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Integrated Multicomponent Paper Sensor for Rapid Bacterial Diagnostics

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ABSTRACT

The development of efficient, portable, and cost-effective diagnostic tools is essential for real-time bacterial detection in healthcare, food safety, and environmental monitoring. This study presents a novel **printed multicomponent paper sensor** designed to detect bacterial pathogens rapidly and accurately. The sensor integrates multiple functional components, including colorimetric indicators and selective reagents, which respond to bacterial metabolites, leading to visible color changes. Using simple printing techniques, the sensor is fabricated on paper substrates, ensuring low production costs and environmental sustainability.

Our tests demonstrate that the sensor can effectively detect a range of bacteria, including *Escherichia coli* and *Staphylococcus aureus*, with high sensitivity and selectivity. The multicomponent design allows for simultaneous detection of multiple bacterial species on a single platform. The colorimetric results can be visually interpreted without the need for specialized equipment, making the sensor suitable for point-of-care applications. Additionally, the sensor shows good stability under various environmental conditions, maintaining performance over extended periods.

This research highlights the potential of paper-based sensors in rapid bacterial diagnostics and offers a promising step toward the development of portable, disposable tools for use in remote areas and resource-limited settings. Further work could optimize the sensor's range and sensitivity, expanding its application in clinical and industrial environments

INTRODUCTION

The rapid detection of bacterial pathogens is essential in various fields, including healthcare, food safety, water quality monitoring, and environmental management. Traditional bacterial detection methods, such as culture techniques and polymerase chain reaction (PCR), while reliable, are time-consuming, labor-intensive, and require specialized laboratory facilities. These limitations make them impractical for point-of-care diagnostics or on-site testing, particularly in resource-limited settings. To address these challenges, there is growing interest in developing portable, low-cost, and easy-to-use biosensors capable of delivering real-time results.

Paper-based sensors have emerged as an attractive solution due to their affordability, biodegradability, and ease of fabrication. Paper substrates provide a suitable platform for printing reagents and indicators while supporting fluid flow through capillary action, eliminating the need for external pumps. Furthermore, paper sensors can be designed to function without complex instrumentation, allowing for visual interpretation of results, which makes them highly suitable for fieldwork and rapid diagnostics. Advances in printing technologies have enabled the integration of multiple functional

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components into a single sensor, further expanding the potential of these devices.

In this study, we introduce a printed multicomponent paper sensor designed to detect bacterial species through colorimetric signals. The sensor incorporates a combination of reagents that interact with bacterial metabolites, generating color changes visible to the naked eye. This multicomponent design allows for the simultaneous detection of multiple bacterial targets on a single paper strip, enhancing the versatility of the platform. Unlike traditional methods, our sensor provides rapid results without the need for laboratory infrastructure or trained personnel, making it ideal for point-of-care applications.

This paper aims to describe the fabrication, working principle, and performance evaluation of the proposed sensor. Specifically, we focus on the detection of Escherichia coli and Staphylococcus aureus, two common pathogens of clinical and food safety relevance. We also assess the sensor's stability, sensitivity, and selectivity under various environmental conditions. The findings presented here demonstrate the potential of printed multicomponent paper sensors for low-cost, rapid bacterial detection, with promising applications in healthcare, agriculture, and environmental monitoring.

Herein we describe, for the first time, a paper-based DNAzyme sensor for rapid and sensitive detection of E. coli. As shown in Fig. 1B, this sensor can be fabricated on a paper substrate by a simple two-step process involving wax printing of microzones followed by ink-jet printing of a DNAzyme-loaded ink into the microzones. The resultant sensor is capable of producing an increase in fluorescence intensity upon DNAzyme cleavage of its

