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Portable Pathogen Diagnostics Using Microfluidic Cartridges Made from Continuous Liquid Interface Production Additive Manufacturing

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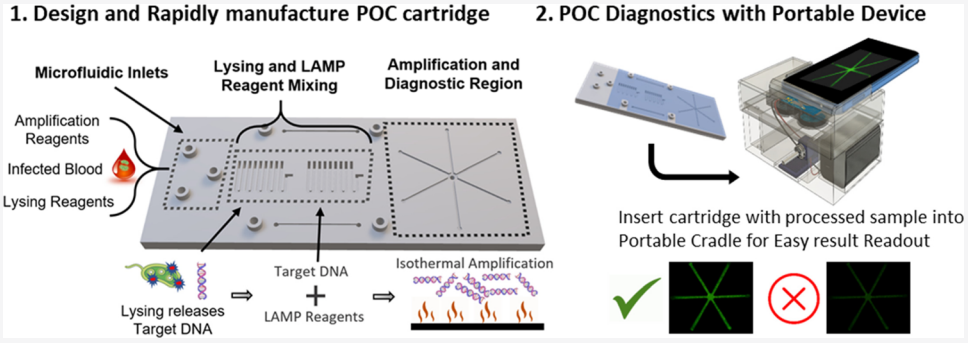

ABSTRACT: Biomedical diagnostics based on microfluidic devices have the potential to significantly benefit human health; however, the manufacturing of microfluidic devices is a key limitation to their widespread adoption. Outbreaks of infectious disease continue to demonstrate the need for simple, sensitive, and translatable tests for point-of-care use. Additive manufacturing (AM) is an attractive alternative to conventional approaches for microfluidic device manufacturing based on injection molding; however, there is a need for development and validation of new AM process capabilities and materials that are compatible with microfluidic diagnostics. In this paper, we demonstrate the development and characterization of AM cartridges using continuous liquid interface production (CLIP) and investigate process characteristics and capabilities of the AM microfluidic device manufacturing. We find that CLIP accurately produces microfluidic channels as small as 400 μm and that it is possible to routinely produce fluid channels as small as 100 μm with high repeatability. We also developed a loop-mediated isothermal amplification (LAMP) assay for detection of *E. coli* from whole blood directly on the CLIP-based AM microfluidic cartridges, with a 50 cfu/μL limit of detection, validating the use of CLIP processes and materials for pathogen detection. The portable diagnostic platform presented in this paper could be used to investigate and validate other AM processes for microfluidic diagnostics and could be an important component of scaling up the diagnostics for current and future infectious diseases and pandemics.

1. INTRODUCTION

Portable biomedical diagnostics based on the processing of small fluid samples offer the potential for rapid identification of pathogens, diagnosis of disease, or identification of a medical condition.[1] Typically, microfluidic diagnostic systems are developed in a laboratory environment where prototype cartridges are fabricated from silicon, silicone or other polymers, and glass. The translation of microfluidic diagnostics for application and deployment requires manufacturing scale up in which the cartridge is manufactured from a polymer, usually made with injection molding. Typically, the development and scale up of injection molding manufacturing of microfluidic devices requires an investment of several years and tens of millions of dollars.[2] Additive manufacturing (AM) can in some situations overcome these obstacles because it requires no tooling. Furthermore, some modern AM processes such as continuous liquid interface production (CLIP)[3] have speed and cost that is comparable to injection molding.

Many disease outbreaks initiated from community transmission spread quickly due to globalization, hence the important need for diagnostic technologies that can both serve as a monitoring system and respond to exponential rates of transmission. This can also lead to an improvement in community health outcomes especially in low-resource

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communities.[4] Additionally, many infectious diseases are critically important in the developing world and play a major role in both diagnostic demand and subsequent burden on healthcare from insufficient monitoring.[5]

For over 20 years, polymerase chain reaction (PCR) and nucleic acid amplification technologies have been the gold standard for diagnosing and identifying the presence of pathogens.[6] First described in 2000 as an alternative to PCR, loop-mediated isothermal amplification (LAMP) was reported by Notomi et al.[7] as a method for nucleic acid amplification. LAMP amplification uses a one-step amplification reaction with high specificity and sensitivity comparable to traditional PCR for target amplification.[7,8] Moreover, LAMP is robust against inhibitors, indicating a strong use-case when processing directly from crude samples without purification.[9,10] LAMP reactions can also be adapted for viral RNA detection with the addition of a heat-stable reverse transcriptase (RT-LAMP).[11,12]

