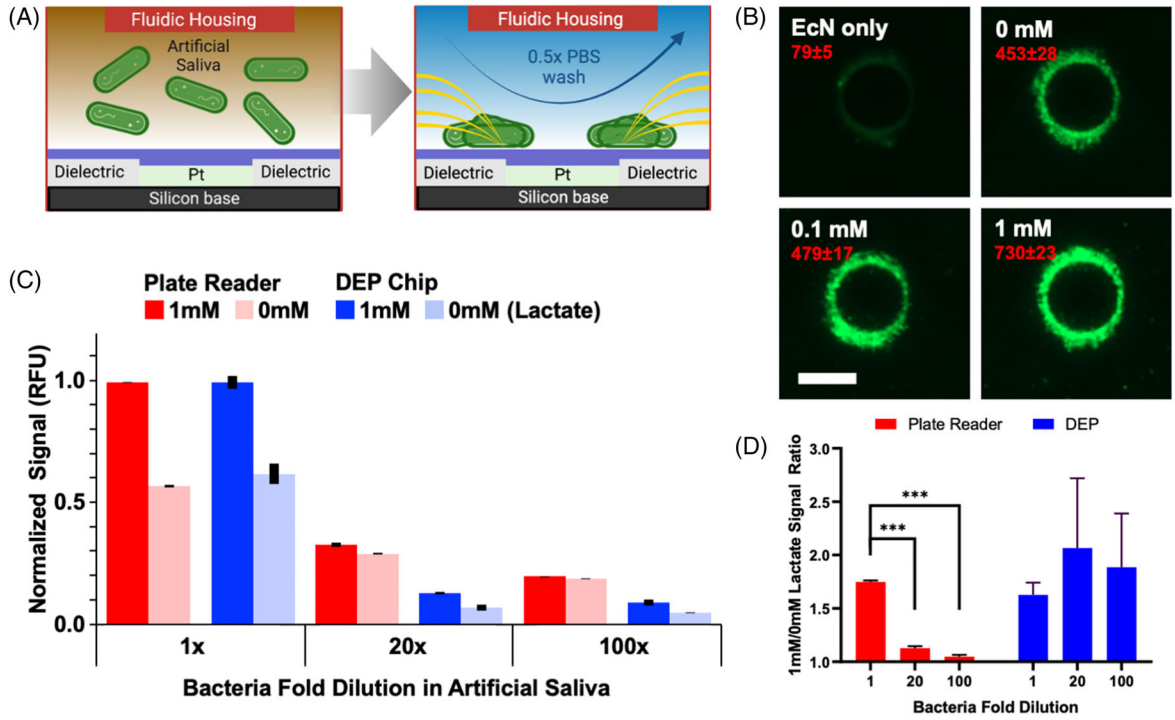

FIGURE 2 Dielectrophoresis (DEP)-based collection of lactate sensing *Escherichia coli* Nissle 1917 bacteria. (A) Cross-sectional schematic of the DEP microfluidic chip showing a single platinum microelectrode from the array. DEP chips have an array of 784 microelectrodes each 60 μm in diameter. A 20 μl of sample containing bacterial probes was deposited in each chip, and bacteria were then drawn through dielectrophoretic force to the corner of each electrode, followed by a purification wash. Imaging and quantification were then done directly on chip. (B) *E. coli* strain Nissle (EcN) transformed with the lactate sensing circuit was grown to mid-log phase in rich media then spun down and resuspended in artificial saliva with the indicated concentration of sodium lactate. Fluorescence microscopy images of the sensing bacteria isolated from artificial saliva on the DEP chip electrodes are shown. Scale bar is 50 μm. Average fluorescence signals (in RFU ± standard error) are shown in red and calculated from whole chips and $n = 3$ replicates. (C) Sensing bacteria were treated with 0 or 1 mM sodium lactate as in panel (B). After 2 h, bacteria were diluted in additional artificial saliva by the indicated amount, and fluorescence was read directly using a plate reader or the DEP chip technique. Fluorescence from bacteria treated with 1 mM sodium lactate is shown in red for the plate reader and blue for DEP. Background signals from 0 mM lactate are shown in lighter shades for both methods. Error bars are standard error, $n = 2$ for 20× and 100× DEP, $n = 3$ for 1× DEP and all plate readings. (D) Signal ratios between 1 and 0 mM lactate from panel C at each bacteria dilution factor. With dilute bacteria, DEP gives an increased signal ratio compared to the plate reader. ***$p < 0.001$ by *t*-test (two tailed, equal variances not assumed)

tailed, equal variances not assumed, $p = 0.624$ for 20× and $p = 0.699$ for 100× compared to 1×).

