Egg beater as centrifuge: isolating human blood plasma from whole blood in resource-poor settings†

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This paper demonstrates that a hand-powered egg beater can be modified to serve as a centrifuge for separating plasma from human whole blood. Immunoassays used to diagnose infectious diseases often require plasma from whole blood, and obtaining plasma typically requires electrically-powered centrifuges, which are not widely available in resource-limited settings. Human whole blood was loaded into polyethylene (PE) tubing, and the tubing was attached to the paddle of an egg beater. Spinning the paddle pelleted the blood cells to the distal end of the PE tubing; the plasma remained as the supernatant. A cholesterol assay (run on patterned paper) demonstrated the suitability of this plasma for use in diagnostic assays. The physics of the system was also analyzed as a guide for the selection of other rotating systems for use in centrifugation. Egg beaters, polyethylene tubing, and paper are readily available devices and supplies that can facilitate the use of point-of-care diagnostics at sites far from centralized laboratory facilities.

Introduction

This paper describes a method for separating blood plasma from human whole blood in polyethylene (PE) tubing using a hand-powered centrifuge (an egg beater). We isolated human plasma using an egg beater by attaching PE tubing containing blood to the paddle of an egg beater, and spinning its handle manually to pellet the blood cells. The plasma remained as the supernatant. The use of the plasma in a diagnostic assay for total cholesterol run on patterned paper demonstrated the practicality of this method. The use of the egg beater as a centrifuge requires little training, and the egg beater is widely available, inexpensive, robust, and completely hand-powered. Diagnostic tools used to detect concentrations of analytes in plasma isolated from whole blood often require expensive, electrically-powered centrifuges. This hand-powered centrifuge offers an alternative that may sometimes be more practical in the resource-poor settings of developing countries.

In developing countries, infectious diseases are responsible for more than 50% of deaths.[1,2] Prompt and accurate diagnosis of infectious diseases would lead to more effective treatment, and lower rates of mortality, and would also ultimately reduce the expense of treating patients and the economic burden of the illness on society.[3] In the innovative developing countries (IDCs),[1] it is often difficult to reach populations that are distributed sparsely over rural areas. Health workers dispatched from centralized hospitals or laboratory facilities to serve these areas face challenges such as difficult terrain, intermittent (or lack of) electricity, poorly-equipped facilities, an unskilled workforce,[4] and limited financial resources. The consequences of these limitations are that diagnostic medical technologies common in industrialized regions are either not usable or not affordable, and that the time required to reach the patient, transport samples to centralized laboratory facilities, and report a diagnosis to the patient can take days to months. Diagnosis of patients in the field enables prompt treatment (Fig. 1); point-of-care diagnosis relies on simple, inexpensive equipment that is available locally.

Medical diagnostics—especially colorimetric assays and many immunological assays—rely upon obtaining clear bodily fluids such as blood plasma to diagnose diseases. Plasma is the liquid component of whole blood; it is usually obtained from whole blood by centrifugation with electrically-powered, bench-top centrifuges at speeds that generate approximately $1000 \times g$ for 15 minutes[5,6] This technique sediments blood cells, which interfere with assays because they scatter light,[7] aggregate, and lyse; lysis of cells releases intracellular components that can contaminate the sample of plasma. Standard centrifuges are impractical for use in severely resource-limited environments:[8] they require a source of electrical power, and are bulky, difficult to repair, and expensive (>$400). Locally available resources such as blenders and record players have been modified for scientific and biomedical use in laboratories, but still require electricity.[9,10] We believe that the hand-powered centrifuge—in combination with paper-based devices for running diagnostic assays[11]—provides a useful capability for diagnostic analyses in regions with limited resources.

Experimental

Equipment and materials

Polyethylene (PE) tubing (inner diameter: 1.57 mm, outer diameter: 2.08 mm) was purchased from VWR (Bridgeport, NJ, USA). Whole human blood (with ethylene diamine tetraacetic acid; EDTA) was obtained from Rockland Immunochemicals (Gilbertsville, PA, USA). Photoresist (SU-8 2010) to pattern the chromatography paper (Whatman No.1; VWR) was purchased