The threat of increases in antimicrobial resistance (AMR) for global health and economic burden suggests a pressing need for developing technologies to detect bacterial pathogens. By 2050, research suggests that AMR will be responsible for up to 10 million deaths each year.[13] The World Health Organization (WHO) began a global plan for addressing AMR, and their report suggests reduction of the misdiagnosis and over prescription of antibiotics, and diagnosis should depend upon rapid and low cost diagnostic tests performed into point-of-care (POC) settings.[14] While some reports have demonstrated detection of bacterial pathogens in a POC system using LAMP, several barriers of these platforms for adequate translation include sample processing prior to on-chip amplification, off-chip amplification prior to POC device read out, or using purified target samples.[15−18]

Several promising research articles demonstrate potential to use AM for POC microfluidic diagnostics.[1,19−23] AM enables novel three-dimensional (3D) geometries for miniature fluidic diagnostics that may provide enhanced functions or new fluid architectures based on 3D fluid channel geometries that can lead to compact systems.[23−26] Most previous publications on AM microfluidics focus on the fabrication of disposable polymer cartridges that would be directly used for the diagnostic, made by stereolithography,[19,23] filament-based deposition,[27,28] or novel processes and materials developed for application to miniature fluidic diagnostics.[29−31] A key issue explored in most publications is whether the AM polymer is compatible with diagnostic chemistries and processes because the chemical processes used for diagnostics can often be very sensitive to the choice of material for the polymer cartridge.[32,33] However, the research literature mostly focuses on AM processes that are slow and not well suited to production scale up. There is a need for research into AM microfluidics that are based on faster and more highly scalable AM processes such as CLIP.[3] Of particular importance are the process resolution, accuracy, and speed as applied to microfluidic diagnostics,[22,34] as well as materials' compatibility with diagnostic chemistries. To our knowledge, there has been no publication that investigates these critical issues for CLIP-based microfluidic diagnostics.

This paper develops a LAMP assay for translation on a CLIP-based AM diagnostic cartridge. We investigate the use of CLIP AM microfluidic diagnostics by characterizing resolution, accuracy, speed, and materials' compatibility with diagnostic chemistries. We first report the validation of a LAMP assay for *E. coli* detection from whole blood. We then investigate manufacturing process characterization for microfluidic geometries. We conclude by demonstrating pathogen detection using the developed assay on an AM microfluidic cartridge in a portable detection system.

2. EXPERIMENTAL SECTION

2.1. DNA and Bacterial Culture Sample Preparation. Pathogen strain for *E. coli*, genomic DNA of *E. coli* (O157:H7) and methicillin-resistant *Staphylococcus aureus* (*MRSA*) strain HFH-30106, NR-10320, and *Staphylococcus aureus* (MSSA), Strain MN8, HM-162D were obtained from BEI Resources. Details for sample preparation and pathogen stocks, which were prepared based on a reported protocol,[35] are available in the Supplementary Material.

2.2. LAMP Reaction Composition. Primer sequences for the LAMP reactions were synthesized by Integrated DNA Technologies. LAMP assays for both off-chip characterization and on-chip experiments consisted of 1 × final concentration of the isothermal amplification buffer (New England Biolabs), 1.4 mmol/L each of deoxyribonucleoside triphosphates, 10 mmol/L of MgSO4 (New England Biolabs), 0.4 mol/L of Betaine (Sigma-Aldrich), and 1 mg/mL bovine serum albumin (New England Biolabs). These reagents were prepared from stocks and stored at −20 °C between experiments.

In addition to the buffer components, 3 μL of primer mix consisting of 0.2 μM F3 and B3, 1.6 μM forward inner primer and backward inner primer, and 0.8 μM LoopF and LoopB, 0.96 U/μL Bst 2.0 WarmStartDNA Polymerase (New England Biolabs) were used. Then, 25 μM EvaGreen (Biotium) or Syto 9 (ThermoFisher) was included in thermocycler or cartridge assays, respectively. Molecular-grade (DNase-free, RNase-free) biology water (0.3 μL) was added to create a 25 μL final reaction volume.

The final reaction solution consisted 8 μL of target sample and 17 μL of LAMP amplification reagents. Samples spiked with whole pathogen are reported as with pathogen concentrations prior to lysing. For reactions on lysed samples, after lysing is performed in a 1:3 ratio, the final reaction solution consisted 8 μL of lysed target sample (2 μL of spiked target and 6 μL of lysing buffer) and 17 μL of LAMP amplification reagents. The composition of lysing buffers used is presented in the Supplementary Information.