When taken as two separate groups, there was a statistically significant difference between the DEP ratios compared to the plate reader ratios indicating that the DEP technique improves the signal-to-noise ratio for bacteria detection from artificial saliva (*t*-test, two tailed, equal variances not assumed, $p = 0.048$).

To determine the source of the DEP background signal remaining after the wash, we isolated EcN bacteria alone (without lactate sensing circuit) and measured the corresponding fluorescence (Figure 2B). Although this bacterial autofluorescence was detectable (79 RFU), the signal was lower than that of EcN with the sensing circuit but without lactate (453 RFU). This suggests that the majority of background signal in the DEP-based assay was from the leaky expression of GFP in the probe bacteria rather than variable saliva components. This leaky expression would have been consistent across different dilution factors and indicates why a significant change in the signal-to-noise ratio was not observed across dilution factors for DEP collection.

4 | CONCLUDING REMARKS

OC continues to be a significant driver of cancer mortality, due in part to a lack of development in early detection for this cancer type. To address this, we have developed a two-part system for detecting excessive lactate production from early oral cancer. The first part is an engineered lactate sensing commensal bacteria that produces a fluorescent signal in response to local lactate concentrations, and the second part is the use of a low-volume microfluidic DEP device for rapid recovery of the bacteria and quan-

tification of the produced signal. The combined system is a low-volume, noninvasive, and rapid diagnostic tool that is ideally suited to enable future point-of-care screening applications.

The advantages of using a commensal EcN as the probing mechanism are twofold. First, using bacteria that can naturally come in close proximity to aberrant cell behavior, where biomarker concentrations are higher, may increase sensitivity compared to a test that measures an analyte diluted into bulk saliva. Second, the easily modifiable commensal used in this technology is highly extendable and could sense multiple analytes to increase the sensitivity or specificity for early oral cancer. The sensing of multiple analytes would not necessarily translate into a more complex analytical platform as multi-detection could be genetically tied to a single output using genetically based gating logic such as AND/OR gates [34]. In addition, this commensal model is intrinsically scalable and inherently biocompatible.

The microfluidic DEP device used here is capable of sensing low quantities of bacteria by exerting a positive DEP force on EcN to collect and concentrate it in specific locations on the electrode array. This enables purification from bulk saliva material by washing away non- or negative-DEP responsive particles along with the biological carrier fluid. This background reduction increases the signal-to-noise ratio compared to bulk measurement, which is especially beneficial in heterogenous human saliva where autofluorescence contributes to background signal. This allows us to more accurately measure the fluorescence indicative of bacterial GFP production. We showed that this version of DEP works directly in undiluted model salvia which is unique in the DEP and microfluidics fields. This reduces sample handling and preparation time and enables greater sensitivity as dilution is unnecessary. DEP collection is simple with few steps making full automation possible which is important for point-of-care applications targeted toward the high-risk oral cancer population. Working with small 20–50 µl volumes, speeds sample loading and washing times. Not needing to fix samples simplifies sample handling, decreases the time for analysis, and eliminates the need for toxic compounds. The application of the electric field and imaging of the chip itself can be accomplished with simple electronics and fluorescence microscopes. These characteristics are needed to enable translation of bacteria-based diagnostics for point-of-care cancer screening.

The DEP device used here is capable of multi-biomarker isolation from a variety of biological fluids in addition to saliva, including whole blood and plasma. Previous work has demonstrated the ability to isolate exosomes from plasma and measure exosomal proteins and internal cargos such as mRNA [20]. The addition of engineered bacterial probes to these analytes further highlights the utility of the DEP chip as a multi-omic platform capable of detecting diverse biological signals from low-volume samples .

ACKNOWLEDGMENTS

This project was supported by funding (Exploratory7420320) from The Knight Cancer Institute's Cancer Early Detection Advanced Research Center (CEDAR) at the Oregon Health and Science University. This project was also supported by the National Institutes of Health grant R01CA258787 to S.D.I. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

CONFLICT OF INTEREST

The authors have declared no conflict of interest.

DATA AVAILABILITY STATEMENT

Data for Figure 2C is available in the Supporting Information section. All other data is available from authors upon reasonable request.

