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## 3D Printed Auto-mixing Chip Enables Rapid Smartphone Diagnosis of Anemia

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### Abstract

Clinical diagnosis requiring a central facility and site visits can be burdensome for patients in resource-limited or rural areas. Therefore, development of a low-cost test that utilizes smartphone data collection and transmission would beneficially enable disease self-management and point-of-care diagnosis (POC). In this paper, we introduce a low-cost  $\mu$ POC<sup>3D</sup> diagnostic strategy which integrates 3D design and printing of microfluidic POC device with smartphone-based disease diagnosis in one process as a stand-alone system, offering strong adaptability for establishing diagnostic capacity in resource-limited areas and low-income countries. We employ smartphone output (AutoCAD 360 app) and readout (color-scale analytical app written in-house) functionalities for rapid 3D printing of microfluidic auto-mixers and colorimetric detection of blood hemoglobin levels. The auto-mixing of reagents with blood through capillary force has been demonstrated in 1 second without requirement of external pumps. We employed this  $\mu$ POC<sup>3D</sup> system for point-of-care diagnosis of anemia using a training set of patients ( $n_{\text{anemia}}=16$ ,  $n_{\text{healthy}}=6$ ), which showed consistent measurements of blood hemoglobin levels (a.u.c.=0.97) and comparable diagnostic sensitivity and specificity, compared to standard clinical hematology analyzer. Capable of 3D fabrication flexibility and smartphone compatibility, this work presents a novel diagnostic strategy for advancing personalized medicine and mobile healthcare.

### INTRODUCTION

Microfluidic point-of-care (POC) diagnosis is an emerging field for advancing healthcare accessibility and affordability, which offers prompt results, timely interventions, and improved efficacy[1–5]. Presently, 3D printing technology has been involved and showed

#### SUPPLEMENTARY MATERIALS

The parameters of Computational Fluid Dynamics (CFD) simulation, the influence of variable contact angles and contact angle measurement conditions, clinical patient sample collection, and parameters of diagnostic ROC, specificity and sensitivity analysis were detailed. See DOI: xxxxxx

tremendous potential for developing microfluidic POC devices, owing to the low-cost, fast and one-step fabrication capability, without the need of laboratory resources[6–8]. Traditional microfabrication of low-cost polymer-based (e.g., PDMS) microfluidic POC devices requires lithographic fabrication of molds to replicate PDMS POC chips[9, 10]. The paper-based microfluidic devices are sensitive to environmental conditions (e.g., humidity) and not durable[11, 12]. In contrast, a consumer-grade 3D printer can produce highly versatile, durable microfluidic POC devices without the need of laboratory settings[13–16]. Application of 3D printing in microfluidic technology has shown tremendous potential in tissue engineering[17–19], sample preparation techniques[20], and cell processing[21–24]. Zhang *et al.* introduced a 3D printed microfluidic chip which utilizes a water and oil interface to filter out the lysate contaminants for extraction of nucleic acids[20]. Chan *et al.* developed movable 3D printed microfluidic chip components, such as torque-actuated pump and valve, rotary valve, and pushing valve, for assembling microfluidic POC devices[25]. The tedious, multiple 3D printed components need to be assembled and employed for protein quantification from artificial urine samples with a smartphone as imaging platform. The 3D printer also has been frequently used for making parts that serve smartphone optical interfaces [26–29]. Dr. Daniel Filippini research group has done pioneer work in developing unibody LOC chip which integrates 3D printed parts as microfluidic POC device with smartphone sensing[30, 31]. In spite of a variable and powerful technology, applications of a 3D printer in microfluidic POC diagnosis with smartphone as a stand-alone system are still need to be explored more broadly [32]. Herein, we introduce a simple and low-cost approach that integrates 3D design and printing of monolithic microfluidic mixers with smartphone-based diagnosis in one process, for developing a stand-alone diagnostic system ( $\mu$ POC<sup>3D</sup> system).

We demonstrated  $\mu$ POC<sup>3D</sup> system for POC diagnosis of anemia. Anemia often causes high fetal mortality and abnormalities, premature deaths, low birth weights for infants, and retarded brain development, which affects over half of pre-school children and pregnant women in developing countries[33, 34]. Although anemia screening is highly recommended for infants and pregnant women by the U.S. Preventive Services Task Force and other developing countries, the bottleneck in control of anemia falls on the accessible screening tools needed to eliminate distance barriers[35]. Clinical diagnosis of anemia by measuring blood hemoglobin levels in rural areas or low-income countries is facing substantial logistic challenges, due to the difficulties in maintaining frequent and costly medical visits. Oftentimes, blood hemoglobin levels need to be known immediately for making decision of blood transfusion in the point of care[36]. Therefore, a stand-alone POC diagnostic system for screening anemia is needed for solving such logistic channelings.