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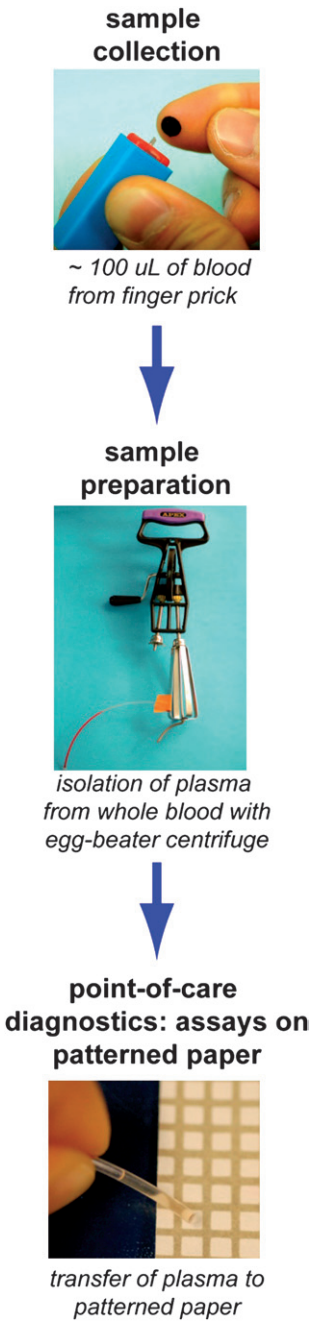

Fig. 1 Point-of-care diagnostics result in rapid, in-field diagnosis. Health workers can extract a small volume of blood from a finger prick and can load the blood into EDTA-coated, PE tubing. To prepare the sample for the diagnostic, plasma is isolated from whole blood using the hand-powered centrifuge.

from MicroChem Corp. (Newton, MA, USA). Solvents were obtained from Sigma–Aldrich (St. Louis, MO, USA) and used as received. A cholesterol detection kit purchased from Invitrogen (Carlsbad, CA, USA) contained the Amplex Red reagents to determine the concentration of cholesterol.

Egg-beater centrifuge

A grocery store in Cambridge, Massachusetts supplied the manual egg beater. We used pliers to remove one paddle of the

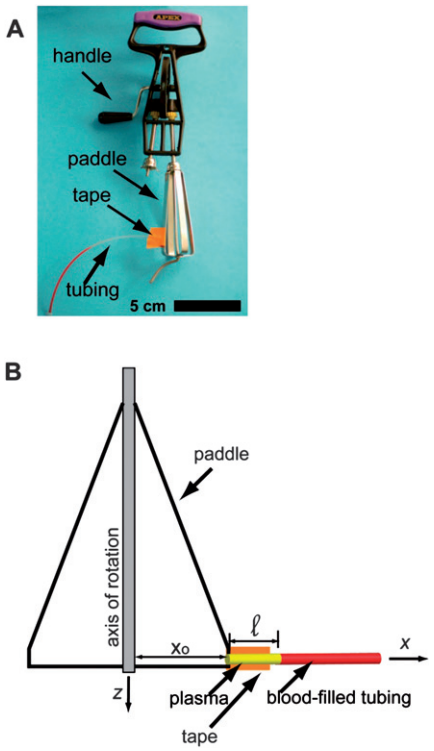

Fig. 2 (A) Photograph of the egg beater. (B) A schematic of the egg beater with the blood-filled tubing attached to the paddle.

egg beater to accommodate the length of the blood-filled, PE tubing, and attached the blood-filled tubing perpendicular to the remaining paddle using a strip of adhesive tape (Fig. 2). Spinning the handle of the egg beater at a comfortably fast pace (this pace varied among users) resulted in a rotational speed of the paddle of at least ~1200 RPM. We found that holding the egg beater with a loose grip in mid air minimized user fatigue.

Filling PE tubing with whole blood

A rubber bulb (VWR) attached to one end of PE tubing (inner diameter: 1.57 mm, outer diameter: 2.08 mm) open at both ends created a vacuum to draw ~100 μL of human whole blood into the tubing. We held each end of the PE tubing over the flame of a candle for one second to melt the end of the tubing, and then pinched the melted end between two rocks, or with pliers, to seal the end of the tubing.

Extracting plasma from PE tubing

To extract plasma from the PE tubing, we used scissors or a knife to cut the tubing just above the interface between plasma and blood cells (Fig. 3C). The two cut segments of tubing contained either the blood cells or plasma. The end of the cut segment that contained the plasma provided direct access to the plasma within the tubing—pressing this end of the tubing against the paper allowed the paper to wick the plasma.