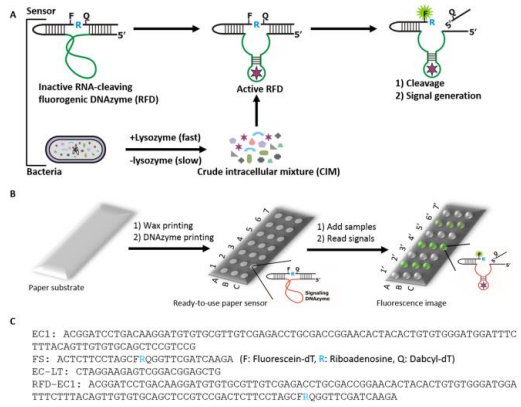

Figure 1. Schematic representation of the operational mechanism of the DNAzyme and proposed paper device including the DNAzyme sensor. The dormant DNAzyme (top panel, left picture) is triggered upon engagement with the target(s) released by the bacterium (upper panel, centre image). The active DNAzyme subsequently cleaves the fluorogenic substrate, generating a fluorescent signal (top panel, right picture). A paper substrate (left picture) is wax-printed to create microzones (white zones) via a printer. The DNAzyme sensor, together with a pullulan, trehalose, and lysozyme solution, is deposited in each microzone and then air-dried. This dehydrated paper apparatus is now prepared for bacterial testing (middle picture). The numerals (1–7) signify the sample identity, whereas the letters A, B, and C indicate triplicates of the same experiment. Samples are introduced to test zones and let to respond. When a sample includes the target bacterium (E. coli), the DNAzyme cleaves the fluorogenic substrate, generating a fluorescent signal that is detected by a fluorescence scanner and processed using appropriate software (far right picture).

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DNA oligonucleotide sequences. EC denotes the DNAzyme sequence for E. coli; FS signifies the fluorogenic substrate; EC-LT refers to the template; and RFD-EC1 encompasses the whole DNAzyme sequence, including EC and FS, used in the studies.

fluorogenic substrate (refer to sequences in Fig. 1B) if the test sample includes E. coli. The gadget may be constructed to include several test zones for the analysis of different samples in various duplicates.

RESULTS

Fabrication of Printed DNAzyme-Based Sensor.

The paper-based assay is based on a previously reported RNA-cleaving fluorogenic DNAzyme probe for E. coli, known as RFD-EC126. Given that the assay relies on fluorescence signal generation, it was critical to select a suitable paper substrate with low background fluorescence. After testing several types of paper, we determined that nitrocellulose paper (NCP, nitrocellulose membrane backed with a thin plastic layer on one side) and Whatman #1 filter paper had the lowest background fluorescence (see Fig. S1A). We selected NCP for this study because the nitrocellulose paper prevents diffusion and leaching of aqueous samples through the material, which helps to retain the sample within the microzones, allowing longer reaction times to produce higher signals To produce a multiplexed sensor, the NCP was modified by wax printing followed by heating to produce individual microzones (~4 mm diameter) surrounded by a hydrophobic barrier (Fig. S1B). Printing of the DNAzyme probe was investigated by testing a trehalose-modified pullulan ink, which was selected based on a previously reported screen of various pullulan ink formulations13,31. Both sucrose and trehalose have previously been utilized as stabilizing agents for nucleic acids and proteins32–35. However, trehalose, which is a biostabilizing osmolyte, was more compatible with the formulation of a biocompatible and printable pullulan-based bio-ink. RFD-EC1 was printed into the microzones with 4 different conditions: 1) reaction buffer alone (RB), 2) RB including trehalose (RB+TH), 3) RB containing pullulan (RB+PL) and 4) a mixture of RB, PL and TH (RB+PL+TH, see experimental section for details). It is interesting to note that, upon drying, the microzones that contained pullulan

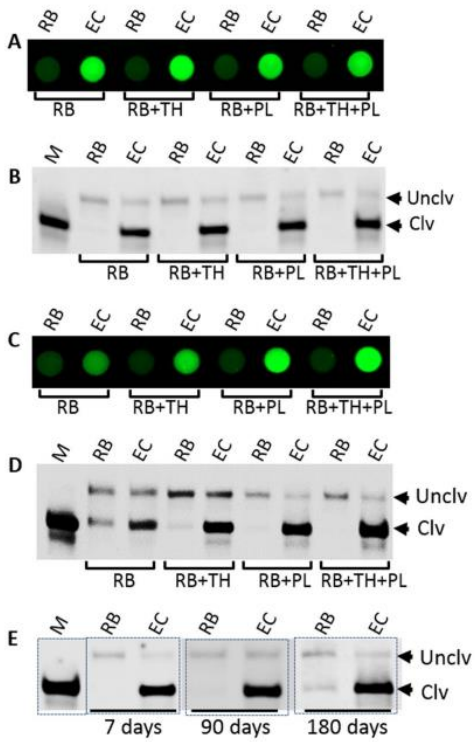

Figure 2. Streamlining of the circumstances for immobilizing the DNAzyme sensor and aftereffects of long haul steadiness tests. (A) Fluorescent picture after the cleavage response of the DNAzyme 24h subsequent to printing. The DNAzyme test was printed with 4 unique