We employ smartphone output (AutoCAD 360 app) and readout (color-scale analytical app written in-house) functionalities for rapid 3D printing of microfluidic auto-mixer and colorimetric detection of blood hemoglobin levels. In addition to generating a POC microfluidic device, the 3D printer can produce a POC chip housing to align with the smartphone camera and optical lens (Fig. 1). The 3D microfluidic simulation was studied to guide computer design and prototyping of POC devices, thereby enabling rapid optimization of various geometries and microstructures for improving mixing performance as needed. The auto-mixing of reagents with blood via capillary force was demonstrated in 1 second for

the first time. Without requiring laboratory setup and external off-chip operation (e.g., pump), 3D printed monolithic microfluidic auto-mixer can introduce oxidizing agents with blood for fast mixing and bioreaction, allowing finger prick blood (~ 5  $\mu\text{L}$ ) assay of hemoglobin levels. Integrated with smartphone image analysis and data transmission (color-scale analytical app written in-house), self-management and POC diagnosis of anemia was successfully implemented, showing consistent results with clinical measurements and good diagnostic accuracy (a.u.c. = 0.97) from testing a training set of anemia patients. Hereby, we introduce a low-cost diagnostic strategy ( $\mu\text{POC}^{3\text{D}}$ ) which enables microfluidic POC device design and fabrication, and disease management integrated in one process as a stand-alone system, offering strong adaptability for establishing diagnostic capacity in resource-limited areas and low-income countries. Our pilot study, focused on anemia diagnosis, represents a key step towards developing variable and advanced mobile healthcare tests which eliminate the expenses of clinic visits and facility resources.

## MATERIALS AND METHODS

### 3D printing of microfluidic chip.

Three-dimensional design is always difficult within conventional microfabrication, due to complicated protocols for accurate alignment and multilayer bonding. Configured with smartphone solid modeling app (AutoCAD 360), we used a laptop-sized 3D printer (D3 ProJet 1200, 30- $\mu\text{m}$  resolution) for fabrication of monolithic microfluidic chips. VisiJet@FTX Clear resin consisting of triethylene glycol diacrylate, sobornyl methacrylate, and 2–3% photoinitiator phenylbis (2,4,6-trimethylbenzoyl)-phosphine oxide was used to produce transparent 3D microfluidic chips per vendor's instruction. Printed 3D devices were cleaned using isopropyl alcohol and blown dry. The uncured resin within microchannel was flushed out by an air compressor. A field emission scanning electron microscope (SEM) characterized printed microstructures using a focus ion beam (FEI Versa) after gold sputtering coating in 20-nm thickness. Ethylene glycol chemistry was used to develop the hydrophilic surface of 3D printed devices: 1.82 M potassium hydroxide (KOH) was mixed into pure ethylene glycol solution (Sigma-Aldrich, anhydrous, 99.8%) and used to soak 3D devices at 55  $^{\circ}\text{C}$  for 2 h. When needed, we also can bond the 3D printed objects with glass materials when 3% of photoinitiator 2-(2-bromoisobutyryloxy)ethyl methacrylate (BrMA, Sigma-Aldrich) was introduced into clear resin and mixed. The Silane-Prep glass slides pretreated with aminoalkylsilane (Sigma-Aldrich) were used to bond the 3D printed layer with open channels and microstructures. The clean 3D layer annealed very well with the glass slides. The two annealed layers were then exposed to 365-UV for 8 min. The bonding was irreversible via covalent chemical bond.

### Auto-mixing assay development

3D computational fluid dynamic (CFD) simulations were performed using ANSYS CFX (R16.1) to guide microfluidic design and achieve effective capillary-driven auto-mixing in the microfluidic mixer. The simulation parameters and conditions were detailed in supplementary information. The visual readout for anemia diagnosis from the view-window in which blood mixed with reagents was based on the 3,3',5,5'-tetramethylbenzidine (TMB) (Sigma-Aldrich) and hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) (Sigma-Aldrich) oxidation-reduction

reaction with hemoglobin as a catalyst. The amount of hemoglobin determined the charge transfer status of TMB, which was displayed in various colors. The 1:1 volume ratio of blood (10 times dilution) and reagents was developed to gauge an assay using 5  $\mu$ L of finger-prick blood. Consistent sample introduction was ensured by using a 3D printed sampling well with “slot-in” placement of chips (see details in supplementary information).

### Smartphone output and readout

An android phone with AutoCAD 360 app was configured with the 3D printer for wireless transmitting solid modeling design. The android phone camera was used for collecting assay readout through a color-scale analytical app written in-house. A 3D printed POC chip housing with a 5 $\times$  gel lens on camera was assembled for capturing and analyzing color-scale results from the chip view-window (Fig. 1). Color-scale image capture and analysis app written in-house was developed to extract RGB peak values in the region of interest (ROI) in the view-window. RGB peak values were then converted to CIE L\*a\*b\* color space values corresponding to Hgb concentrations via calibration, using the MATLAB mathematic model[37]. The CIE L\*a\*b\* color space is a color representation model which maps a three-dimensional integer space for device-independent digital representation of color [37].

### Clinical blood samples

Study protocols and clinical sample collection protocols were approved by the IRB committee of Kansas State University and the University of Kansas Medical Center and Hospital. The inclusion criteria include aged 25 to 39 years on the day of inclusion and resident of the site zone, and being able to attend anemia screening (no exclusion criteria). A total of 22 clinical blood samples from adult patients and healthy donors in outpatient clinics of the University of Kansas Hospital and Biorepository were collected, tested, and analyzed using our smartphone *i*POC<sup>3D</sup> system (see details in supplementary information). All samples were acquired under informed consent. Results from smartphone *i*POC<sup>3D</sup> system were compared to patients' clinical Complete Blood Count (CBC) tests which measure hemoglobin levels by hematology analyzer through 2 mL of venous blood draw. The study revealed that patients benefited from much less blood sampling and rapid data readout.

