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# Rapid and even spreading of complex fluids over a large area in porous substrates

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## ABSTRACT

Rapid and even spreading of complex fluids over a large area on substrates like paper is required for chemical and biological sensing applications. Non-Newtonian flow behavior and the presence of multi-phase components pose a significant challenge to uniform flow in porous media. Specifically in the case of blood, for biosensing applications, fast spread on a large area is required to avoid coagulation and non-uniform component spread. In this work, we have developed a filter paper-based device to resolve this spreading challenge. We sandwich the filter paper between a matrix of nanofibrous membrane backed by polyethylene terephthalate (PET) sheets, forming a multi-scale porous network: one within the filter paper and the other between the PET sheet and the filter paper. By doing so, we decrease the overall resistance to flow while maintaining the same capillary suction pressure to obtain a quick, uniform spread of dyed liquids, milk solutions, and whole blood. The device design and concepts used here can be used in paper microfluidic applications and to develop devices for dried blood spot analysis, which utilize this fast flow while maintaining even spreading over a large area.

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Lab-on-chip devices have emerged as useful tools for applications in rapid and efficient analytical chemistry, sensing and diagnostics, and bioengineering.<sup>1–3</sup> These devices primarily depend on a power source to drive small volumes of liquid samples in specially designed micro/nanochannels to achieve their objectives. Here, paper microfluidics has emerged as a viable alternative to create lab-on-chip devices, which can drive liquids passively using capillary suction.<sup>4</sup> The advantages offered by paper-based devices are that they are simple to operate, amenable to scale-up manufacturing, easily deployable in resource constraint places, and cost-effective.<sup>5</sup> To develop devices for applications in point-of-care diagnostics,<sup>6</sup> it is important to develop devices that can passively drive complex fluids, like blood and saliva. Here, fluid properties like non-Newtonian flow behavior and interaction of active and passive biomatter with the interconnected pores of a paper present significant variability in flow behavior.

Several works have addressed fluid flow enhancements in porous substrates,<sup>7</sup> primarily for Newtonian fluids, by either altering the base porous material properties<sup>8,9</sup> or novel device designs.<sup>10,11</sup> These methods primarily alter the fundamental passive transport properties, such

as increasing capillary suction pressure or reducing resistance to fluid flow. Strategies such as the use of alternate materials (e.g., fibrous paper,<sup>8</sup> hydrogels,<sup>12</sup> and cellulose<sup>13</sup>) pore interconnectivity,<sup>14</sup> designed pore gradients,<sup>15,16</sup> evaporation-assisted pumping,<sup>17,18</sup> integration of hydrophilic backing materials,<sup>19</sup> and layered substrate designs<sup>20,21</sup> have shown significant success in transporting Newtonian fluids, with a potential of extrapolation to complex fluids. However, very few designs address flow nature of complex fluids like blood,<sup>22–24</sup> semen,<sup>25</sup> and saliva,<sup>26</sup> which have significant implications in bioanalysis for diagnostic and pharmaceutical applications.

In this work, we present a device that enhances the capillary suction of complex colloidal liquids in a normal filter paper. We utilize a multi-scale porous network design that retains the capillary suction of the filter paper while easing the flow of fluid through an integrated relatively large pore network. We show that this device enhances the imbibition for both Newtonian and non-Newtonian liquids by using dyed water, skimmed milk solution in water, and whole blood. The device not only allowed an enhanced flow rate but also enabled even spreading of the colloidal liquids. We explain these observations using