ORCID

Sean Hamilton https://orcid.org/0000-0001-6740-290X
Delaney Shea https://orcid.org/0000-0001-8180-3937
Stuart Ibsen https://orcid.org/0000-0001-8619-3832
Michael Brasino https://orcid.org/0000-0001-5264-6442

REFERENCES

1. Siegel RL, Miller KD, Fuchs HE, Jemal A. Cancer statistics, 2022. CA Cancer J Clin. 2022;72(1):7–33.
2. Song X, Yang X, Narayanan R, Shankar V, Ethiraj S, Wang X, et al. Oral squamous cell carcinoma diagnosed from saliva metabolic profiling. Proc Natl Acad Sci USA. 2020;117:16167–73.
3. Blatt S, Voelxen N, Sagheb K, Pabst AM, Walenta S, Schroeder T, et al. Lactate as a predictive marker for tumor recurrence in patients with head and neck squamous cell carcinoma (HNSCC) post radiation: a prospective study over 15 years. Clin Oral Investig. 2016;20:2097–104.
4. Brizel DM, Schroeder T, Scher RL, Walenta S, Clough RW, Dewhirst MW, et al. Elevated tumor lactate concentrations predict for an increased risk of metastases in head-and-neck cancer. Int J Radiat Oncol Biol Phys. 2001;51:349–53.
5. Warburg O. On the origin of cancer cells. Science. 1956;123:309–14.
6. Bhat MA, Prasad K, Trivedi D, Rajeev BR, Battur H. Pyruvic acid levels in serum and saliva: a new course for oral cancer screening? J Oral Maxillofac Pathol. 2016;20:102–5.
7. Zheng D, Liwinski T, Elinav E. Interaction between microbiota and immunity in health and disease. Cell Res. 2020;30:492–506.
8. Belkaid Y, Hand T. Role of the microbiota in immunity and inflammation. Cell. 2014;157:121–41.
9. Zheng JH, Min J-J. Targeted cancer therapy using engineered *Salmonella typhimurium*. Chonnam Med J. 2016;52:173–84.