2.3. Reaction Platforms. All the off-chip LAMP assay characterization was carried out in 0.2 mL PCR reaction tubes in a QuantStudio 3 real-time PCR system. The tubes were incubated at 65 °C for 50 min, and fluorescence data were recorded every 1 min. For on cartridge experimentation, the spiked whole blood sample, lysing buffer, and LAMP reagents were loaded into 1 mL syringes and flown using syringe pumps (Harvard Apparatus) at constant flow rates of 2, 6, and 17 μL/min, respectively. After processing and loading the sample, the diagnostic cartridge was sealed with adhesive to prevent leakage and evaporation during amplification. The cartridge is placed on a heating element for 50 min at 65 °C. The reaction occurs directly on-chip, and detection was performed in the reported portable detection system.

2.4. CLIP-Based AM Cartridge Fabrication. The microfluidic cartridges were fabricated from RPU 70 using a Carbon M2 printer.[36] The main criteria for material selection were accuracy and fidelity to repeatably produce the desired features; mechanical and chemical stability at the amplification temperature; and low water absorptivity. The print orientation was with the narrow edge of the part attached to the build tray,

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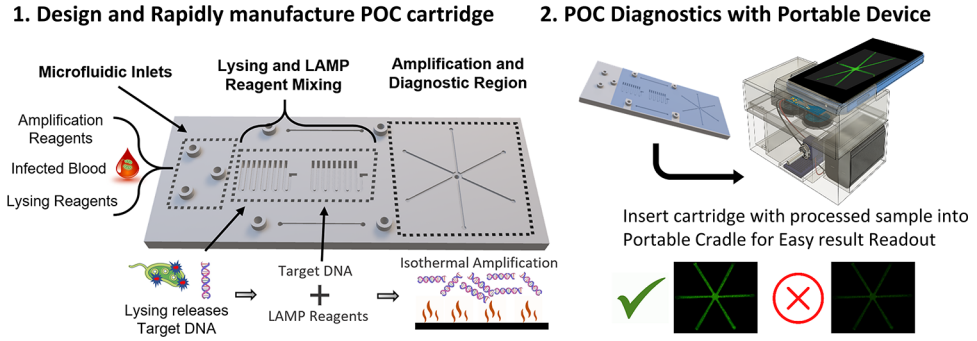

Figure 1. Concept for on-demand microfluidic devices for pathogen detection. (1) Pandemic/disease outbreak requires design and rapid manufacturing of the CLIP-based AM diagnostic cartridge for detection of pathogens. (2) Cartridge is inserted into a portable POC smartphone detection system for simple diagnostic readout.

the longest dimension of the part parallel to the build tray, and the large flat faces with microchannels facing orthogonal to the build tray. We were able to pack about 25 parts to be packed onto a single build tray, with ample room between parts for resin flow during printing and resin removal after printing. The print time using standard settings on the machine was ∼90 min. A detailed description of device dimensions and a diagram detailing the design regions for assay operation on the diagnostic cartridge are provided in the Supplementary Information (Figure S1).

2.5. Portable Diagnostic Module Fabrication. Our portable system for imaging the amplification performed on the cartridge is based on our previous studies, and a detailed explanation of the optical components and imaging conditions is available in the Supplementary Material.[8,37]

2.6. Image and Data Analysis. All on-chip fluorescence images were recorded using IP Webcam and PRO settings (1000ISO, 1 s exposure) on the smartphone and saved in JPG format. Fluorescence intensity and baseline fluorescence were analyzed on Image-J by measuring mean pixel intensity from the star channels or pie pools. The fluorescence intensity across each replicate was measured, averaged, and plotted on GraphPad Prism 7. All off-chip RT-LAMP data were analyzed and plotted in MatLab and GraphPad Prism 7. All amplification curves were fitted with a sigmoidal 5-point parameter model. The reported threshold times were defined by an amplification curve reaching 20% of its maximum intensity.

3. RESULTS

3.1. Overview of the Approach. Figure 1 shows the process flow and motivation for addressing the unmet need of rapidly produced diagnostic tests using CLIP-based AM cartridge fabrication. Exemplified by the 2019 corona virus pandemic, lack of diagnostic tests and contact tracing results in preventable healthcare costs and economic burden. In the case of a disease outbreak or temporary surge in diagnostic testing demand, manufacturing advantages of CLIP-based AM devices allow significantly reduced translation time compared to traditional lab-based microfluidic tests. An occurrence of diagnostic demand initiates the production of pathogen- and specimen-specific diagnostic cartridges. Maintaining a robust catalog of validated assays for various pathogen and specimen types enables readiness to a wide range of potential diagnostic demands. Small changes in primer design can allow the response to mutations and phenotypically similar pathogens while maintaining foundational processing techniques for different types of raw samples such as blood, urine, saliva, or nasal fluid.