Patterning paper

To embed SU-8 2010 photoresist into Whatman No. 1 chromatography paper, we poured ~2 mL of photoresist onto a piece

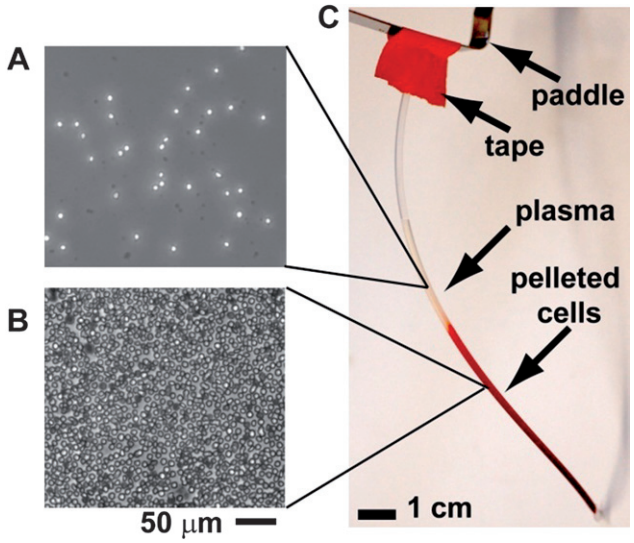

Fig. 3 Phase contrast images of plasma obtained by spinning blood in PE tubing that were taped to the egg beater (A) and of whole blood (B). The total number of cells remaining in the plasma after centrifuging with the egg beater was ~50 000 cells per mL. (C) PE tubing containing plasma that has been separated from whole blood using the egg-beater centrifuge.

of chromatography paper (8 × 8 cm) and spread the photoresist uniformly into the paper with a wooden rolling pin. We then baked the paper at 95 °C on a hotplate for ten minutes, and exposed the baked paper for four minutes to 365 nm UV light through a transparency mask that contained a grid pattern. After baking the UV-exposed paper at 95 °C on a hotplate for another ten minutes, soaking the paper successively in baths of propylene glycol methyl ether acetate for 15 minutes and isopropyl alcohol for 15 minutes removed uncrosslinked photoresist from the unexposed portions of the paper. After rinsing with isopropyl alcohol, treating with air plasma (Structure Probe, Inc., West Chester, PA, USA) for ten seconds prior to loading reagents removed any remaining organic solvents within the paper and increased the hydrophilicity of the paper.

Spotting reagents that detect cholesterol onto patterned paper

We spotted 1 μL of a solution containing Amplex Red (300 μM in dimethylsulfoxide (DMSO)), cholesterol oxidase (2 U mL^{-1} in 0.1M potassium phosphate, 0.05 M NaCl, 5 mM cholic acid, 0.1% Triton X-100), and horseradish peroxidase (2 U mL^{-1} in 0.1M potassium phosphate, 0.05 M NaCl, 5 mM cholic acid, 0.1% Triton X-100) in PBS, onto the paper that was patterned with a grid of hydrophobic lines of SU-8. The hydrophobic grid demarcated square regions of hydrophilic paper; after spotting, the reagents were allowed to dry for five minutes.

To determine the calibration curve for the intensity of Amplex Red, we added 1.5 μL of a solution of water-soluble cholesterol (Sigma; in PBS). After a five-minute incubation of the paper with dilutions of the solution of cholesterol in PBS at room temperature, we used a digital camera (Nikon D40) to capture images of the paper and export them to Adobe Photoshop in which we converted the images to grayscale and extracted the intensity of Amplex Red in each square region on the paper. We used KaleidaGraph© to plot and fit the data.

Results and discussion

Blood-filled polyethylene tubing

Pieces of PE tubing served as vessels to hold human whole blood. PE tubing is appropriate for use in point-of-care diagnostics in developing countries because it is (i) lightweight, (ii) flexible, (iii) suited for transport by field workers over rough roadways and paths, (iv) easily disposable (*e.g.*, does not require sharps containers, and can be incinerated with trash), (v) safer than syringes, needles, and glass capillaries (*e.g.*, poses little risk of injury from puncture since the tubing is soft and pliable), (vi) easily sealed (by melting or pinching), (vii) transparent (enabling visual inspection of plasma), (viii) easily cut into multiple segments for multiple assays, (ix) flexible and tough, and therefore essentially unbreakable if it hits a surface while being spun, and (x) self-aligning in the direction perpendicular to the axis of rotation. By centrifuging multiple sealed tubes at once, one can increase the number of samples analyzed at a time, and thus decrease the physical effort required per sample. We have centrifuged up to 20 tubes at once.