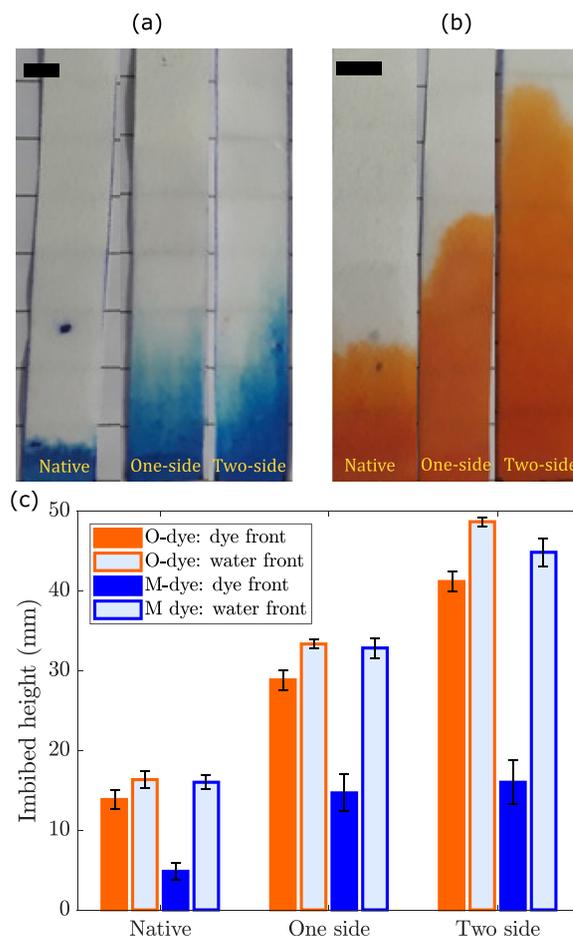
an analytical model employing Darcy's law and a hydraulic circuit analogy. The proposed concept and designs have implications in increasing the sensitivity and reliability of paper-based sensors and diagnostic devices.

A polymer solution of polycaprolactone (PCL) and porcine skin gelatin was prepared for electrospinning. First, two separate solutions of PCL and gelatin in 2,2,2 trifluoro ethanol solvent were prepared (12 wt. %/vol). Both the solutions were kept for overnight stirring at room temperature (IKA C-MAGHS7 digital). After the overnight stirring, both solutions were mixed followed by an addition of 50  $\mu\text{l}$  of pure glacial acetic acid to generate a clear, transparent PCL/gelatin solution. The polymer solutions were fed into a 5 ml clinical syringe, and electrospinning was carried using a computer-controlled electrospinning apparatus (ESPIN NANO, Physics Instruments Co.) with the following parameters: flow rate: 0.5 ml  $\text{h}^{-1}$ , voltage: 10 kV, needle gauge: 24, and distance between electrodes: 150 mm. The nanofibers were deposited on a thin cellulose acetate (PET) sheet, which were then laid over a thin strip of Whatman filter paper (width: 10 mm and height: 60 mm. This method was followed to prepare the one-sided and two-sided samples. The dye solution of methylene blue dye (M-dye) and orange food dye (O-dye) in water (1 wt. %/vol) was prepared to study the thin layer chromatography (TLC) effect in filter paper. The simulated blood sample was prepared by adding milk (skimmed milk powder) and de-ionized water to form solutions of different densities (0–50 wt. %/vol) to study the capillary rise through filter paper. The solutions with different milk concentrations were sonicated for 10 min to obtain a homogenous solution.<sup>27</sup> Gift blood samples were received from a pathology lab owned by Molecular Solutions Care Health LLP, Bangalore.

For the spreading studies, the devices were held vertically with the help of a clamp stand. The liquid reservoir was placed on a lab jack for manual vertical movement. The reservoir is slowly raised to contact the device. After 60 s from the time of first contact, the reservoir is promptly lowered and the height achieved by liquid in the filter paper is noted. This experiment is repeated multiple times for each of the liquids (O-dyed and M-dyed water, milk solutions, and whole blood) for each of the three device designs: native filter paper, filter paper supported on one side by an electrospun nanofiber sheet (one-sided), and filter paper sandwiched between electrospun nanofiber sheets (two-sided).

Figure 1 shows the imbibed length of dyed water in the different substrate designs after 1 min. The sandwich designs are clearly seen to drive a higher volume of liquid compared to the bare filter paper substrate. The dyed solutions undergo a chromatographic separation, wherein the water wetting front advances further leaving a separate dye front. The methylene blue dye has a higher molecular weight and, therefore, imbibes a shorter distance compared to the orange dye. As a result, the difference in the imbibed height of the wetting front and the dyed front is less significant for the orange dye compared to the methylene blue dye, as seen in Fig. 1.