## RESULTS AND DISCUSSIONS

### Principle and 3D simulation for capillary-driven auto-mixing

Flow behavior in several 3D microfluidic channels and microstructures were studied in order to develop an effective capillary force driven auto-mixing in a 3D printed microfluidic mixer. The flow in microfluidic channels is characterized by low Reynold's numbers, which indicates the flow regime is laminar. For incompressible, laminar flow in a circular microscale tube (microfluidic channels), the flow can be represented by the Hagen-Poiseuille equation[38]. The surface tension forces (capillary force) and the viscous forces (frictional drag) are the governing forces[39], and the gravitational force can be ignored due to low bond (Bo) number in microscale tube[40]. Thus, the auto-mixing flow rate is determined by a force balance between the capillary force and the frictional force. The capillary force can be represented as below equation by the principle of Young-Laplace[41]:

$$\Delta P = \frac{4\sigma\cos\theta}{D}$$

where  $P$  is pressure drop,  $\sigma$  is surface tension,  $\theta$  is the contact angle, and  $D$  is the channel diameter. The frictional drag force for laminar flow in a round channel can be represented by [42]:

$$\Delta P = \frac{64}{Re} \times \frac{L}{D} \times \frac{\bar{V}^2}{2} \times \rho$$

where  $Re$  is the Reynold's number ( $\rho VD/\mu$ ) [43],  $\mu$  is the dynamic viscosity of the fluid,  $L$  is wetted length,  $V$  is flow velocity, and  $\rho$  is density. By equating frictional force to capillary force based on above two equations, we can determine the auto-mixing velocity by the following relation:

$$\bar{V} = \frac{D\sigma\cos\theta}{8\mu L}$$

Therefore, the capillary-force induced flow rate is governed by geometry, surface properties and fluid profile properties. At a given fluid, geometry or characteristic dimension of microfluidic channel in given surface chemistry dominates the capillary-force induced flow rate. The velocity of capillary flow is fast at short wetted lengths while slow at long wetted lengths. Contact angle affects the capillary flow rate under varied surface conditions (e.g., chemistry and roughness) (See Figure S1) with higher flow rate induced by smaller contact angle. The flow rate will increase with larger channel diameters, until gravitational force and inertial forces become dominant. On the other hand, at small diameters, the flow rate tends to be extremely slow due to the drag force overpowering the surface force. Hence, the theoretical analysis gives an applicable dimension regime of microchannels for a capillary force driven flow, ranging from  $\sim 100 \mu\text{m}$  to 2 mm in diameter with Reynold's numbers below 500. The typical capillary flow rate in a microchannel with 500  $\mu\text{m}$  in diameter and 22 mm in wetted length is about 20  $\mu\text{L/s}$ .

We initially designed three microfluidic mixers with characteristic size of 500  $\mu\text{m}$  in microchannel diameter: split and recombination (SAR) (Fig. 2a), serpentine channel (Fig. 2b), and ring channel (Fig. 2c). All three of these designs have Bo numbers less than 1, indicating capillary force is dominant, and have Reynold's numbers ranging from 11 to  $\sim 450$ . A Y-shaped channel was used to introduce the two flows into the mixing unit and a round circular chamber served as a view-window. The SAR micromixer was adapted from published work by Lee et. al. [44] who fabricated SAR device using PDMS by complicated multilayer alignment and bonding. In contrast, we fabricated SAR device by one-touch 3D printing. In the SAR micromixer, the split and recombination process increased flow interfaces exponentially by continually laminating interfaces along the channel, resulting in fluidic mixing. Although serpentine channel is a classic design [45] used for microfluidic mixing, the basic serpentine is not suitable for fast mixing, due to diffusion dominated mixing process which requires relatively longer mixing time. By combining the split and

recombination structure with the serpentine structure, we designed a ring-shaped mixing channel (Fig. 2c).

We established a CFD model to simulate blood flow mixed with aqueous solution under capillary force in three micromixers (see details in supplementary information). As shown in Fig. 2d straight flow-through channel, the two flows were unable to mix. The sharp boundary between two flows demonstrated the time frame for diffusive mixing which was much longer than the fluid residence time at the given capillary driven flow rate. By inclusion of SAR units in the straight flow-through channel, blood flow can mix completely with the aqueous solution after passing into view-window of SAR micromixer. We also simulated the blood mixing profile in ring-shaped micromixer. Although ring channel directed flow to split and recombine, two flows were unable to crossover, and continue to flow side-by-side. The initial simulation results led us to a novel design by rotating the ring structure 90 degrees in the x-y plane with respect to the “Y” inlet (see Fig 2d, fourth figure). Thus, two flows can be split in a manner that causes interfaces to travel through the ring section. Due to the large amount of chaotic advection occurring in the ring channels, fast mixing (~1 sec) can be achieved in a more compact 3D micro-structure. Along with the supplementary CFD simulation video 1 and 2, the rapid prototyping was demonstrated in Fig. 2e and f. We used food dyes in red and blue to experimentally demonstrate the auto-mixing process, and the experimental results are consistent with simulation results (Fig. 2e and f). For serpentine micromixer, due to the long channel length (~ 255 mm), the capillary-driven flow rate was relatively slow and required more time for fluids to flow through the device (~ 1 minute), which consequently provides ample time for diffusive mixing. On the contrary, the SAR and ring micromixers have relatively short lengths (~ 30 mm and 22 mm), resulting in high capillary-driven flow rates and shorter mixing times (less than a second). In order to develop quick mixing of blood with reagents, we chose the rotated ring channel micromixer for following tests.

