Microfluidics made of yarns and knots: from fundamental properties to simple networks and operations†

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We present and characterize cotton yarn and knots as building blocks for making microfluidic circuits from the bottom up. The yarn used is made up of 200–300 fibres, each with a lumen. Liquid applied at the extremity of the yarn spontaneously wets the yarn, and the wetted length increases linearly over time in untreated yarn, but progresses according to a square root relationship as described by Washburn’s equation upon plasma activation of the yarn. Knots are proposed for combining, mixing and splitting streams of fluids. Interestingly, the topology of the knot controls the mixing ratio of two inlet streams into two outlet yarns, and thus the ratio can be adjusted by choosing a specific knot. The flow resistance of a knot is shown to depend on the force used to tighten it and the flow resistance rapidly increases for single-stranded knots, but remains low for double-stranded knots. Finally, a serial dilutor is made with a web made of yarns and double-stranded overhand knots. These results suggest that yarn and knots may be used to build low cost microfluidic circuits.

Introduction

Yarn and textile can be traced back five millennia and were used for clothing and for other practical and decorative purposes. One of the primary functions of textiles is to isolate the carrier from the environment, and in particular from rain and water, and many treatments have been developed to make hydrophobic, water repellent yarns and fabrics. Hydrophilic textiles have also been developed, primarily as microfibre-based fabrics for fast wicking and sweat venting. Natural cellulose cotton fibres can also be made hydrophilic using wet and dry (e.g. plasma activation) processes.

Natural fibres have been used for making microfluidic flow transport in the form of lateral flow devices, which rely on the wicking properties of nitrocellulose membranes (sheets) to transport liquid in predetermined ways. By optimizing the flow speed of the sample based on the biomolecular reaction rates, rapid and highly sensitive bioassays have been developed. This lateral flow technology has been packaged into simple microfluidic devices and over-the-counter diagnostics notably in the form of rapid pregnancy tests. More complex microfluidic circuits have recently been made using chromatography paper and tape. Microchannels were formed by defining hydrophobic barriers in the paper followed by stacking different layers so as to form three dimensional circuits. These 3D paper-based microfluidics are built based on a top-down paradigm and functional elements are patterned on a flat substrate using a sequence of processing steps as in semiconductor microfabrication.

We and others recently proposed yarn as a microfluidic carrier and illustrated its use for making simple microfluidic devices from the bottom up. Li et al. demonstrated the fabrication of three-dimensional microfluidic devices by sewing cotton yarn into polymer sheets, transporting and mixing fluids using tapes and carrying out simple colorimetric assays. Reches et al. independently developed similar devices and studied different treatments to make the yarn hydrophilic, build simple devices by encapsulating the yarn within tapes, or by sewing it into different materials including band aids, and used it for multiplex assays of ketones, glucose, and proteins.

Here, we report our findings on the flow properties of yarn and on using knots for differential mixing and building complex microfluidic networks. We measured the flow resistance of the yarn and the knots and show that the knot topology can be used to control the mixing ratio between two inlets and two outlets. Finally, we built a serial dilutor by iterative combining of mixing and splitting of fluids using a knotted web, analyze the fluid distribution using network analysis concepts developed for electrical circuit analysis, and compare the theoretical and experimental results.

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Materials and methods

Yarn and chemicals

The yarn used here was a white sewing thread obtained from Tehran (Iran) and from mercerized stores locally (100% Cotton yarn Ne 30/1, Mahabad Riss Co, Iran). They were tested for suitable wicking properties before usage. The yarns were first dried in an oven, and made hydrophilic using an air plasma (Plasmaline 415, Tegal Inc., US) for two minutes at 250 mTorr at a power of 150 mW. To obtain the cross-sectional micrographs of the yarns, a 2 cm length of the yarn was soaked with blue dye and encased into poly(dimethylsiloxane) (PDMS; Sylgard 184, Dow Corning), degassed in a desiccator for 6 h, and cured at 65 °C for 8 h in an oven (Lindberg Blue M, Fisher Scientific). A razor blade (single edged razor blade, Fisher Scientific, Canada) was used to cut thin slices.

