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Self-powered disposable prothrombin time measurement device with an integrated effervescent pump

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ABSTRACT

Coagulation is an essential physiological activity initiated by the interaction of blood components for clot formation. Prothrombin time (PT) measurement is a clinical test for the assessment of the extrinsic/common pathways of coagulation cascade. Periodic measurement of PT is required under numerous conditions including cardiovascular disorders. We present a self-powered microfluidic device for quantitative PT measurement from 50 µl whole blood. The entire platform is disposable and does not require any external pumping, power, or readout units. It consists of a 3D-printed effervescent pump for CO_2 generation from a chemical reaction, a cartridge for two-channel fluid flow (blood and water), and a grid for the quantification of fluid migration distance. Following the introduction of the fluids to the corresponding channel inlets, marking the coagulation start, an acid-base reaction is triggered for gas generation that drives the fluids within the channels. When the blood coagulates, its flow in the channel is halted. At that point, the distance water has travelled is measured using the grid. This distance correlates with PT as demonstrated through clinical tests with patient samples. This single-unit device has a potential for rapid evaluation and periodic monitoring of PT in the clinical settings and at the point-of-care.

1. Introduction

Blood coagulation is a dynamic hematological activity necessitating the interplay of numerous plasma proteins, cells, and coagulation factors for the formation of cross-linked fibrin networks to prevent bleeding [1,2]. The coagulation cascade is triggered by either extrinsic or intrinsic pathways through the activation of tissue factors or surface, respectively, and leads to a common pathway where stable fibrin strands are formed from fibrinogen [3,4]. Prothrombin time (PT) test is frequently performed in clinics for the evaluation of the extrinsic and common pathways of the coagulation cascade [5]. Sensitive and periodic measurement of this parameter is critical for patients who are under pre-, peri-, and post- operative monitoring or undertaking anticoagulant therapy for the regulation of the clotting status by the blood-thinning drugs such as a vitamin K antagonist Coumadin® [6,7]. People with coagulation disorders, atrial fibrillation, pulmonary embolism, myocardial infarction, and several other cardiovascular diseases as well as heart valve prosthesis need lifelong anticoagulant therapy [8,9]. However, the medications for the therapy have narrow therapeutic windows [10]. Therefore, the required dose varies among individuals and fluctuates for the same patient on a daily basis because of diet and genetic background [10,11]. The failure to receive maintenance doses may result in either hemorrhage or thrombo-embolism, which are both life-threatening [12,13]. The conventional practice to monitor the course of the therapy is hospital visits at fixed intervals for PT measurements by benchtop devices. This practice, on the other hand, is time-consuming and expensive, and delayed visits may end up with unfortunate consequences.

Point-of-care devices have been developed to address the issues associated with the benchtop counterparts in clinical settings [14]. Commercial coagulation time measurement devices have been launched to decrease the test turnaround times [15]. Different measurement principles are being utilized including electrochemical detection (CoaguChek XS), mechanical measurement by a cantilever (CoagMax), or optical detection of the cease of blood flow (Hemochron Signature) [16,17]. These products consist of a main device and disposable test cartridges that contain the necessary reagents, electrodes, magnetic particles, or MEMS structures depending on the measurement principle. Even though these products have a potential for point-of-care testing to facilitate timely intervention in case of emergency, the costs of the main devices make them unaffordable for the majority. Since the health insurances in most of the countries (including the authors' homeland)

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have limited coverage for such devices, the patients are dependent on clinics or anticoagulation centers for their periodic PT measurements. The tests are even more inaccessible for those living in resource-poor regions. The microfluidics community is aiming to develop novel methodologies for the PT measurements such as impedimetric [18], aptamer-based [19], paper-based [20], cantilever-based [21], quartz crystal microbalance (QCM) [22], centrifugal [23] and fluorescence-based [24]. These studies, on the other hand, generally have a long way to compactness and lack clinical validation tests, which are necessary for commercialization.

The requirement to employ external components such as pumps, power sources, and control circuitry increases the cost and complexity of lab-on-a-chip platforms while limiting their portability [25]. Miniaturized integration of these components is desired to make these platforms appropriate for point-of-care use [26]. Apart from the frequently used pressure or syringe pumps, the liquid handling is aimed through micropumps categorized as non-mechanical (electrowetting, electrokinetic, electrochemical) and mechanical (rotary, peristaltic, diaphragm) [27,28]. Of the first category, chemical pumps have recently become popular for the construction of self-powered platforms due to their simple fabrication and integration [29]. Examples include the decomposition of H_2O_2 or N_2H_4 in the presence of metal substrates (e.g., Ag, Au, Pt, Pd) for self- electrophoresis [30] or electroosmosis [31], respectively. Methanol is decomposed in a microfluidic device to generate CO_2 for fluid pumping [32]. Differing from this trend, chemical pumps can also be designed without any need for microfabricated substrates. Effervescent tablets used in medical treatment are composed of an acid and a base. Before intake, these tablets are dissolved in water generating CO_2 bubbles. As shown in this work, effervescent micropumps can employ acid-base neutralization reactions in aqueous solutions that generate enough CO_2 for long durations to drive fluids in microfluidic systems for a variety of applications. These effervescent micropumps yield predictable pressures, with rapid response times and long durations at steady-state values, that are firmly controlled by the type and the molarity of the reactants used in the reactions [33].