### **Fabrication of 3D microfluidic POC chips**

SEM imaging was performed to characterize 3D printing quality. Wireless printing was conducted through desktop size D3 ProJet 1200 3D printer which has a build area of 43 mm × 27 mm × 150 mm and provides a physical pixel size of 56 μm × 30 μm. After the printing was complete, the 3D object was washed with isopropyl alcohol, and uncured resin was flushed out by an air compressor. We printed a number of basic structures and three micromixer designs to evaluate printing capability and quality, as shown in Fig. 3. The SAR micromixer with eight mixing units was readily printed out in less than 10 min, and the chip was completely transparent for visualizing colored reagents in the microchannel (Fig 3a). Note that PDMS-fabricated SAR micromixer requires complicated alignment and multilayer bonding, which takes more than one day to complete. The rotated, ring-shaped channels were prototyped in 10 min, without requiring additional equipment, as shown by the angled view in Fig. 3b. The serpentine channel was also successfully printed, as shown by SEM imaging in Fig. 3c. A smooth surface was observed in the microchannel and circular chamber. Other basic structures often used in microfluidic chips were also characterized, including post arrays (Figs. 3d and e), microwells (Fig. 3f), and a D-shaped hollow microchannel. The cost for printing each 3D microfluidic chip is less than 50 cents. Close

examination of SEM images revealed additional printing properties, and printing layers were observed due to the minimum pixel resolution (30  $\mu\text{m}$ ), such as the “stair”-looking of the D-shaped microchannel shown in Fig. 3g (diameter of 200  $\mu\text{m}$ ). Although a smaller channel could be printed, complete removal of uncured resin was difficult, due to high backpressure for cleaning[13].

We also developed a bonding protocol to attach a 3D printed open channel layer to a glass slide (Fig. 3h). An open channel with a diameter of 50  $\mu\text{m}$  can be printed and attached to a glass slide, resulting in a closed hollow microchannel. The bonding protocol follows the principle of saline chemistry by forming covalent chemical bonds between the glass surface and resin surface. As shown in Fig. 3h, the ring-shaped channel in 50- $\mu\text{m}$  deep and wide was bonded to a glass slide without leakage observed, which was proven by running through a red dye solution. This protocol could extend 3D printer capability for printing smaller hollow microchannels below 200  $\mu\text{m}$  i.d.

The cured resin was slightly hydrophobic, as indicated by measuring contact angles (Fig. 4a,  $\theta = 64^\circ$ ), so we developed a simple chemistry protocol for treating 3D printed objects into hydrophilic property. The 3D printed micromixer chip was immersed into a pure ethylene glycol solution containing 1.82 M KOH and incubated at 55  $^\circ\text{C}$  for 2 h[46]. The contact angle of deionized (DI) water was measured to note the hydrophilicity of 3D printed microfluidic chip as compared to PDMS and glass materials. As shown in Fig. 4a, hydrophilicity was significantly improved ( $\theta = 32^\circ$ , RSD = 4.6%, n = 10), providing a stable capillary-driven force for auto-mixing. Hydrophilically treated 3D chip also can be stored in water solution for reuse for several days, thereby maintaining a stable surface chemistry. We further characterized the surface chemistry using Fourier transform infrared spectroscopy (FTIR). Untreated surface spectra failed to show an OH stretch between 3000 and 3750  $\text{cm}^{-1}$  (Fig. 4b) compared to treated 3D printing material which exhibits strong OH stretches. Such hydrophilic surface provides a stable capillary force required for auto-mixing of blood with test reagents.

### Rapid POC diagnosis of anemia

Utilizing capillary-driven auto-mixing in a 3D-printed microfluidic mixer, we developed a color-scale assay for POC diagnosis and self-management of anemia. Hemoglobin (Hgb) level is the primary laboratory parameter for the clinical diagnosis of anemia[47]. The current gold standard is a complete blood count (CBC) using a hematology analyzer via venous blood, which requires a blood draw by a trained phlebotomist and needs electrical power to operate in centralized hospitals or clinics[48]. Several portable devices for anemia diagnosis have been developed[49, 50], such as HemoCue-B Hemoglobin[51], STAT-Site® MHgb[52], Rainbow® signal extraction technology (SET), and SpectOLight™ Occlusion[53]. However, few of these tools are well-suited for transferring to low-resource areas, due to their costs and sophisticated designs that require professional operation. Simple and disposable platforms have recently been developed to test blood Hgb levels[37, 54, 55], including the WHO Hb color scale (HbCS) test[56], but these platforms require fabrication facilities in scientific laboratories or skilled technicians for mass production and technology

transfer, thereby offering limited adaptability for establishing study capacity in rural areas or low-income countries.