Food dyes (McCormick & Co., MD, USA) were obtained from local stores. Experiments were performed at a relative humidity of ~30% and at the room temperature of 23 ± 2 °C. Unless indicated otherwise, the yarn was positioned horizontally for all experiments and a sheet of hydrophobic polytetrafluoroethylene (PTFE) was used as a substrate so as to avoid wicking of liquid in the gap between the yarn and the substrate.

Imaging

Images of the flow were captured using a stereomicroscope (Leica MZ8, Leica Microsystems, Switzerland) outfitted with a CCD camera (DS-Fi1, Nikon, Japan). A scanning electron microscope (S-3000N, Hitachi, Japan) was used for structural analysis following coating with a thin Au layer. Cross-sectional images were acquired with an inspection microscope (LV150A, Nikon).

Flow resistance measurement

Polyamide monofilament precision fabrics (Sefar, Lumberton, NJ, USA) with the pore opening of 110 μm and the thickness of 75 μm were employed as a capillary pump because it generates a constant and well defined capillary pressure. The fabric was plasma activated to make it hydrophilic before the measurement. A measurement setup was built by clamping the extremity of the yarn with the fabric between two microscope slides (Fisher Scientific, Canada), and dipping the other extremity of the yarn into a small reservoir. A known volume of solution (typically 9 μl) was added to the reservoir, and the time needed to drain the liquid from the reservoir was measured. Multiple measurements were performed by repeatedly adding solutions of different colours to the reservoir.

Image analysis

To establish the mixing ratios of the dyes by the knots, we used the Image J software (NIH) to extract the hue colour intensity and compared it to a calibration curve established with known mixtures of the different dyes, see Fig. S1 in the ESI†.

Results and discussion

Yarn as a flow carrier

Cotton yarn is widely available, low-cost, and like paper, the main component is cellulose. The results reported in this study were obtained with regular sewing thread made of cotton, Fig. 1A. The yarns are 500 ± 34 μm in diameter and made up of ~200 fibres each 12–15 μm thick, as measured from SEM images, Fig. 1B and C. Imaging of encased cross-sections of yarn reveals that the fibers comprise a lumen, akin to wood cellulose, Fig. 1D and E.

The wicking action of natural fibres is well known, and a red food dye solution spontaneously flowed along a cotton yarn wetting 5 mm after 40 s when using it as received, Fig. 2A and Movie S1†. The flow speed was slow, advanced stochastically, and was sensitive to the relative humidity (data not shown). Yarns are not optimized for capillary flow, and indeed for instance sewing threads are often coated with wax and other additives that improve gliding and prevent tearing of the yarn, but which render it hydrophobic. When using the same yarn following activation and hydrophilization using an air plasma, the liquid advanced 32 mm during the same time, Fig. 2B. Capillary forces generated in the gaps between the fibres drive the flow, and the wetted length L as a function of time t is thus expected to follow Washburn’s equation:  

\[
L = \sqrt{\frac{\gamma \cos \theta D t}{4 \mu}} [m]
\]

where γ is the interfacial tension, θ is the contact angle between the liquid and the yarn surface, D is the effective capillary diameter (yarn comprises multiple parallel, open flow paths), and μ is the viscosity of the liquid. We recorded L as a function of t for both as-received and plasma activated cotton, and fitted the data with a square root function, Fig. 2C, and Movies S1 and S2 in the ESI†. The regression factors are \( R = 0.98 \) for hydrophilic yarn and for yarn used as received \( R = 0.92 \). However, a linear fit yields \( R = 0.99 \) for the as-received yarn indicating that filling progresses linearly over time. The linear filling may be accounted by the complex processes that underlie wetting of cellulosic fibres, and indeed non-Washburn filling speeds have also been reported in the wetting of fibrous materials notably due to swelling of the porous medium. We measured that a liquid volume \( V = 2.5 \mu l \) wetted a yarn with a diameter of ~500 μm over a length of 43 ± 2 mm. Assuming that the geometry of the yarn is cylindrical with a diameter of 500 μm, 70% of the yarn is made of fibres and only 30% are gaps filled with liquid. Moreover, having eqn (1), and the relationship between \( V \) and \( L \), the flow rate of the liquid, \( Q \), may be expressed as:  

\[
Q(t) = \frac{\partial V}{\partial t} = 2.9 \times 10^{-11} \sqrt{\frac{\gamma \cos \theta D}{4 \mu t}} [m^3s^{-1}]
\]

This equation is only valid for the (hydrophilic) yarns for which filling obeys Washburn’s equation.