A rubber bulb drew commercially-available human blood (containing EDTA) into PE tubing. (In the field, whole blood obtained from a finger prick (~100 μL) can be drawn into PE tubing; anticoagulants are not required when only plasma from whole blood is desired). Commercially-available blood provided a large volume (50 mL) of single-donor blood for use in multiple experiments, and made it possible to carry out replicate experiments.

Sealing both ends of the PE tubing by holding each end over the flame of a candle for one second, and pressing the melted ends between two rocks, or with pliers, for three seconds, prevented leakage of blood from the PE tubing. A 2 cm × 2 cm strip of adhesive tape (Fig. 2B) attached the filled PE tubing to the egg beater (perpendicular to the paddle).

Centrifugation of whole blood with an egg beater

A hand-powered egg beater separated plasma from whole blood in PE tubing by centrifugation. (The particular type of egg beater we used is sold widely in India for 105 rupees—approximately $2.50 USD.) The manual egg beater represents a suitable device for use in IDCs because it (i) is low-cost (and therefore unlikely to be stolen from health workers), (ii) requires no electricity, (iii) is sturdy enough to be sterilized and cleaned with boiling water, (iv) does not require extensive training or instruction for use, (v) is locally available, and (vi) can be repaired easily without specialized tools.

The egg beater comprises a large gear that is connected to smaller gears that spin two paddles. We removed one paddle in order to attach the PE tubing to the remaining paddle (Fig. 2A). In our egg beater, the large gear had 72 teeth and the small gear had 12 teeth. Rotating the handle of the egg beater at 200 revolutions per minute (RPM), which most users found to be a comfortable pace, caused the tube containing the blood to rotate at 1200 RPM (~280 × *g* at the most distal point of tubing, which is 12.5 cm away from the axis of rotation). The cells were initially distributed uniformly throughout the blood. Human

blood cells are more dense (1.097 g mL^{-1})[12] than the human blood plasma (1.023 g mL^{-1}),[13] so, after ten minutes of centrifugation, a pellet of cells formed at the distal end of the tubing, and the plasma (Fig. 3C) remained as the supernatant.

Mathematical solution for the time required to sediment cells by centrifugation

We modeled the sedimentation of human blood cells due to centrifugation as the motion of a spherical particle suspended in a rotating vessel. In a rotating vessel, two forces act on a spherical particle of radius R (m) and density ρ_p (kg m^{-3}), that is suspended in a medium of density ρ_m (kg m^{-3}), and dynamic viscosity η (kg m^{-1} s^{-1}) (Fig. 2B). These forces are gravity, $\vec{F}_g$, and the Stokes force, $\vec{F}_s$, which is due to the viscous drag exerted by the suspending medium on the particle. The balance of forces is given by eqn (1),[14] where m (kg) is the mass of the particle and $\vec{a}$ (m s^{-2}) is its acceleration in an *inertial* frame of reference.

$$m\vec{a} = \vec{F}_g + \vec{F}_s \tag{1}$$

The balance of forces (eqn (1)) in the frame of reference rotating with the vessel at an angular velocity of $\vec{\omega}$ (rad s^{-1}) is given by eqn 2, where $\vec{v}^*$ is the velocity, and $\vec{a}^*$ is the acceleration of the particle in the rotating frame of reference, and $\vec{g}$ is the acceleration of gravity.[14,15]

$$m\vec{a}^* = \frac{4\pi}{3}(\rho_p - \rho_m)R^3\vec{g} - 6\pi\eta R\vec{v}^* - \frac{4\pi}{3}(\rho_p - \rho_m)R^3 \left(2\vec{\omega} \times \vec{v}^* + \vec{\omega} \times (\vec{\omega} \times \vec{r}) + \frac{d\vec{\omega}}{dt} \times \vec{r}\right) \tag{2}$$