We developed a color-scale assay combined with smartphone to ascertain Hgb blood levels using only ~5  $\mu\text{L}$  blood from a finger prick. The diluted drop of blood and oxidizing agents were held uniformly and tightly in micro-wells (~ 20  $\mu\text{L}$  volume) in 3D printed sampling platform, due to the surface tension (Fig. 5a). Therefore, consistent sample introduction was ensured by using a “slot-in” placement of chips in this sampling platform (Fig. 5b). The sampling platform also can be printed as an array format. The visual readout via smartphone camera from the micromixer reaction chamber was based on the 3,3',5,5'-TMB oxidation-reduction reaction with blood Hgb as a catalyst. The amount of Hgb determines the charge transfer status of TMB (charge transfer TMB complex with one-electron is in blue, two-electron in yellow)[37, 57]. Excess hemoglobin yields a red color. Thus, depending on the ratio of several color species in the mixture, the resultant visual colors span blue, green, yellow, orange, and red with increased hemoglobin concentration (Fig. 5c and d). The color images collected from an android phone with a micromixer housing system were consistently obtained in order to define ROI and quantitative RGB analysis. We used a rotated ring channel design to rapidly mix blood with oxidizing agents in 1 s and observe color development. The standard hemoglobin protein was spiked in 100-time diluted blood with concentrations of 0, 5.5, 8.0, 9.0, 12.5, and 16.0 g/dl, which was used to establish calibration curve for correlating color-scales ( $R^2=0.99$ ,  $RSD= \sim 2.5\% - 5\%$ , Fig. 5d). The visual readout from the micromixer was also calibrated by comparing to photospectrometer measurements for quantitative measurement of Hgb concentrations (Fig. 5e). Absorbance peaks of Hgb at 540 nm and 575 nm showed linear increase with increased Hgb concentrations. In contrast, the absorbance peak of TMB at 630 nm decreased with high Hgb levels due to TMB oxidization. The analysis of blood from a healthy donor showed Hgb level in line with the WHO recommended cutoff as a positive reference.

In order to further validate the clinical usability of developed smartphone  $i\text{POC}^{3\text{D}}$  system, we started a clinical training set under IRB approval. A total of 25 patients were assigned, but three patients did not show up on the day of collection. Thus, total of 16 from adult patients and 6 from healthy donors in outpatient clinics of the University of Kansas Hospital and Biorepository were collected and analyzed using both standard hematology analyzer and smartphone  $i\text{POC}^{3\text{D}}$  system. Fig. 5f demonstrated the capability of  $i\text{POC}^{3\text{D}}$  system for diagnosing clinical blood samples from anemic patients and healthy individuals, which showed high correlation between clinical measurements and our smartphone  $i\text{POC}^{3\text{D}}$  measurements ( $r= 0.8$ ,  $p < 0.0001$ ). Blood from healthy donors showed Hgb levels in line with the WHO-recommended cutoff (>12 g/dl) (Table s1). Severe anemia (<8 g/dl) and mild anemia (<12 g/dl, and >8 g/dl) can be clearly differentiated in distinctive color readout. Based on color RGB analysis via an app written in house, direct output of Hgb levels from a smartphone can prevent potential variations due to human color vision, thereby it is suitable for color-blind patients. The diagnostic accuracy of smartphone  $i\text{POC}^{3\text{D}}$  system with the area under ROC curve (a. u. c.) is 0.97, which is comparable with standard clinical measurements (a.u.c. = 1.0) (Fig. 5g and Table s2). Smartphone  $i\text{POC}^{3\text{D}}$  system for detecting anemia gives the coefficient of variation in the range of ~ 0.2% - 5% which is less than 0.4 g/dl Hgb (Fig. s2). The diagnostic sensitivity is 81.2% for detecting severe anemia,



and 100% for mild anemia. The diagnostic specificity is 100% for detecting severe anemia, and 83.3% for mild anemia (Table 1), yielding comparable diagnostic sensitivity and specificity to standard clinical measurements and reported POC test[37]. These data highlighted the clinical usability of smartphone *i*POC<sup>3D</sup> system for diagnosing anemia.