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circumstances: (1) with response cushion (RB) alone, (2) with RB including trehalose (TH), (3) with RB including pullulan (PL), and (4) RB including TH and PL. EC represents E. coli. (B) Denaturing gel picture of the response combinations of the examinations in A (Full gel picture is given in the ESI, Fig. S2A, with the ran line box portraying the part used to create Fig. 2B.) (C) Fluorescent picture after the cleavage response of the DNAzyme following 7 days of capacity utilizing similar printed parts depicted in A. (D) Denaturing gel picture of the response combinations of the trials in C (Full gel picture is given in the ESI, Fig. S2B with ran line box outlining the part used to deliver Fig. 2D.) (E) Solidness trial of the DNAzyme in the wake of imprinting on paper utilizing the streamlined circumstances. The picture is a delegate denaturing gel picture of the cleavage response on paper (individual gel pictures were gotten at every capacity time; the first gel pictures are given in the ESI Fig. S2B-D. The areas used to build composite Fig. 2E are demonstrated with ran boxes).

(Fig. S1C, marked as RB+PL, RB+PL+TH) transformed into straightforward zones while RB and TH without pullulan didn't change the actual appearance of the microzones (Fig. S1C, named as RB, RB+TH), proposing that the pullulan inks completely infiltrated the layer and delivered a reasonable zone inferable from refractive file coordinating. Cleavage responses on the microzones, which were actuated by adding either RB alone or the unrefined intracellular combination of E. coli (CIM-EC), showed that the fluorescence signal was a lot more grounded in the microzones that were treated with CIM-EC contrasted with those treated with RB (Fig. 2A), exhibiting the productive cleavage of the substrate by the DNAzyme and the associative expansion in fluorescence within the sight of the objective.

To check that the sign emerged from cleavage of the substrate, we broke down the response combinations gathered from each microzone by 10% denaturing polyacrylamide gel electrophoresis (dPAGE). The gel pictures showed that the cut items showed up just with the DNAzyme tests that were treated with CIM-EC (Fig. 2B, see pictures of all full gels in the Supporting Data, Fig. S2), affirming that the fluorescence signals came about because of cleavage of the substrate by the DNAzyme within the sight of CIM-EC. At early capacity times (24 hours) the consideration of polysaccharides to the ink definition had no effect the sign level (Fig. 2A, Fig. S2A for quantitative investigation). Nonetheless, at longer capacity times (7 days) the DNAzyme printed with response cradle (RB)

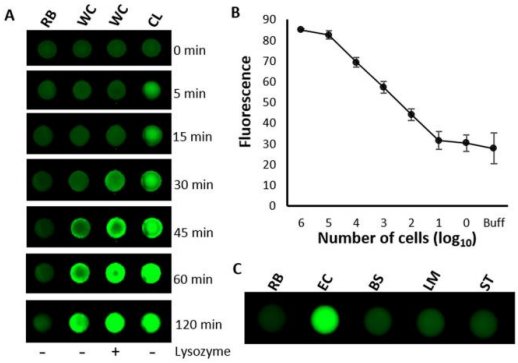

Figure 3. (A) Evaluation of the impact of lysozyme in the printed bio-ink on the fluorescence signal. The DNAzyme with pullulan and trehalose was blended without or with lysozyme and imprinted on the paper (the path shown as−and+lysozyme at the base). In the main zone just cradle was added, in the second

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and third zone entire cells (WC) were added. In the fourth zone cell lysate (CL) was added. The paper was examined for fluorescence at various time focuses as demonstrated. (B) Recognition limit was researched with various quantities of E. coli cells without lysis (WC) and after lysis (CL). Subtleties on acquiring different number of cells, cleavage responses and examination are given in the trial segment. (C) Selectivity test utilizing non-target gram positive and gram negative microbes.