We validated the assay development by detecting pathogens in whole blood samples that can be collected at the POC, processed, and tested on disposable cartridges with a compatible handheld cradle for test read out. Our cartridge contains three main components: microfluidic inlets, sample processing by microfluidic mixing, and an amplification and diagnostic region. The three microfluidic inlets introduce infected blood, lysing buffer, and LAMP amplification reagents. The LAMP reaction stability in the presence of inhibitors allows a chemical lysing step and removes the need for traditional purification techniques and macroscale instrumentation for processing. After lysing and lamp reagent mixing, the hands-free processed sample directly loads into the amplification and diagnostic region for isothermal amplification at 65 °C directly on-chip. Our portable detection system uses a traditional smartphone and compact optical instrumentation for fluorescence imaging, which can differentiate positive amplification vs a control sample in under 1 h.

3.2. Assay Development and Characterization. The first step toward amplifying and detecting the presence of *E. coli* in whole blood in the diagnostic platform required assay validation prior to cartridge translation. We targeted the malB gene in *E. coli* for amplification, which is present among the majority of pathogenic *E. coli* strains, using previously reported LAMP primers.[38] We characterized the developed LAMP assay off-chip using a standard benchtop thermocycler. First, the sensitivity and specificity of the primer set were tested on purified *E. coli* DNA in isolation and in the presence of off-target pathogen genomic contamination using MRSA- and MSSA-purified DNA (Figure S2). Several reported approaches have attempted to miniaturize lysis methods in microfluidics for molecular detection of pathogens, including ultrasonic,[39] mechanical,[40,41] thermal,[42] and chemical methods.[43,44] Ultrasonic and mechanical lysing of bacteria often requires various types of complex electronic instrumentation or the addition of beads, neither of which are compatible with microfluidics nor applications at the POC. Thermal lysis temperatures require 90 °C that are incompatible with LAMP amplification enzymes or require downstream purification of the target. Chemical lysis is ideal for our microfluidic application because of reagent stability and minimal modifications to system design.[43,45,46]

We then characterized and optimized a chemical lysis buffer. Detergents are known for their ability to break down biological membranes for interrogation of internal DNA.[47,48] We compared reported lysis formulas on threshold times using

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saponin,[49,50] ACK, SDS,[47,48] and Triton X-100.[46] The lysis buffer compositions are listed in the Supplementary Material. In a 1:3 ratio of sample to lysis buffer, a 1×10^3 cfu/μL *E. coli* sample was mixed with each lysis buffer before adding LAMP amplification reagents. The saponin formulas demonstrated a faster amplification threshold time of around 25 min, and Triton X and SDS had threshold times ~30 min, while all samples are amplified by 45 min. All lysis compositions maintained sensitivity with no false positive amplification among the controls (Figure 2a; Figure S3).

The saponin lysis formula was tested against an *E. coli* standard curve in buffer, and we achieved an LOD of 10 cfu/μL after 50 min of amplification (Figure S4). To test the specificity of our developed assay, the saponin lysis formula was tested against an *E. coli* standard curve in buffer containing 1×10^4 cfu/μL off-target bacterial contamination and an LOD of 10 cfu/μL after 50 min of amplification was maintained (Figure 2b; Figure S5). We then tested our assay against whole blood samples. Whole blood was spiked with the corresponding *E. coli* concentrations for a standard curve with both low (Figure S6) and high concentrations of off-target contamination and then lysed with the validated saponin-based lysis buffer. The raw fluorescence data were analyzed with a sigmoidal fit (Figure S7) to calculate the threshold times of the assay (Figure 2c). The assay achieved a sensitivity of 50 cfu/μL. The matrix effect from whole blood likely caused the drop in sensitivity compared to the validation experiments performed in buffer.