## CONCLUSIONS

We implemented 3D microfluidic simulation-guided computer design for developing capillary-force enabled auto-mixing in the 3D printed microfluidic chip. Without requiring external power or pump, auto-mixing of oxidizing agents with blood in the micromixer was achieved in 1 s, thereby allowing the development of finger prick blood-based assay for POC diagnosis of anemia, combining with the smartphone as colorimetric sensing platform. The correlation analysis between *i*POC<sup>3D</sup> measurements and standard clinical measurements showed significance of  $p < 0.0001$  and the correlation coefficient  $r = 0.8$ , demonstrating good diagnostic capability. A desktop 3D printer costs ~ \$4,000 with a resin cartridge (~ \$45) for producing more than 100 chips. The overall cost per test is ~ 50 cents that covers resin consumed and oxidizing agents. Once the design is optimized and proven proof-of-concept, the manufacturing-scale 3D printer can be used for mass production without requirement of specialized infrastructure. Such low-cost *i*POC<sup>3D</sup> diagnostic strategy integrates design, fabrication and disease management in one process as a stand-alone system, offering strong adaptability for establishing diagnostic capacity in resource-limited areas and low-income countries. Set as a pilot study, we will continue to seek coherent large-scale clinical study for validating clinical usability, as well as the efficacy as an anemia screening tool. Although this pilot study focuses on POC diagnosis of anemia, the strong adaptability enables the development of other blood-based diagnostic tests, representing a novel research and diagnostic model.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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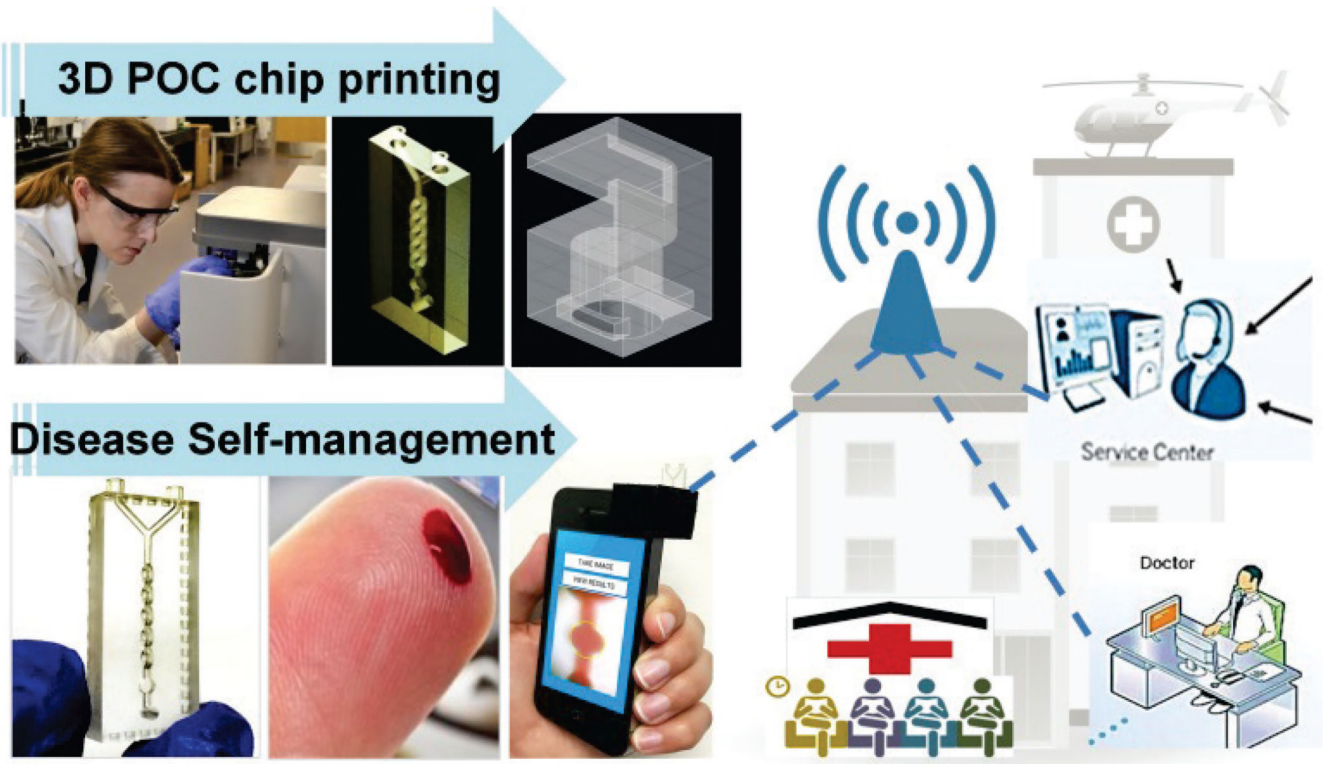
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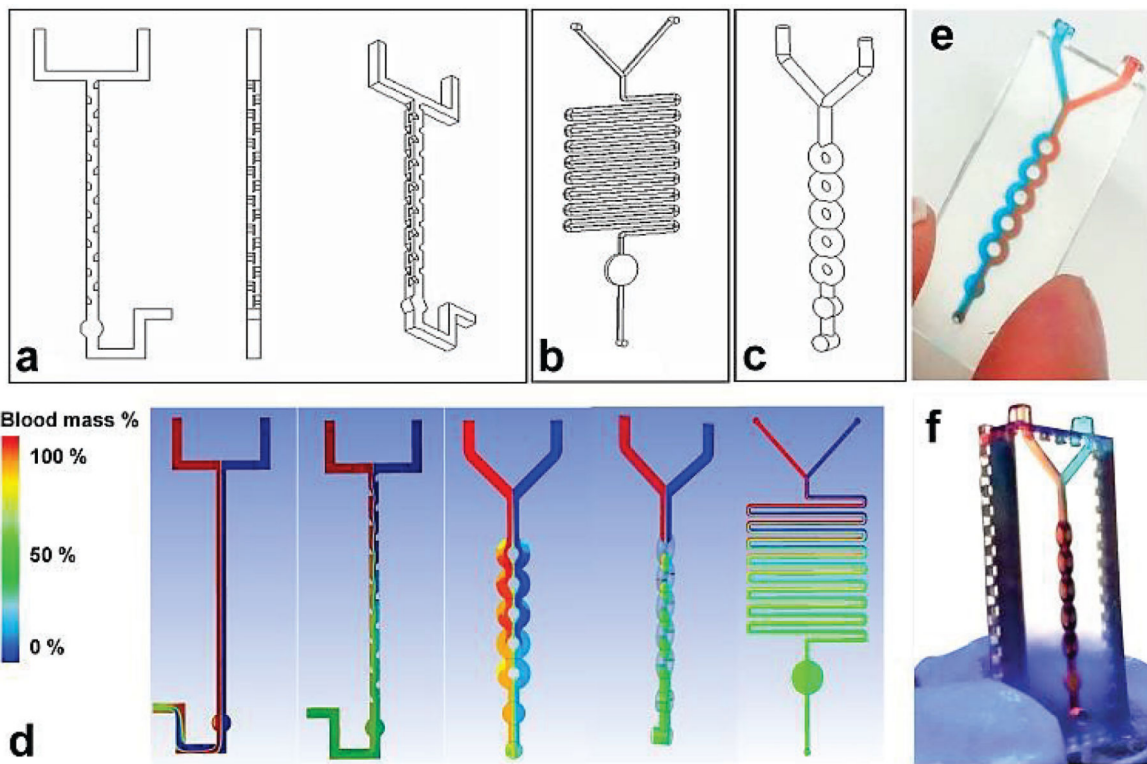
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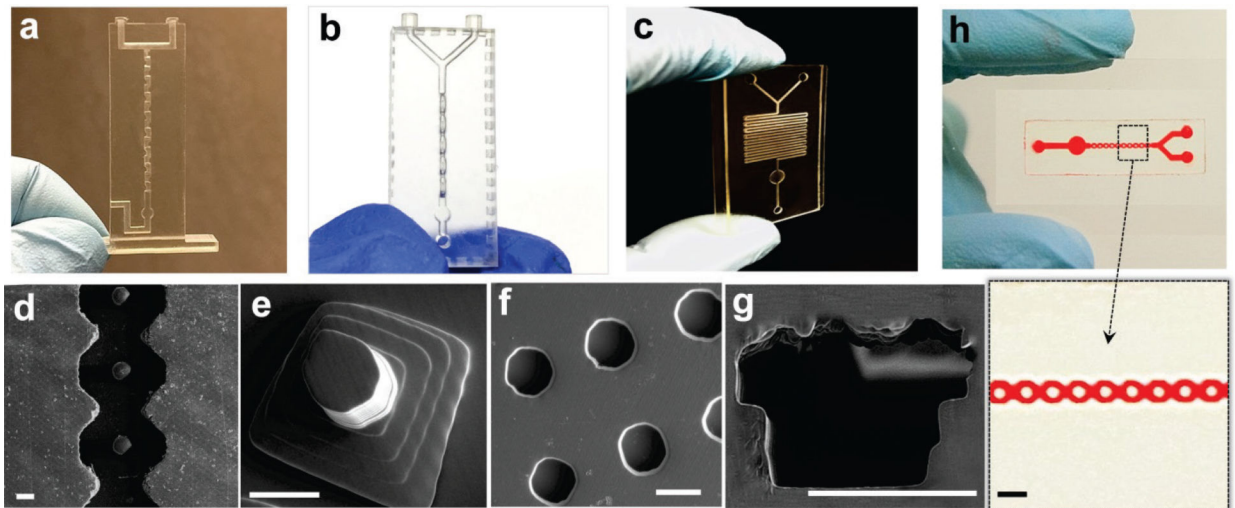
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**Figure 1.** Low-cost, smartphone-based, 3D-printed POC microfluidic chip (smartphone  $POC^{3D}$  system) for rapid diagnosis of anemia in 60 seconds.