10. Forbes NS. Engineering the perfect (bacterial) cancer therapy. Nat Rev Cancer. 2010;10:785–94.
11. Danino T, Prindle A, Kwong GA, Skalak M, Li H, Allen K, et al. Programmable probiotics for detection of cancer in urine. Sci Transl Med. 2015;7:289ra84.
12. Panteli JT, Forkus BA, Van Dessel N, Forbes NS. Genetically modified bacteria as a tool to detect microscopic solid tumor masses with triggered release of a recombinant biomarker. Integr Biol (Camb). 2015;7:423–34.
13. Kruis W, Frič P, Pokrotnieks J, Lukás M, Fixa B, Kascák M, et al. Maintaining remission of ulcerative colitis with the probiotic *Escherichia coli* Nissle 1917 is as effective as with standard mesalazine. Gut. 2004;53:1617–23.
14. Haukioja A, Loimaranta V, Tenovuo J. Probiotic bacteria affect the composition of salivary pellicle and streptococcal adhesion *in vitro*. Oral Microbiol Immunol. 2008;23:336–43.
15. Hoshino T, Kawaguchi M, Shimizu N, Hoshino N, Ooshima T, Fujiwara T. PCR detection and identification of oral streptococci in saliva samples using GTF genes. Diagn Microbiol Infect Dis. 2004;48:195–99.
16. Meurman JH, Antila H, Salminen S. Recovery of *Lactobacillus* strain GG (ATCC 53103) from saliva of healthy volunteers after consumption of yoghurt prepared with the Bacterium. Microb Ecol Health Dis. 1994;7:295–98.
17. Tominaga T. Rapid detection of coliform bacteria using a lateral flow test strip assay. J Microbiol Methods. 2019 160:29–35.
18. Gustafson KT, Huynh KT, Heineck D, Bueno J, Modestino A, Kim S, et al. Automated fluorescence quantification of extracellular vesicles collected from blood plasma using dielectrophoresis. Lab Chip. 2021;21:1318–32.
19. Ibsen S, Sonnenberg A, Schutt C, Mukthavaram R, Yeh Y, Ortac I, et al. Recovery of drug delivery nanoparticles from human plasma using an electrokinetic platform technology. Small. 2015;11:5088–96.
20. Ibsen SD, Wright J, Lewis JM, Kim S, Ko SY, Ong J, et al. Rapid isolation and detection of exosomes and associated biomarkers from plasma. ACS Nano. 2017;11:6641–51.
21. Sarno B, Heineck D, Heller MJ, Ibsen SD. Dielectrophoresis: developments and applications from 2010 to 2020. Electrophoresis. 2021;42:539–64.
22. Hinestrosa JP, Searson DJ, Lewis JM, Kinana A, Perrera O, Dobrovolskaia I, et al. Simultaneous isolation of circulating nucleic acids and EV-associated Protein biomarkers from unprocessed plasma using an AC electrokinentics-based platform. Front Bioeng Biotechnol. 2020;8:581157.
23. Pethig R. Dielectrophoresis: theory, methodology and biological applications. Chichester: John Wiley & Sons; 2017.
24. Fernandez RE, Rohani A, Farmehini V, Swami NS. Review: microbial analysis in dielectrophoretic microfluidic systems. Anal Chim Acta. 2017;966:11–33.
25. Lewis JM, Vyas AD, Qiu Y, Messer KS, White R, Heller MJ. Integrated analysis of exosomal Protein biomarkers on alternating current electrokinetic chips enables rapid detection of pancreatic cancer in patient blood. ACS Nano. 2018;12:3311–20.
26. Suehiro J, Yatsunami R, Hamada R, Hara M. Quantitative estimation of biological cell concentration suspended in aqueous medium by using dielectrophoretic impedance measurement method. J Phys D: Appl Phys. 1999;32:2814–20.
27. Wang H-C, Nguyen N-V, Lin R-Y, Jen C-P. Characterizing esophageal cancerous cells at different stages using the dielectrophoretic impedance measurement method in a microchip. Sensors. 2017;17:E1053.
28. Graham KA, Mulhall HJ, Labeed FH, Lewis MP, Hoettges KF, Kalavrezos N, et al. A dielectrophoretic method of discrimination between normal oral epithelium, and oral and oropharyngeal cancer in a clinical setting. Analyst. 2015;140:5198–204.
29. Shah SS, Senapati S, Klacsmann F, Miller DL, Johnson JJ, Chang HC, et al. Current technologies and recent developments for screening of HPV-associated cervical and oropharyngeal cancers. Cancers (Basel). 2016;8:E85.
30. Chiu T-K, Chou W-P, Huang S-B, Wang HM, Lin YC, Hsieh CH, et al. Application of optically-induced-dielectrophoresis in microfluidic system for purification of circulating tumour cells for gene expression analysis- cancer cell line model. Sci Rep. 2016;6:32851.
31. Sambrook J, Russell DW. Molecular cloning: a laboratory manual. New York: Cold Spring Harbor Laboratory Press; 2001.
32. Aguilera L, Campos E, Giménez R, Badía J, Aguilar J, Baldoma L. Dual role of LldR in regulation of the lldPRD operon, involved in l-lactate metabolism in *Escherichia coli*. J Bacteriol. 2008;190:2997–3005.
33. Kelly JR, Rubin AJ, Davis JH, Ajo-Franklin CM, Cumbers J, Czar MJ, et al. Measuring the activity of BioBrick promoters using an *in vivo* reference standard. J Biol Eng. 2009;3:4.
34. Chien T, Harimoto T, Kepecs B, Gray K, Coker C, Hou N, et al. Enhancing the tropism of bacteria via genetically programmed biosensors. Nat Biomed Eng. 2022;6:94–104.
35. Goers L, Ainsworth C, Goey CH, Kontoravdi C, Freemont PS, Polizzi KM. Whole-cell *Escherichia coli* lactate biosensor for monitoring mammalian cell cultures during biopharmaceutical production. Biotechnol Bioeng. 2017;114:1290–300.
36. Kaur J, Jacobs R. Combination of autofluorescence imaging and salivary protoporphyrin in oral precancerous and cancerous lesions: non-invasive tools. J Clin Exp Dent. 2015;7:e187–91.
37. Luna R, Heineck DP, Bucher E, Heiser L, Ibsen SD. Theoretical and experimental analysis of negative dielectrophoresis-induced particle trajectories. Electrophoresis. 2022;43:1366–77.

SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: Hamilton S, Shea D, Ibsen S, Brasino M. On-chip dielectrophoretic recovery and detection of a lactate sensing probiotic from model human saliva. Electrophoresis. 2023;44:442–449.
https://doi.org/10.1002/elps.202200214