The enhancement in flow in the sandwiched designs compared to the native filter paper designs can be qualitatively explained by observing the porous network structure in the design (Fig. 2). The small pores in the filter paper generate a high capillary pressure, while also restricting the flow, which leads to a slow moving wetting front. By placing a nanofibrous membrane (with larger pore sizes than the filter paper), the sandwich designs retain the high suction pressure of the filter paper and provide an easier pathway to the flow within the fibrous membrane structure, supported by a hydrophilic backing



**FIG. 1.** Vertical imbibition results with dyed water: (a) methylene blue dye (M-dye) in de-ionized water and (b) orange dye (O-dye) in de-ionized water; the scale bar is equivalent to 5 mm (c) position of the wetting front recorded after 60 s from the start of imbibition for the native filter paper and one-sided and two-sided sandwiched filter paper designs. The plot shows the dye front and the water wetting front due to chromatographic separation of the dye.

material. Subsequently, through this multi-scale porous network, we obtain a much faster spread in the sandwich designs as compared to the native filter paper.

To quantify the difference in imbibition characteristics of the three designs, we use Darcy's law for characterizing the flow in the porous media,<sup>28</sup>

$$v = \frac{k}{\mu\phi} \nabla p, \quad (1)$$

where  $v$  is the velocity of the wetting front,  $k$  is the permeability of the liquid in the substrate,  $\mu$  is the dynamic viscosity of the liquid,  $\phi$  is the porosity of the substrate, and  $\nabla p$  is the pressure gradient driving the flow. In our vertical imbibition setup (against gravity), the flow is driven by the capillary pressure in the pores of the filter paper ( $p_c$ ). As a result, Eq. (1) can be written as

$$\frac{dh}{dt} = \frac{k}{\mu\phi} \frac{(p_c - \rho_l g h)}{h}, \quad (2)$$

where  $h$  is the height of the wetting front,  $\rho_l$  is the density of the liquid,  $g$  is the acceleration due to gravity, and  $p_c = \rho_w g h_c$ , with  $\rho_w$  being the density of water and  $h_c$  the capillary pressure head.<sup>18</sup> Assuming the density difference to be comparable, i.e.,  $\rho_l \approx \rho_w$ , the solution to Eq. (2) is obtained as

$$\frac{h}{h_c} + \log\left(1 - \frac{h}{h_c}\right) = -\frac{k g \rho_l}{\phi h_c \mu} t. \quad (3)$$

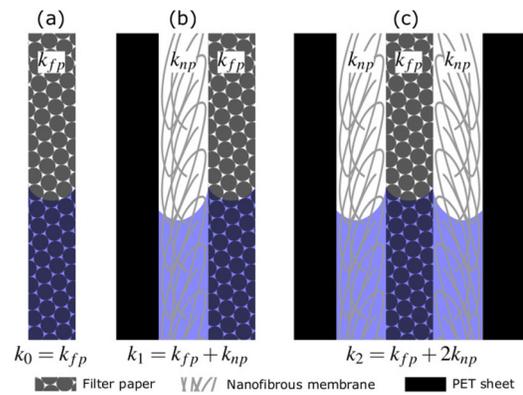
By using Eq. (3), we can obtain the value of permeability  $k$  for the three substrate designs. For an appropriate design comparison across different complex liquids, it is important to isolate the value of  $k$  from the other parameters. However, isolating this value of  $k$  accurately is extremely challenging, as evaluating the other parameters requires measuring additional material properties ( $\phi, h_c$ ) and liquid properties ( $\rho_b, \mu$ ) accurately through different experiments, which are highly sensitive in their setup and measurement.<sup>29,30</sup> We also avoid the use of equivalent models, like the Kozeny–Carman capillary model<sup>31,32</sup> to calculate and compare the permeability for these designs as such models are approximations and require information about the porous medium’s microscopic properties like pore geometry and tortuosity, which are difficult to measure accurately.<sup>33,34</sup> Instead, we use Eq. (3) with our experimental data to calculate the parameter  $K_i = k_i g \rho_l / (\phi_i h_c \mu)$ , where  $i = 0, 1$ , and  $2$  for the native filter paper, single-sided substrate, and double-sided substrate, respectively. For a given liquid, this apparent permeability ( $K_i$ ) is an indirect method of comparing the ease of flow of liquid through the different substrates, calculated from the following equation:

$$\frac{h}{h_c} + \log\left(1 - \frac{h}{h_c}\right) = -K_i. \quad (4)$$

In Table I, we calculate the apparent permeability ( $K_i$ ) for the three substrates for the liquid and dye wetting front for the two dyes shown in Fig. 1 and clearly observe higher values for the sandwich designs. We can understand the increase in apparent permeability for the sandwich designs using the principle of equivalent hydraulic resistance. As shown in Fig. 2, the sandwich designs can be considered as two different porous media arranged in parallel, where the permeability of flow in the native filter paper is  $k_0 = k_{fp}$  and that in the nanofibrous channel is  $k_{np}$ .  $k_{np}$  is obtained from the equivalent permeability of the one-sided design ( $k_1 = k_{fp} + k_{np}$ ), which can then be used to predict the permeability of the two-sided design by  $k_2 = k_{fp} + 2k_{np}$ . This equivalence can also be extended to the parameter  $K_i$  when using data for the same imbining liquids. As seen from the data in Table I, this simplistic equivalent resistance argument (i.e.,  $K_2 \approx K_0 + 2K_1$ ) agrees well with the experimental values for all the wetting fronts

**TABLE I.** Apparent permeability ( $K_i$ ) of the native filter paper ( $K_0$ ) and one-sided ( $K_1$ ) and two-sided ( $K_2$ ) sandwich designs obtained from Eq. (4).

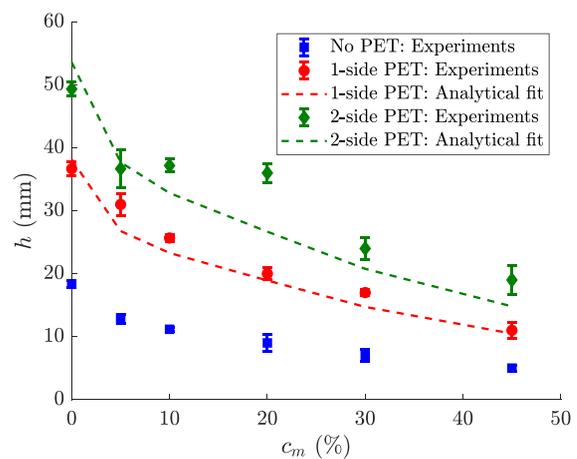
Liquid		$K_0 (\times 10^6)$	$K_1 (\times 10^6)$	$K_2 (\times 10^6)$
O-dye	Dye front	$1.61 \pm 0.07$	$7.07 \pm 0.62$	$14.53 \pm 0.90$
	Liquid front	$2.25 \pm 0.29$	$9.47 \pm 0.33$	$20.40 \pm 0.49$
M-dye	Dye front	$0.19 \pm 0.01$	$1.84 \pm 0.59$	$2.2 \pm 0.77$
	Liquid front	$2.16 \pm 0.23$	$9.19 \pm 0.71$	$17.28 \pm 1.37$



**FIG. 2.** Depiction of the devices used in our experiments and their associated permeability relations: (a) native filter paper, (b) one-sided sandwich design, and (c) two-sided sandwich design.

except the methylene blue dye front (due to early chromatographic separation).