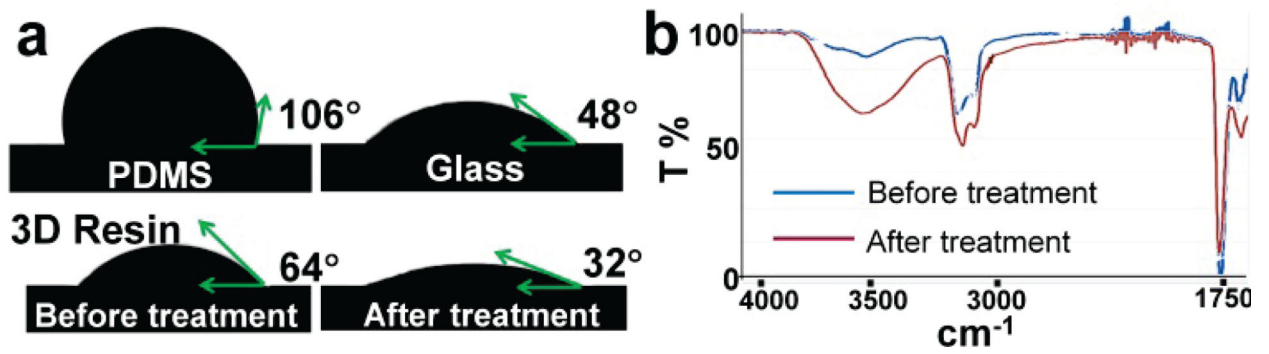


**Figure 2.** Design of three microfluidic mixers: (a) SAR, (b) serpentine channel, and (c) ring-shaped channel. Channel diameter was  $\sim 500 \mu\text{m}$ . (d) 3D CFD simulation of blood mixing profile in three micromixers: Red flow is blood mixed with aqueous solution (blue); green color indicates complete mixing. (e) Experimental image of 3D printed planner micromixer showing incomplete mixing under capillary force using colored dye solutions. (f) Experimental image of 3D printed, 3D-structured micromixer showing efficient mixing within 1 s using colored dye solutions.