Evaporation of water from the surface of the yarn

Eqn (2) does not consider evaporation, which is negligible initially, but becomes significant as the wetted length increases...
and the (capillary) flow rate diminishes. Water evaporation from a surface without air flow may be written as:

\[ Q_E = \frac{7.84 \times 10^{-8} \times (P_w - P_a) \times A}{\Delta H_v} \left[ \text{m}^3 \text{s}^{-1} \right] \] (3)

where \( Q_E \) is the volumetric flow rate of water being evaporated from the surface, \( A \) is the surface area of water in contact with air, \( P_w \) is the saturation vapour pressure at the water temperature, \( P_a \) is the saturation vapour pressure at the air dew point, and \( \Delta H_v \) is the latent heat of water at the specific temperature [kJ kg\(^{-1}\)]. To calculate the mass transfer from the surface of the yarn, we used above mentioned equation, assuming that the surface area of the yarn can be approximated by multiplying the wetted length by its circumference; however, in reality, the liquid fills both the gaps between the fibres, and wets each fibre individually, and thus likely represents a larger surface area, and the calculation using the simple circumference represents a lower limit. The values for the parameters in eqn (3) were taken from the thermodynamic tables provided in ref. 22 for a temperature of 20 °C, and a relative humidity of 30%. The evaporation rate thus becomes:22

\[ Q_E = 8.8 \times 10^{-11} \times L \left[ \text{m}^3 \text{s}^{-1} \right] \] (4)

For the yarn used here, the capillary flow and evaporation rate become equivalent for a wetted length of ~90 mm; this constitutes the maximal length that the yarn may be wetted with a humidity of 30%. If we arbitrarily set the maximal allowable ratio between evaporation and capillary flow to 1/3, the maximal useful length of the yarn is ~30 mm. Using the results from the measurements shown in Fig. 2 and on the evaporation rate given in eqn (4), we calculated the theoretical filling speed in the absence of evaporation (see Fig. S2 in the ESI† for more details). Whereas in our experiments 38 were needed to fill 30 mm, only 34 s would be needed in the absence of evaporation, and the yarn could be wetted over much greater length.

For many biological applications, long incubation times are required, and if the flow stops in a yarn with a 500 μm diameter, it will only take 18.3 min to evaporate 50% of the liquid contained in the yarn with a relative humidity of 30% at 20 °C under the assumption that the circumference remains constant. From these results it is clear that evaporation needs to be taken into account when designing yarn based microfluidics. For bioanalytical applications, a long incubation time is often required to achieve a high sensitivity. As a result, in yarn based microfluidics the flow rate will need to be optimized to maximize the sensitivity for a given volume by either keeping the flow path short while continuously resupplying liquid, or increasing the relative humidity of the environment to allow using longer yarns, or then sheathing the yarn with an impermeable coating.

Flow resistance of the yarn

The resistance to flow in a capillary driven conduit can be expressed in terms of the liquid flow rate and the capillary pressure difference \( \Delta P \) between the inlet and the outlet of the channel as:23

\[ R = \frac{\Delta P}{Q} \left[ \text{Pa s m}^{-3} \right] \] (5)

where \( R \) is the flow resistance of the yarn, and \( Q \) is the flow rate. The particularity of capillary flow is that the fluidic conduit also
reached a height of 133

it between two glass slides and measuring the capillary rise, which

pressure of a plasma activated fabric was measured by clamping

flow resistance was used as a capillary pump. The capillary

fabric with a well defined capillary pressure and with a negligible

flow resistance of yarns and knots, a polyamide monofilament

measuring the time needed to flow 9

flow resistance of 25 mm of yarn was determined by repeatedly

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methods section, and found to be:

l of solution through the

outlet yarns; however, when it is rotated 90°, using the two ends

of one of the yarns as inlets, and the other yarn as outlets, the

mixing is almost entirely suppressed, Fig. 3D–F and Movies S5

and S6†. A thin line of green colour appears on the right yarn

outlet as in (A) as-received and (B) plasma activated, hydrophilic cotton yarn.