In this equation, the first term on the right-hand side of the equation is the force of gravity corrected for buoyancy, and the second term is the Stokes force. The third term in eqn (2) is a sum of three *fictitious* forces (centrifugal, Coriolis, and Euler forces) arising from our use of a non-inertial, *accelerating* reference frame (the frame of reference rotating with the vessel) to describe the motion of the suspended particle.[15] In this equation, we also accounted for the effect of these inertial forces on the suspending medium, as we did with the buoyant force and gravity. The component form of eqn (2) is shown by eqn (3), where we assume that the particle is traveling with its terminal velocity ($\vec{a}^* = 0$), and that the angular velocity of the rotating vessel, $\vec{\omega}$, does not change with time.

$$\begin{pmatrix} -6\pi\eta R v_x^* - \frac{4\pi}{3}(\rho_p - \rho_m)R^3(2\omega v_y^* - \omega^2 x) \\ -6\pi\eta R v_y^* - \frac{4\pi}{3}(\rho_p - \rho_m)R^3(-2\omega v_x^* - \omega^2 y) \\ \frac{4\pi}{3}(\rho_p - \rho_m)R^3 g - 6\pi\eta R v_z^* \end{pmatrix} = \vec{0} \tag{3}$$

Eqn (4) shows the solution of the X and Y components of eqn (3) for v_x^*—the velocity of the particle along the X axis, coaxial with the long axis of the rotating vessel:

$$v_x^* = \frac{\alpha\omega}{2(1+\alpha^2)}x - \frac{\alpha^2\omega}{2(1+\alpha^2)}y, \text{ where } \alpha = \frac{4}{9\eta}(\rho_p - \rho_m)R^2\omega \tag{4}$$

We can estimate the dimensionless parameter α ($\alpha = \frac{2}{9}\sqrt{Ta}$, where Ta is the Taylor number characterizing the importance of inertial forces relative to viscous forces) using the actual parameters of the system: $\eta = 4.02 \times 10^{-3}$ (kg m^{-1} s^{-1}),[13] $\rho_p = 1097$ (kg m^{-3}), $\rho_m = 1023$ (kg m^{-3}), $R = 3.5 \times 10^{-6}$ (m),[16,17] and $\omega = 126$ (rad s^{-1}) (or about 1200 RPM), as shown in eqn (5).

$$\alpha = \frac{4}{9\eta}(\rho_p - \rho_m)R^2\omega \approx 6.4 \times 10^{-6} \tag{5}$$

Because parameter α for our system is so small (eqn (5)), we can safely neglect the second term of eqn (4) that is proportional to α^2; eqn (4) then becomes eqn (6).

$$v_x^* = \frac{\alpha\omega}{2}x, \text{ where } \alpha = \frac{4}{9\eta}(\rho_p - \rho_m)R^2\omega \tag{6}$$

We can use eqn (6) to predict the amount of plasma we can obtain by centrifuging a piece of tubing (inner diameter, $d = 1.57 \times 10^{-3}$ (m), total length, $L = 0.05$ (m)) filled with ~100 μL of whole blood at 1200 RPM (angular velocity, $\omega = 126$ (rad s^{-1})) for a set period of time, t_0 (s). To perform this calculation, we integrate eqn (6) in time to find the distance, l (m), a suspended particle—a red blood cell—would be able to travel from its initial position, x_0 (m), at the top of the tubing (closest to the axis of rotation) towards the distal end of the tubing (eqn (7)).

$$l = x_0\left(\exp\left\{\frac{2}{9\eta}(\rho_p - \rho_m)R^2\omega^2 t_0\right\} - 1\right) \tag{7}$$

The volume of cleared plasma, V_{plasma} (m^3) is then given by eqn (8) and the fraction of cleared plasma, $f_{cleared}$, is given by eqn (9).

$$V_{plasma} = \frac{\pi}{4}d^2x_0\left(\exp\left\{\frac{2}{9\eta}(\rho_p - \rho_m)R^2\omega^2 t_0\right\} - 1\right) \tag{8}$$

$$f_{cleared} = \frac{x_0}{L}\left(\exp\left\{\frac{2}{9\eta}(\rho_p - \rho_m)R^2\omega^2 t_0\right\} - 1\right) \tag{9}$$