In this manuscript, we present a portable and entirely disposable microfluidic device for point-of-care PT measurement from 50 µl whole blood in less than 2 min. The device is lightweight (50 g) and does not require any external components. Even though the device is designed for self-evaluation of the test result, integration of a cell phone makes it an ideal telemedicine application if needed. The key element of this self-powered system is an integrated effervescent pump where an acid-base neutralization reaction generates CO_2 gas that supplies pressure for liquid flow within the two-channel test cartridge. The sample channel is for whole blood, whereas the reference channel is for DI water. The assay principle is based on the measurement of the distance travelled by water using an integrated grid upon the cease of the blood flow due to coagulation. We explained the fabrication steps of the 3D-printed effervescent pump and characterization results of the generated pressure and settling time of the pump for different acid and base molarities. Comparative tests were performed at a local clinic using a conventional benchtop device to show the potential of our system for reliable and quantitative PT measurements.

2. Experimental section

2.1. Materials

We obtained ethical approvals from the Ethics Committees of both Yildirim Beyazit University Medical School and Bilkent University. We received informed permission from all the blood donors. We acquired 3 ml intravenous whole blood from each volunteer into vacuum tubes containing 3.2% sodium citrate anticoagulant at most 6 h before the tests. The samples were incubated at 37 °C before use. Thromborel S Human Thromboplastin was purchased from Siemens as the coagulation activator reagent. 47.5 mg Thromborel S Human Thromboplastin was dissolved in 250 µl DI water at room temperature. 10 µl of the solution was pipetted into the inlet of the sample channel of each cartridge, and the cartridges were left at −80 °C for 2 h. The reagents in the cartridges were then lyophilized in a freeze-dryer (Labconco, US) for 6 h, and the cartridges were stored at 4 °C until their use.

Citric acid and sodium bicarbonate were purchased from Sigma-Aldrich and stored at room temperature. Coverslips (20 mm x 20 mm, thickness: 0.15 mm) were purchased from neuVitro. Silicone (ID: 1/32 in. and tygon (ID: 3/32 in. tubings were purchased from Cole Parmer. Blood lancet was purchased from Hema-Lab Ltd. Dual bulb exact volume pipettes (50 µl) were purchased from Alpha Laboratories Ltd. Liquid sodium citrate as an anticoagulant was passed through the exact volume pipettes, and the pipettes were left to dry at room temperature overnight.

2.2. Cartridge fabrication

The test cartridge design was drawn in a CAD software (Autodesk, AutoCAD 2016). Transparent 3 mm thick polmethyl methacrylate (PMMA) sheet was obtained from a local supplier, and the cartridges were fabricated out of PMMA using a 30 W CO_2 laser cutter (Epilog, Zing) [34]. The feed rate, power, and frequency of the cutter were set to 2.6 cm/s, 2.4 W, and 1000 Hz, respectively. The cartridge has a 34 mm x 127 mm rectangular structure. It has two inlets for two separate serpentine channels. One channel is for the reference fluid (DI water), and the other channel is for the sample fluid (whole blood). The inlet chamber volumes are 85 mm^3, and the outlet chamber volume is 16 mm^3. The serpentine channels have 80 µm height and 250 µm width.

The Hagen-Poiseuille law for a steady, laminar, and Newtonian flow states that

$$\Delta P = QR \tag{1}$$

where ΔP is the pressure difference, Q is the volumetric flow rate, and R is the channel resistance [35]. For rectangular channels, the hydrodynamic resistance for $0 < h/w < 1$ is approximated as

$$R = \frac{12\eta L}{wh^3}\left[1-0.63\frac{h}{w}\right]^{-1} \tag{2}$$

where η is the dynamic viscosity, L is the channel length, w is the channel width, and h is the channel height. Also,

$$Q = \frac{wh\,\Delta L}{\Delta t} \tag{3}$$

where t is the time. Plugging Eqs. (2) and (3) into Eq. (1), we have

$$L = \sqrt{\frac{th^2\Delta P}{12\eta}\left[1-0.63\frac{h}{w}\right]} \tag{4}$$

DI water dynamic viscosity is lower than whole blood apparent viscosity, so the water flows faster in a channel in a given time interval [36]. Therefore, the length of the serpentine channels is found by taking the water flow into consideration. The clotting time for a whole blood sample is around 110 s at max. The channel length should be sufficiently long so that water does not reach the outlet until the coagulation, i.e., $t = 110\,s$. ΔP generated by the effervescent pump is $195\,mbar$ as explained in the next section. Also, $h = 80\,\mu m$ and $\eta = 8.9x10^{-4}\,Pa.\,s$. Plugging these parameters in Eq. (4), the length of the serpentine channel is calculated as 1100 mm.

2.3. Effervescent pump fabrication

The effervescent pump consists of four polymer layers and a coverslip as shown in Fig. 1. The four layers were designed in a CAD software (SolidWorks 2016) and fabricated by a 3D-printer (M200, Zortrax) with 100% infill. The filament was acrylonitrile butadiene styrene (ABS), a thermoplastic polymer used as a

common 3D printing material [37]. The 1st printed layer contains a