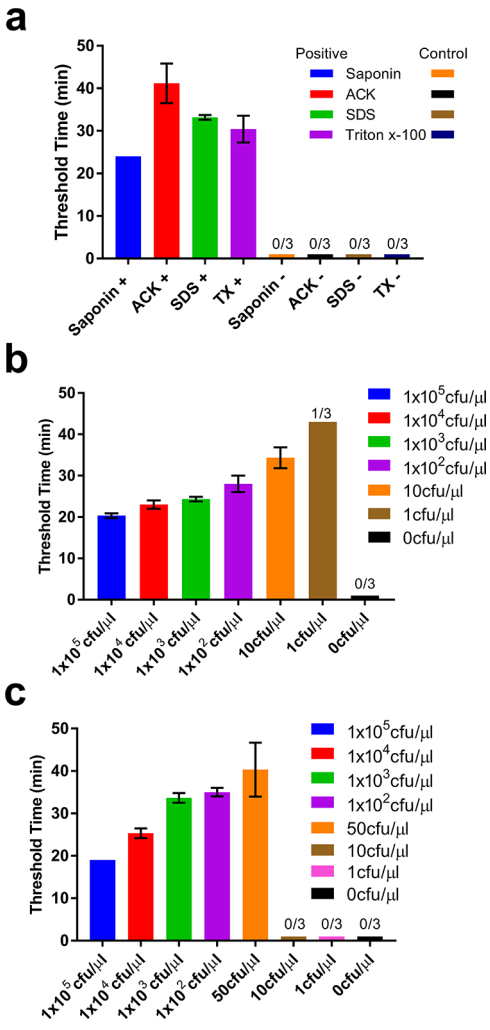

Figure 2. Thermocycler-based *E. coli* LAMP assay characterization. (A) Baseline-subtracted amplification threshold data for lysing composition characterization on (n = 3) samples of 1×10^3cfu/μL *E. coli* in buffer. (B) Baseline-subtracted amplification threshold data for the detection of *E. coli* using saponin-based lysing buffer with 1×10^4 cfu/μL off-target contamination in the sample for testing specificity. The assay maintains a detection limit of 10 cfu/μL with off-target contamination. (C) Baseline-subtracted amplification threshold data from amplification curves with sigmoidal fitting (n = 3) for the detection of *E. coli* in whole blood samples with 1×10^4 cfu/μL off-target contamination in the sample for testing specificity.

3.3. Manufacturing Characterization for CLIP-Based AM Microfluidics. There are trade-offs between manufacturability, print orientation, and build time.[51] We investigated the accuracy and resolution of CLIP for small fluid channels by measuring the 3D geometry of the devices using an Alicona InfinteFocus microscope. This scanner offers <100 nm vertical resolution and <1 μm lateral resolution.[52] We fabricated accuracy test parts with microchannels of widths varying over the range of 100 to 1000 μm and constant depth, as well as through holes with diameters ranging from 300 to 1000 μm. Six versions of this part were designed, with a thickness of either 2 or 3 mm and microchannel depth set to 400, 600, or 800 μm uniformly across the part (Figure S8).

An important parameter for AM is how the part is oriented during printing. We explored three different part orientations. The first orientation ("flat") configured the part with the large area surface with microchannels facing away from the build tray. The large area surface opposite from the microchannels was attached to the tray. The second orientation ("parallel) configured the part with its narrow edge attached to the tray and the large area surface with microchannels faced orthogonal to the tray, and the microchannels were oriented parallel to the surface of the tray. The third orientation ("orthogonal") configured the part with its narrow edge attached to the tray and the large area surface with microchannels faced orthogonal to the tray, and the microchannels were oriented orthogonal to the tray.

We investigated the effect of microchannel width and print orientation on microchannel depth for three different depths. We measured microchannel depth vs target microchannel width for target depths of 400, 600, and 800 μm (Figure S9). The microchannel width and the print orientation have a significant effect on the microchannel depth. The measured microchannel depth is consistently smaller than the target depth for widths <500 μm. For wider microchannels, the measured depth is less than the target depth by approximately 50−100 μm. For the smallest features, the resolution limits of the printer affect the ability to make small features. For larger features, however, the dimensions may be affected by inaccuracies in the build instructions within the printer software and postprocessing steps.

The process accuracy can be characterized as the difference from target (DFT), which is the difference between the measured and designed microchannel feature dimension. Figure S10a shows these results. For most features, width DFT is within ± 50 μm, which is approximately the expected accuracy of the CLIP process.[53] Figure S10b shows DFT for microchannel width for three different print orientations representing all depth levels. The summary of manufacturing parameters by print orientation can be found in Table S1.