We examined the volume of plasma obtained as a function of time for tubing filled with 100 μL of whole blood placed at $x_0 =$ 7.5 cm. Fig. 4 is a plot of the volume of plasma obtained *versus* time of centrifugation (experimental and predicted values from eqn (8)). The error bars represent one standard deviation. The volume of plasma obtained experimentally followed closely the predicted volumes; the difference between predicted and experimental values was primarily due to the inconsistent RPM between the users. For $x_0 = 7.5$ cm and tubing filled with 100 μL of blood, we obtained a maximum of ~58 μL of plasma after 8 minutes of centrifugation. This maximum value is consistent with the maximum amount of plasma we would expect to obtain from 100 μL of whole blood (plasma is ~55–60% of whole blood by volume). Our theoretical model cannot predict a maximum volume of plasma because it assumes an infinitely long tube containing an infinite amount of blood. Without centrifugation (sedimentation only by gravity), we obtained ~27 μL of plasma after 4 hours.

The total cell count in the plasma after centrifugation with the egg beater was ~50 000 cells mL^{-1} (Fig. 3A and 3B); this

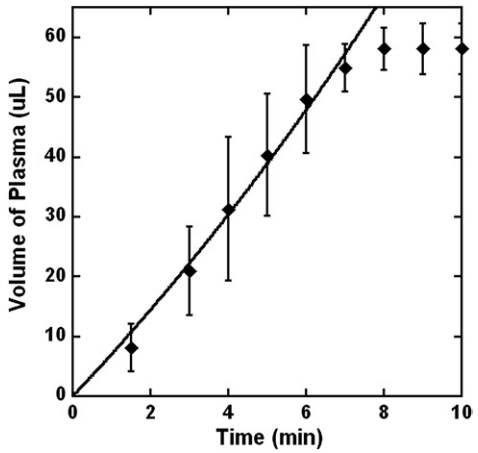

Fig. 4 Length of time of centrifugation required to obtain plasma from 100 mL of human blood. A maximum volume of plasma (~60 mL) is obtained after 8 minutes of centrifugation. The line represents the volume of plasma obtained as calculated by the model. Diamonds ($x_0 = 7.5$ cm) represent experiment results.

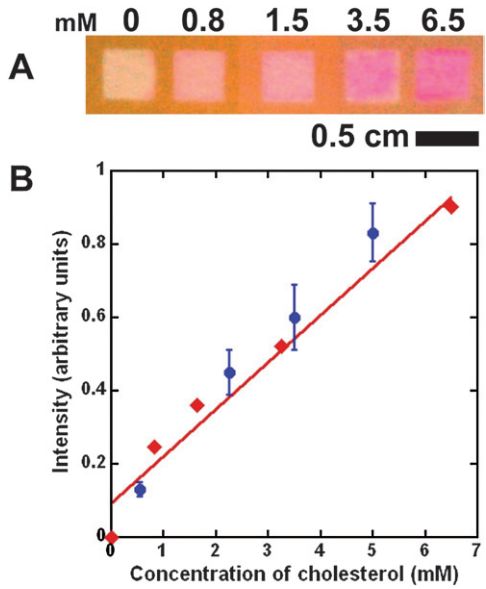

Fig. 5 Colorimetric calibration curve. (A) Photograph of patterned paper that contained samples of cholesterol of known concentration and Amplex Red and cholesterol oxidase. (B) A plot of the concentration of cholesterol *versus* intensity of Amplex Red. The samples of cholesterol in PBS ranged from 0–6.5 mM (red diamonds). The line represents the curve fit. The blue dots represent the intensity of the Amplex Red for plasma that was doped with additional aliquots of cholesterol. The error bars represent one standard deviation.

quantity is comparable to the density of cells obtained by centrifuging whole blood for 5 minutes at 150 × *g* with an electrically-powered, bench-top centrifuge.

Cholesterol assay on patterned paper

Assays on paper are appropriate for resource-poor settings because paper is inexpensive, widely available, and easy to store and to transport. Paper-based devices absorb aqueous solutions by capillary action, and therefore, do not require an external power source to be filled. Also, disposal of the assay can be achieved by burning. Martinez and coworkers have detected protein and glucose content in artificial urine using regions of hydrophilic paper, separated by lines of a hydrophobic polymer.[11] We used a similar method to pattern a grid of hydrophobic lines on paper.