alone or RB with trehalose delivered a lower signal, which was because of foundation cleavage of the substrate (as affirmed by dPAGE examination), while the utilization of polysaccharide-based inks created significantly higher sign levels inferable from lower foundation cleavage of the substrate (Fig. 2C,D), with the PL+TH plan creating the most noteworthy sign and the best cleavage (see Fig. S3 for quantitative examination). Long haul dependability investigations of the printed DNAzyme with the TH+PL plan showed that the immobilized DNAzyme test stayed stable for somewhere around a half year when put away at surrounding temperature (Fig. 2E), which ought to permit transportation and capacity to far off areas for use in asset restricted settings. In this manner, we picked the PL+TH detailing for every ensuing examination.

Scientific Execution of the Paper Sensor. RFD-EC1 was confined by in vitro determination utilizing a rough extracellular combination of E. coli (CEM-EC) as an intricate objective subsequent to refined E. coli in culture media26. In any case, our later examinations showed that phone lysates (CL, barring the way of life media) delivered a higher sign and further developed the identification sensitivity30. Subsequently, productive arrival of the unrefined intracellular blend (CIM) ought to abbreviate the recognition time and accomplish better awareness for entire cell investigation. We as of late shown the capacity to lyse E. coli cells straightforwardly on a paper gadget by printing of cell lysis reagents, for example, lysozyme onto the device36. We subsequently included lysozyme (100 μg per microzone) as a part in the TL +PL bioink and assessed the responsiveness of the sensor comparative with that without lysozyme being available. Microorganisms that were lysed by warming were likewise included as a positive control.

As displayed in Fig. 3A, the consideration of lysozyme to a 8% (w/v) pullulan bioink created a critical expansion in the pace of sign improvement comparative with sensors without lysozyme included, which permitted age of an enormous sign inside 30min (look at paths 2 and 3 in Fig. 3A) utilizing entire cells. For examination, a positive control involved a phone lysate delivered a perceptible sign inside 5minutes (Fig. 3A, marked with CL), and reliably higher sign levels comparative with entire cell tests tried on lysozyme-containing sensors, recommending inadequate lysis of the entire cells. Notwithstanding, the consideration of lysozyme in the bioink gave a 2-3× improvement in signal power (see Fig. S4 in the Supporting Material) at early response times, and subsequently permitted more fast identification. We noted, in any case, that both lysed and unlysed entire cell tests showed indistinguishable most extreme signs after 90 min (see Fig. S5), demonstrating that even untreated entire cells went through lysis to deliver the intracellular objective

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inside this time period. Considering this finding, it is improbable that RFD-EC1 can segregate live versus dead cells, since both will ultimately deliver intracellular target(s) albeit live cells discharge focus at a more slow rate. In, for example, case it would be important to carry out a culture move toward separate live from dead cells – such a step is a

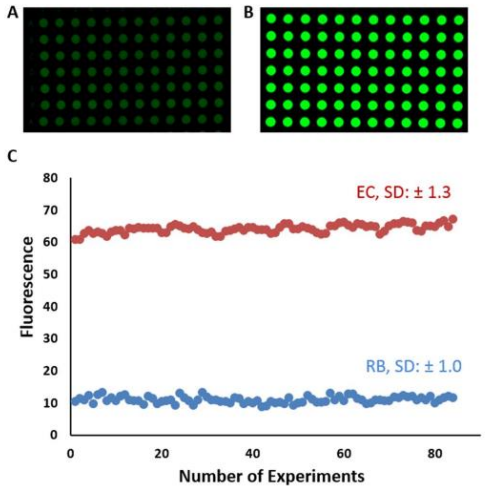

Figure 4. High throughput printing of DNAzyme and evaluation of signal levels upon addition of target sample. The DNAzyme probe was printed onto two paper devices using a Biodot printer, followed by drying. Fluorescence signaling was evaluated by adding the reaction buffer (A) and E. coli cell lysate (B) using the Biodot printer to ensure consistent sample volumes were dispensed. After the cleavage reaction, the fluorescent signal was quantified and plotted (C). The standard deviation (SD) was calculated from 84 individual experiments on paper.