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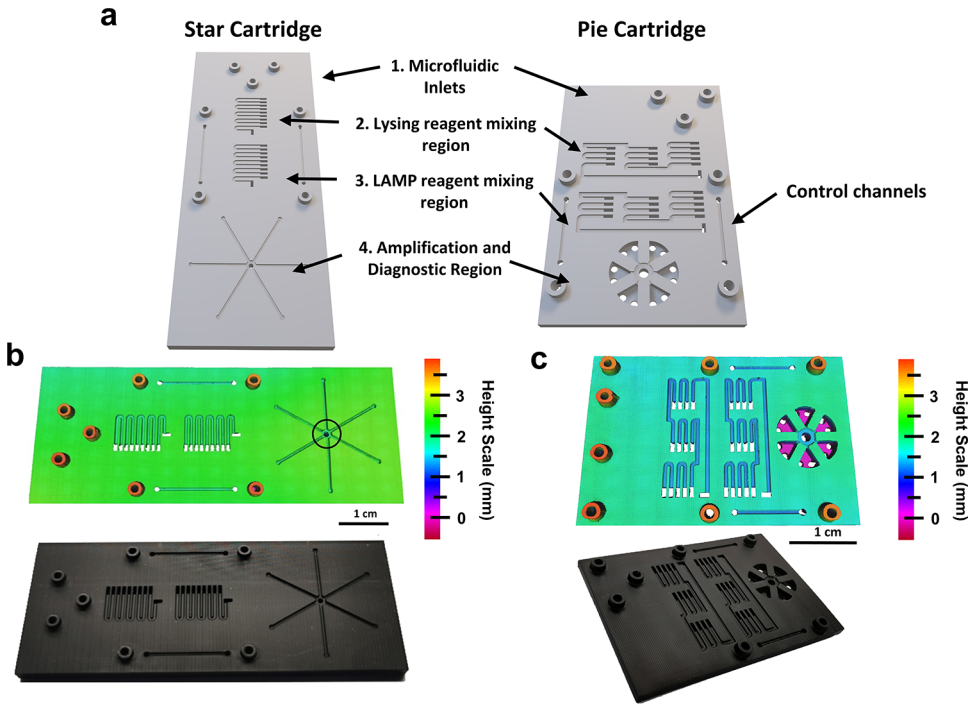

Figure 3. CLIP-based AM cartridge for portable pathogen diagnostics. (a) Assay operation on the star and pie diagnostic cartridges. Microfluidic inlet ports intrude the sample, lysing reagents, and LAMP amplification reagents into the device. The lysing reagent mixing region mixes the sample and lysing reagents before introduction to the LAMP reagent mixing region. The LAMP reagent mixing region mixes the lysed sample with final reaction components required for isothermal amplification of the target. The amplification and diagnostic region of the device distributes the final reaction mix among six regions, allowing for isothermal amplification and imaging of the reaction products directly on the device. (b,c) Three-dimensional scans and photographs of the star and pie diagnostic cartridges demonstrating printing resolution.

3.4. CLIP-Based AM Cartridge for Portable Pathogen Diagnostics. We designed and characterized two disposable diagnostic cartridges, with a star-cartridge or a pie-cartridge design, to address all the elements needed for whole blood processing and amplification based on the developed assay (Figure 3a). Both cartridges use three microfluidic inlets to mix and process the sample. First, infected blood is mixed with lysis buffer to free the pathogenic DNA for amplification. The lysed sample containing free *E. coli* DNA is mixed with LAMP amplification reagents. Both cartridges have 3D serpentine microfluidic channels for mixing efficiency. The star-cartridge integrated 3D mixing to improve a 2D-based PDMS and silicon-based prototype from a microfluidic system validated previously.[8] We further reduced the total device volume with two design changes to create the pie cartridge. We minimized the total device area by taking maximum advantage of 3D geometric capability. The pie-cartridge mixing regions are composed of three serial mixing units across the device width, and the star-shaped amplification region was replaced with pie pools that reduce the required width and total material compared to the star cartridge. After the serial combination of solutions, the processed sample is loaded into a star- or pie-shaped amplification region with six equal sections. This design enables potential multiplexed detection by preloading primers of interest into the channels. The channels are equidistant from the single inlet port to allow uniform sample loading. 3D scans were performed on the microfluidic cartridges to confirm that the manufactured devices achieve the target accuracy and printing resolution for the designed microfluidic features (Figure 3b,c). The cartridge geometries were measured to be accurate to within 20 μm, which is the limit of the CLIP process. The roughness of the devices was $Ra < 10$ μm, much smaller than the smallest device features. This small surface roughness and the low flow rates ($Re \sim 10$) ensured that the roughness would not affect fluid flow through the cartridge. Figure S11 shows device packaging and fluid connections and pictures of sealed and packaged devices after manufacturing prior to experimentation.

3.5. *E. coli* Real-Time Detection from Whole Blood in an AM Microfluidic Cartridge. The cartridge is designed for the direct amplification on-chip and smartphone compatible fluorescence readout using compact and portable optical instrumentation. The portable detection system is based on previous work from our group on portable handheld diagnostics.[8,37] The design and characterization of the portable detection system for operation and imaging characterization on the AM cartridges are available in the Supplementary Material (Figure S.12).