We analyzed the concentration of cholesterol in the plasma using paper patterned with a grid of hydrophobic lines. We detected cholesterol in plasma using a colorimetric assay based on the oxidation of cholesterol by cholesterol oxidase.[18] The reaction of cholesterol oxidase and cholesterol yields hydrogen peroxide (H_2O_2) and 4-cholesten-3-one; the reaction of horseradish peroxidase (HRP) and Amplex Red (Invitrogen), in the presence of H_2O_2, results in a pink color. The intensity of the pink color is proportional to the amount of H_2O_2 produced, and thus to the amount of cholesterol. An increase in the concentration of cholesterol (above normal levels) and of its precursors in human plasma is indicative of medical conditions, such as cardiovascular disease,[19] diabetes mellitus,[20] and severe birth defects (*e. g.*, Smith–Lemli–Optiz syndrome).[21]

To create a calibration curve, we diluted a solution of 6.5 mM cholesterol in the phosphate-buffered saline (PBS), and spotted 1.5 μL of each dilution onto the hydrophilic square regions of patterned paper that were previously pre-spotted with reagents for the colorimetric assay for cholesterol (Fig. 5A). After incubation of cholesterol on the square regions of paper for 5 minutes, we then photographed the paper, imported the images to Adobe Photoshop, and extracted the intensity of each square. Normalization of the intensity was performed by subtracting the intensity of the Amplex Red reagent from the square containing no cholesterol (only PBS) and dividing all values by the maximal value of intensity. The intensity of the Amplex Red reagent increased linearly with the concentration of cholesterol (Fig. 5B): $Intensity = 0.103 + (0.126)C$, where C is the concentration of cholesterol (in mM).

After centrifuging whole blood using the egg beater for 10 minutes, we used scissors to cut the tubing at the interface between the supernatant and the plasma, and then extracted plasma from the tubing by bringing the PE tubing into contact with patterned paper that was pre-spotted with reagents for the cholesterol assay—this method of extraction did not require expensive pipettes and pipettors. The segment of tubing remained in contact with the paper to allow the paper to wick the plasma (< 1 second). We then allowed the plasma to incubate on the paper for 5 minutes at room temperature, imaged the paper with a digital camera, and extracted the intensity of squares in the patterned paper.[22] The calibration curve determined the concentration of cholesterol in the commercially-available whole blood (~0.67 mM).

We also added aliquots of cholesterol into human whole blood and compared the actual concentration of cholesterol with the expected concentration by using the calibration curve. The added cholesterol to the blood increased the expected concentrations of cholesterol to ~2.5 mM, 3.5 mM, and 5 mM. We spotted the cholesterol-doped plasma onto reagent-loaded, square regions of paper, allowed it to incubate for five minutes, and then imaged the squares using a digital camera. We extracted the intensity in Adobe Photoshop and compared those intensities to the

theoretical concentration of cholesterol for a given intensity of Amplex Red. The measured intensity of Amplex Red of the cholesterol-doped plasma increased with the concentration of cholesterol and corresponded to the expected concentration of cholesterol for a given intensity (Fig. 5B).

Conclusions

This paper demonstrates the separation of plasma from whole blood using a hand-powered centrifuge. Plasma isolated with centrifuges that do not require electrical power enables the detection of analytes in plasma in a field setting (away from laboratory facilities). We used the plasma in a cholesterol assay run on paper. The plasma can also, in principal, be used in assays to diagnose other infectious diseases, such as Hepatitis B[23] and cysticercosis,[24] which depend on plasma for rapid diagnostic tests.

Isolation of plasma typically requires the transportation of samples of blood to central health facilities and the use of an electrically-powered centrifuge to remove blood cells. This hand-powered centrifuge enables health workers to isolate plasma in the field, and to use existing rapid, diagnostic tests that depend on plasma. This rapid diagnosis at the point of care decreases the amount of time required to return a diagnosis to a patient and decreases the delay between diagnosis and treatment. This hand-powered centrifuge also reduces the burden upon health workers, who are often limited by an inadequate workforce and insufficient medical supplies. The isolation of plasma at the point-of-care enables health workers to travel to rural communities without spending additional time traveling back to centralized laboratory facilities to obtain a diagnosis. The egg beater is a non-traditional medical supply that is widely available. Point-of-care diagnosis also increases access to villagers who would be otherwise unable to travel to central health facilities to receive diagnosis and treatment. Although more point-of-care diagnostics are needed in the field, we believe a tool to isolate plasma from whole blood enables further development of diagnostics that rely upon plasma.

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