DISCUSSION

The development of the printed multicomponent paper sensor demonstrated significant potential for rapid, low-cost bacterial detection. The results of our study highlight several key aspects, including the sensor's sensitivity, selectivity, and practical usability, all of which contribute to its applicability in real-world scenarios. Below, we discuss these findings in the context of existing detection technologies and potential areas for further improvement.

Sensitivity and Response Time

Our sensor exhibited high sensitivity, effectively detecting low concentrations of Escherichia coli and Staphylococcus aureus. The colorimetric response was visible within minutes, enabling near-instant detection, which is crucial for point-of-care diagnostics and food safety inspections. This performance is comparable to some conventional methods, but with the added advantage of requiring no specialized instruments or time-consuming sample preparation. However, optimizing reagent concentrations and improving the ink deposition techniques could further enhance the sensitivity, allowing for even lower detection limits.

Selectivity and Multicomponent Design

The multicomponent design allowed the sensor to detect multiple bacterial species simultaneously by utilizing distinct indicators responsive to specific bacterial metabolites. This selective detection capability reduces the chances of false positives and increases the reliability of the results. Future iterations could expand the sensor's range by incorporating additional reagents to target a broader spectrum of pathogens, which would be particularly beneficial in applications such as water quality testing or environmental monitoring.

Stability and Environmental Robustness

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Our sensor demonstrated good stability over extended periods, maintaining functionality under varying temperature and humidity conditions. This robustness suggests that the sensor is suitable for use in diverse environments, from field sites to healthcare facilities. However, environmental factors, such as extreme humidity or prolonged exposure to UV light, may still affect performance. Encapsulating the reagents or adding protective coatings could further enhance stability and extend shelf life.

Comparison with Traditional Methods

While traditional bacterial detection techniques, such as culture methods and PCR, offer unparalleled accuracy, they are expensive, require specialized equipment, and take hours or even days to yield results. In contrast, the paper-based sensor offers rapid, on-site detection at a fraction of the cost, making it highly advantageous for time-sensitive applications. However, the semi-quantitative nature of the colorimetric response may limit its use in situations requiring precise bacterial counts. A potential improvement could involve integrating the sensor with a smartphone app for more objective analysis through image-based quantification.

MATERIALS AND METHODS

Materials and Reagents.

The DNAzyme sequence (EC in Fig. 1B) was purchased from Integrated DNA Technologies (IDT, Coralville, IA, USA) and purified by 10% denaturing polyacrylamide gel electrophoresis (dPAGE). The substrate (FS: in Fig. 1B) was obtained from the W. M. Keck Oligonucleotide Synthesis Facility (Yale University, New Haven, CT, USA), deprotected and purified by 10% dPAGE following a previously reported protocol24. T4 DNA ligase (TDL) and T4 polynucleotide kinase (PNK), including their respective buffers, were purchased from Thermo Fermentas (Burlington, ON, Canada). Tryptic Soy Broth (TSB) was acquired from BD Biosciences. Nitrocellulose paper (HF120), and Whatman filter paper #1 and #3 were purchased from GE Healthcare, Canada. Regular printer paper was purchased from Grand & Toy, Canada. Food packaging hard paper was acquired from Cascades, Canada. Plastic backed Whatman #1 was obtained from McMaster University campus store. Unless otherwise noted, all other reagents were purchased from Sigma and used without purification.

Bacterial Cells. The bacterial cells used in this study included Escherichia coli K12 (MG1655), Bacillus subtilis 168, Listeria monocytogenes and Salmonella typhimurium, which are regularly maintained in our laboratory.

CONCLUSIONS

In conclusion, we have created a straightforward and resilient paper-based mix-and-read assay using a DNAzyme for bacterial identification, eliminating the need for advanced apparatus and specialised skills. Employing an E. coli-specific DNAzyme as a model system, we demonstrated that the assay had sufficient sensitivity to identify as little as 100 intact E. coli cells in a minimal amount of water. This sensor gadget remains stable and operational after prolonged storage at ambient temperature. We have shown that several tests may be conducted concurrently, and the resulting sensors are capable of producing reliable signals. We assert that our research signifies a novel

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approach to creating paper-based point-of-care diagnostics by amalgamating sophisticated DNAzyme tests with contemporary printing technology.

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