Figure 4 shows the translation of our developed assay on the additively manufactured diagnostic cartridges. The imaged results with the fabricated portable detection show complete agreement with off-chip thermocycler-based characterization. We performed amplification reactions and imaged directly on-chip: endpoint measurements on the star cartridge and real-time measurements on the pie cartridge. The spiked whole blood, lysing solution, and amplification reagents were loaded into syringes and flown through the diagnostic cartridge at the established ratios detailed in off-chip characterization. The cartridges were heated at 65 °C for 50 min, and images were collected using the cradle. After on-chip sample processing, we amplified and performed endpoint measurements of whole blood samples spiked with *E. coli* across a concentration range of 1×10^5 cfu/μL−50 cfu/μL with 1×10^4 cfu/μL MRSA/MSSA background (Figure 4a). The assay performance on the

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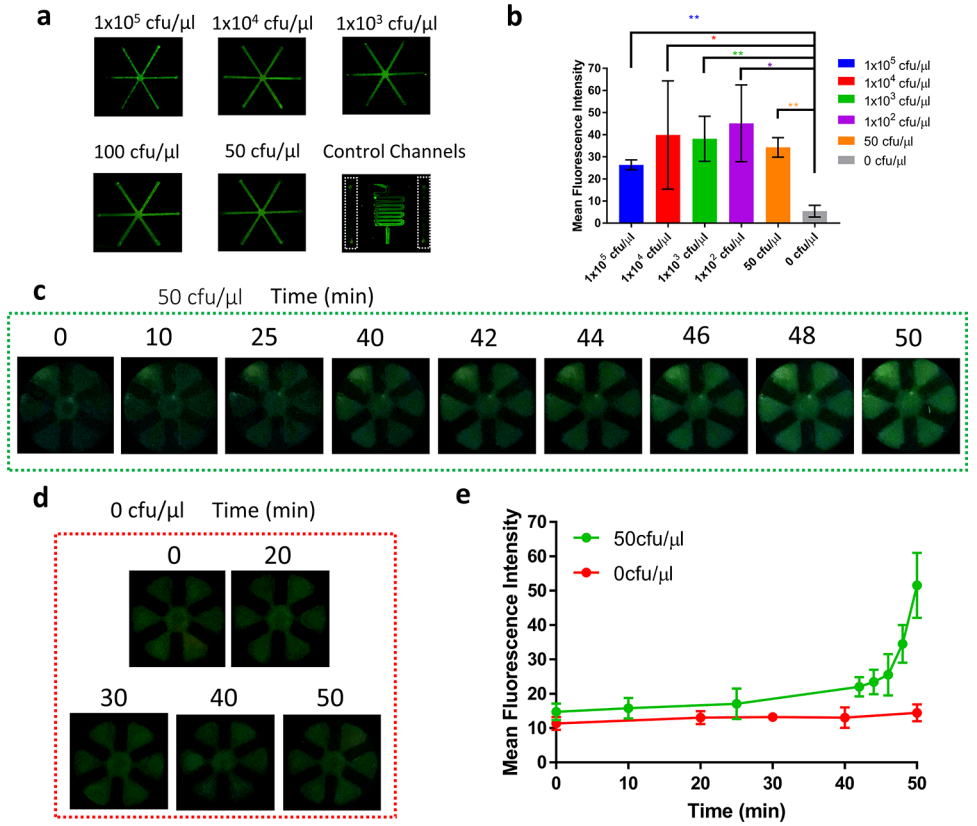

Figure 4. Detection of *E. coli* in whole blood using an additively manufactured microfluidic cartridge and portable detection system. (a) Postamplification fluorescence images of *E. coli* LAMP analysis at the endpoint of detection on the additively manufactured microfluidic diagnostics chip. (b) Mean fluorescent intensity at time = 50 min (for three cartridge replicates) for concentrations of *E. coli* in whole blood. The bar graphs show mean and standard deviation of the fluorescence intensity for each condition, and the *P* values indicate the significance of each condition compared to the no-target control group. (c) Real-time fluorescent imaging timepoints for a 50 cfu/μL sample highlighting times amplification in 2 min intervals (d) Real-time fluorescent imaging timepoints for a 0 cfu/μL control sample without amplification after 50 min. (e) Fluorescent intensity values of each well from real-time measurement images.

cartridge matched off-chip characterization and maintained a sensitivity of 50 cfu/μL. The endpoint image fluorescence intensities were analyzed using FIJI, and the fluorescence intensity for three replicates per condition was plotted against negative controls (Figure 4b).