**Figure 3.**

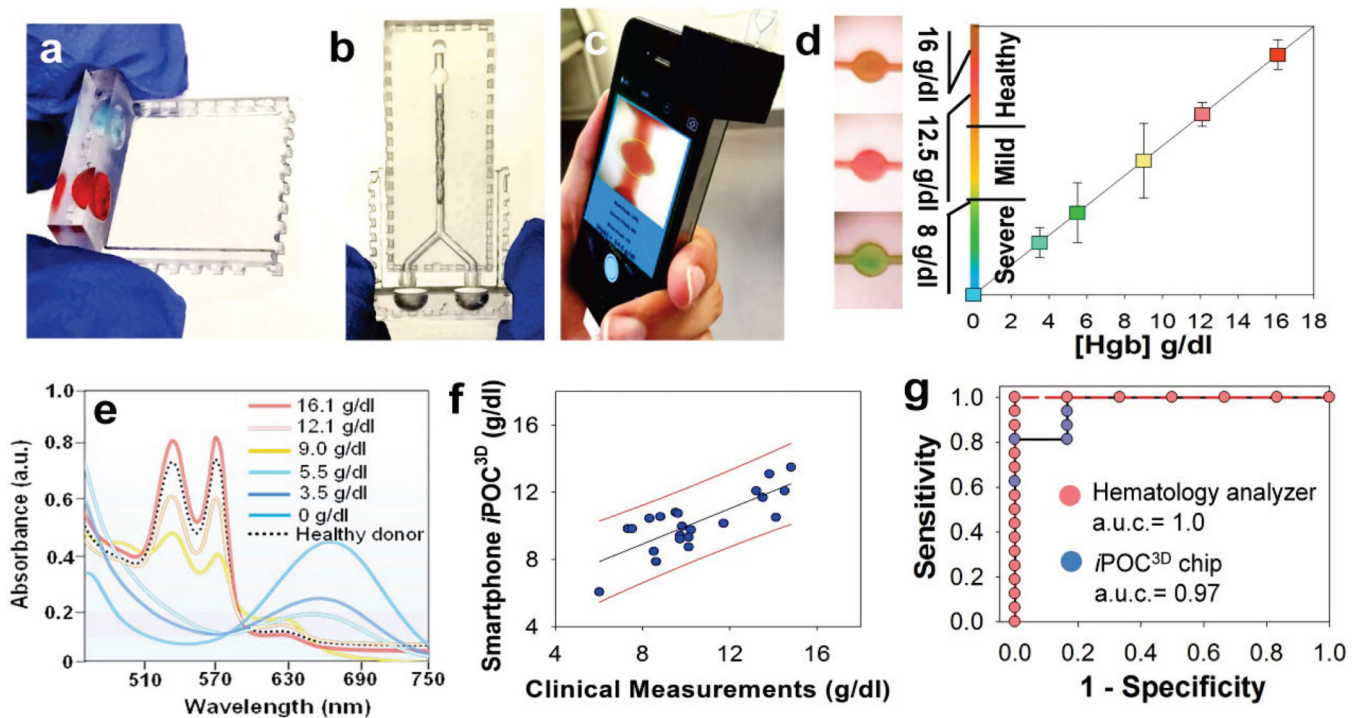
Characterization of 3D printing for fabricating microfluidic mixers: (a) SAR microfluidic mixer, (b) 3D ring-shaped micromixer, (c) and serpentine micromixer. Channel diameter is  $\sim 500 \mu\text{m}$ . SEM imaging was used to characterize variable 3D-printed microstructures: (d) posts in curved microfluidic channel, (e) pyramid post arrays, (f) microwells, and (g) a D-shaped hollow microchannel. Scale bar:  $200 \mu\text{m}$ . (h) The bonded layer of a 3D-printed open channel with a glass slide.



**Figure 4.**

(a) Hydrophilic surface treatment of 3D-printed microfluidic chip for capillary-driven auto-mixing; the contact angle ( $\theta$ ) of DI water shows the hydrophilicity of PDMS, glass, and printed resin before and after treatment. (b) FTIR analysis of surface chemistry of 3D printed resin before and after surface treatment.





**Figure 5.**

(a) 3D printed sampling platform. (b) Consistent introduction of blood with oxidizing agents for colorimetric assay. (c) Smartphone sensing system with color-scale analytical app written in-house. (d) Quantitative measurement of Hgb levels for color-scale anemia diagnosis using smartphone *iPOC*<sup>3D</sup> system *via* capillary-driven auto-mixing of finger-prick blood. The calibration curve is established by measuring standard hemoglobin proteins spiked in 100-time diluted blood in 0, 5.5, 8.0, 9.0, 12.5, 16.0 g/dl, and correlated with color scale. (e) Spectrophotometric analysis of Hgb levels in blood reaction mixture for validating readout from smartphone *iPOC*<sup>3D</sup> system; Hgb peaks are at 540nm and 575nm, and TMB peak is at 630 nm. Blood from a healthy donor is indicated in the black dashed line. (f) Pilot study of anemia diagnosis ( $n_{\text{anemia}}=16$ ,  $n_{\text{healthy}}=6$ ) and the correlation of smartphone *iPOC*<sup>3D</sup> measurements with clinical standard hematology analyzer measurements using Durbin-Watson Statistic Normality test with 95% confidence interval ( $r=0.8$ ,  $p < 0.0001$ ). (g) ROC analysis of diagnostic accuracy using smartphone *iPOC*<sup>3D</sup> system, compared with standard clinical measurements using hematology analyzer.

**Table 1.**

The diagnostic sensitivity and specificity comparison between standard clinical measurements, Smartphone-based iPOC3D measurements, and reported method.

Anemia Status	Standard Clinical Meas.		Smartphone-based iPOC <sup>3D</sup> meas.		Reported method <sup>[37]</sup>	
	Spe.	Sen.	Spe.	Sen.	Spe.	Sen.
Severe Anemia	100%	87.5%	100%	81.2%	93.9%	100%
Mild Anemia	83.7%	100%	83.3%	100%	91.1%	79.2%

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