This enhancement in permeability to flow by our sandwich designs is not only limited to Newtonian liquids but also applicable to complex liquids exhibiting a non-Newtonian flow behavior, as shown in Figs. 3 and 4 for milk and blood, respectively. Blood is a complex fluid with multiple particulate and active biomatter, while milk has been shown to exhibit complex non-Newtonian flow behavior at different concentrations in water.<sup>27,35,36</sup> Estimating the flow characteristics of these liquids becomes challenging in porous media as imbibition speed, presence of particulate matter, and protein interaction affect their viscosity and, by extension, resistance to flow. In particular, in blood sampling applications, like dried blood spot analysis, the general variability in hematocrit volume (volume percentage of red blood cells) of blood among individuals poses a challenge during formation of consistent size and uniformity of blood spots. This leads to poor adoption of the dried blood spot technique for any quantitative



**FIG. 3.** Imbibed height  $h$  for different concentrations of milk powder in water ( $c_m$ ) for different substrate types: native filter paper, one-sided sandwiched paper, and two-sided sandwiched paper.

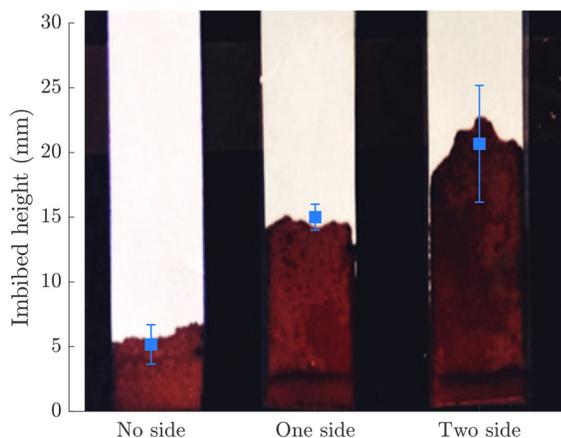


FIG. 4. Comparison of vertical imbibition of blood in the three paper designs.

analysis in pharmaceutical and diagnostic fields.<sup>37</sup> Hence, a qualitative blood flow characterization provides sufficient utility than an accurate quantitative flow characterization for diagnostic applications as such accuracy is very difficult to achieve. Therefore, in order to compare the enhancement in ease of flow of complex fluids in our designs, we retain the above presented simplistic Newtonian approach.

To demonstrate the enhanced permeability of the sandwich designs for each of the liquids with different milk concentrations, we obtain the apparent permeability for the filter paper design, i.e.,  $K_0$ , from their imbibed height after 1 min. Then, using the factors obtained from Table I ( $K_1 \approx 4.3K_0$  and  $K_2 \approx 2K_1$ ), we obtain  $K_1$  and  $K_2$  for each of the liquids (with different milk concentrations) and analytically calculate the imbibed height after 1 min using Eq. (3), and we compare it with the experimental data in Fig. 3. As seen in Fig. 3, the permeability enhancements obtained for dyed water also agree with a wide range of milk solutions, spanning Newtonian and non-Newtonian fluid flow behaviors. Similar agreement is obtained for the devices with blood flow (Fig. 4), where  $K_0 = 0.20 \pm 0.11 \times 10^{-6}$ ,  $K_1 = 1.90 \pm 0.55 \times 10^{-6}$ , and  $K_2 = 3.88 \pm 0.93 \times 10^{-6}$ .

Our presented sandwich device design utilizes a multi-scale porous network to provide an enhanced capillary suction pressure (because of smaller pores) and a low resistance to liquid flow (due to the larger porous matrix). The speed and uniformity of liquid suction in a porous substrate depend on the nature of the liquid. We have shown that our sandwich designs pump both Newtonian and non-Newtonian liquids uniformly at high speeds without significant chromatographic effects. Our initial results on pumping and spreading complex colloidal fluids, like milk and blood, in a filter paper are promising to develop a paper-based diagnostic sensor using the whole blood sample. The potential future of this work lies in integrating this multi-scale porous network design concept in devices for dried blood spots and biosensors. Integration with sensor electronics presents additional questions on the effect of charges on flow and particle behavior. For example, the presence of charges on the paper will increase the hydrophilicity for an enhanced capillary pumping and also provide platforms for bioconjugation of antibodies for sensing applications.<sup>38</sup> The microporous filter paper can also be coated with nanofibrous polymers that can provide additional surface with desired charges for bioconjugation.<sup>39</sup>

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#### DATA AVAILABILITY

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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