We loaded and processed whole blood samples on the pie cartridge with the LOD of our endpoint measurements for real-time detection. Figure 4c shows real-time fluorescent images across 50 min of amplification spiked whole blood samples containing 50 cfu/μL *E. coli* and 1 × 10^4 cfu/μL MRSA/MSSA background. We used images at 2 min intervals after the start of the amplified signal. Figure 4d shows real-time fluorescent images across 50 min of a control sample containing 0 cfu/μL *E. coli* and 1 × 10^4 cfu/μL MRSA/MSSA background. We analyzed fluorescence intensities of relevant time points from the real time on chip experimentation using FIJI. For the positive samples, the acquired real-time amplification intensity matches the expected threshold range achieved off-chip at ~40−50 min (Figure 2c), while the sample containing 0 cfu/μL did not amplify (Figure 4f). Both endpoint and real-time measurements achieved an LOD of 50 cfu/μL *E. coli* compared to off-chip controls after translation to our developed POC system. We observed no degradation of the AM material or change in shape through the experiments and were able to detect no chemical interaction between the AM material of the cartridge and chemicals used in the experiments. These results validate the compact mixing performance and amplification reaction compatibility of the presented CLIP-based AM cartridges.

4. DISCUSSION

The economic and social burden of infectious disease will continue to impact low-resource communities and populations without proper recourse for widespread diagnostic assessment. The current gold standard for pathogen detection is based on PCR, which often requires RNA/DNA extraction and purification. While PCR laboratory tests can be designed to run at scales that match testing demand in regions equipped with the infrastructure and specialized equipment, the inadequate resources for and disease monitoring in low-resource settings present an urgent need to diagnose infections at the POC.

This research reports the rapid detection (<60 min) of *E. coli* directly from whole blood in a portable diagnostic cradle directly on additively manufactured cartridges at an LOD of 50 cfu/μL. The assay uses a LAMP-based protocol for amplification and detection directly on the cartridge. By integrating hands-free processing of whole blood with on-chip chemical lysing and removing macroscale instrumentation, this assay removes a key barrier toward adopting and translating a system for diagnostic use in low-resource settings or where testing should be performed with sample collection. While chemical lysing offers certain advantages for crude sample processing, a direct dilution of the starting sample without a

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subsequent purification step leads to a reduction in assay sensitivity. Future improvements to the lying chemistry will require optimizing the tradeoff of using highly concentrated buffers without denaturing or inhibiting the amplification enzymes.[54]

The use of AM for cartridge manufacturing compares well with the traditional approach of injection molding. The AM material performs as well as an injection molded polymer for this application, and in particular, the AM material is stable during the extended exposure to 65 °C during the amplification step. The processes and materials used in this study for prototyping can be scaled up for production with no changes because we can use the same material equipment in prototyping and production. This contrasts with injection molding, for which the scalable production would use different processes and tooling than those used in prototyping. By eliminating the need to validate new processes as part of the manufacturing scale up process, we can eliminate a key source of cost and risk for translation of microfluidic diagnostics. We further show microfluidic device designs and packaging concepts that are suitable for CLIP- and resin-based AM processes. The microchannels are mostly distributed on the device surface, although they can be linked together through holes. These geometries cannot be easily made using injection molding. The geometry of the ports is made possible by the high resolution of the CLIP process. The device can be packaged quickly, within a few minutes in a manual process, and could be readily adapted to automation for example in a pick and place machine. Furthermore, we showed that microfluidic cartridges made from additively manufactured RPU 70 are compatible with chemical lysing, amplification based on LAMP chemistry, and fluorescence detection by demonstrating detection of *E. coli* bacteria directly from whole blood using these process steps.

5. CONCLUSIONS

We developed and characterized an isothermal-based LAMP assay to detect *E. coli* directly from whole blood. We report a saponin-based lysing approach for processing crude whole blood samples directly for amplification, which subsequently allows a portable translation of our assay on CLIP-based microfluidics at POC. We investigated the capability of CLIP AM for microfluidic diagnostics including manufacturability of microchannel geometries using CLIP, materials' compatibility with common diagnostic chemistries, and ability to support 3D designs. We found that microchannels can be accurately produced with width and depth as small as 400 μm. For the smallest features, the print orientation has the largest effect on feature size and process accuracy. The results of this study could be used to inform the development of chemical lysing of crude samples for amplification-based assay development and the design and manufacturing of other types of microfluidic devices made using resin-based AM processes.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.analchem.1c00654.

DNA and bacterial culture sample preparation; lysing buffer compositions; portable diagnostic module fabrication; design features for performing the developed LAMP assay on the diagnostic device; diagnostic device dimension details; baseline-subtracted raw fluorescence amplification curves; AM device fabrication and feature analysis extraction; three-dimensional scan; process accuracy of microfluidic channels; summary of manufacturing parameters by print orientation; packaging of the diagnostic cartridge; design and characterization of a portable detection system; and portable detection system and imaging characterization (PDF)

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Author Contributions

†J.B. and M.Y.A. contributed equally to this work.

Notes

The authors declare the following competing financial interest(s): W.P.K. is a cofounder and Chief Scientist at Fast

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Radius Inc., where the additively manufactured cartridge was produced.

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