(C) The wetted length scales as \( L \propto \sqrt{t} \) for the hydrophilic yarn and obeys a Washburn’s relation, and \( L \propto t \) for the as-received yarn reflecting a constant filling speed irrespective of filled length and increasing flow resistance. See also Movies S1 and S2 in the ESI†.

acts as the pump via capillary effects. As the fluid advances, the flow resistance increases as determined by the flow resistance of the conduit. For yarn, neither the flow resistance per unit length, nor the capillary pressure are known, and owing to the various scales of porosity and swelling of yarn the capillary pressure is not well defined nor constant over time.29 Thus, to measure the flow resistance of yarns and knots, a polyamide monofilament fabric with a well defined capillary pressure and with a negligible flow resistance was used as a capillary pump. The capillary pressure of a plasma activated fabric was measured by clamping it between two glass slides and measuring the capillary rise, which reached a height of 133 ± 8 mm corresponding to a capillary pressure of \(-1303 \pm 78 \text{ Pa} \) (three replicate experiments). The flow resistance of 25 mm of yarn was determined by repeatedly measuring the time needed to flow 9 µl of solution through the yarn using the measurement setup described in the materials and methods section, and found to be:

\[
R = \frac{1303 \pm 78}{(3.05 \pm 0.05) \times 10^{-11}} = (6.8 \pm 0.28) \times 10^{16} \text{ [Pa m}^{-3}] \quad (6)
\]

The capillary pressure of the plasma activated yarn was calculated based on the resistance of the yarn and the flow rate as shown in Fig. S2 in the ESI† and found to be \( \Delta P = 1192 \text{ Pa} \).

Due to the gradual swelling of fibres inside the yarn while they are in contact with the liquid, the current technique gives more accurate and reproducible values for both the flow resistance and the capillary pressure of the yarn than those obtained from the capillary rise experiments.24

Knots as functional fluidic elements

Yarn can easily be knotted, and knots lend themselves to splitting and merging liquid streams on yarns, and may thus be used as a functional element for making microfluidic networks. A simple three-way splitter with a blue dye being distributed into outlet yarns is shown in Fig. 3A and Movie S3†. Merging and mixing of two streams is illustrated with a blue and a yellow dye merging into a green one (Fig. 3B and Movie S4†). The flow speed at the time of imaging in the outlet yarn was \(~2 \text{ mm s}^{-1} \). Using the cross-section of an individual yarn fibre (~15 µm) as a crude approximation of the characteristic radius of the conduit, the Reynolds number becomes \( Re = 0.03 \). Given the low \( Re \) number and hence laminar flow conditions, the relatively large diameter of the yarn and the short mixing length (the knot), the mixing of the two streams, visible by the homogeneous green colour found immediately downstream of the knot, is surprisingly efficient. The entanglement of the fibres and the torsion in the yarn and knot may contribute to enhance mixing. This was observed reproducibly over multiple experiments using hand-made knots.

We reasoned that depending on the knot topology, the mixing ratio between two outlets may be altered, and we tested this idea using the overhand and the hunter’s knots. Images from the outlet yarns were recorded using a CCD camera, and the mixing ratios were determined by comparing the colour to a reference palette of yarns soaked with dyes mixed at different ratios (ESI, Fig. S1†). We assessed the colour at the outlet of each knot by comparing the hue to a calibration curve that was established using known ratios of blue and yellow dye (see Fig. S1 in the ESI†). The overhand knot results in equal mixing ratios in both outlet yarns; however, when it is rotated 90°, using the two ends of one of the yarns as inlets, and the other yarn as outlets, the mixing is almost entirely suppressed, Fig. 3D–F and Movies S5 and S6†. A thin line of green colour appears on the right yarn after a while, which reflects slightly unequal flow rate in each branch and which arises due to inhomogeneities in the yarn and variation in wicking speed.

The hunter’s knot shows much less intertwining of the two yarns (Fig. 3G), and results in almost no mixing with a 95 : 5 ratio of the two fluids in each outlet, Fig. 3H. Conversely, after rotating it by 90°, the mixing ratio was 70 : 30 between the two outlets, Fig. 3I. The results of 5 replicate experiments for different knots and orientations are shown in Fig. 3J, and the pictures are shown in Fig. S3 in the ESI†. These results illustrate that knot topology can be used to conveniently control mixing of different fluids, and based on the large variety of knots that exist,25 many more mixing combinations should be feasible.

The flow resistance of knots

When the knots are fastened, the fibres inside the yarn are compressed and/or twisted leading to a change in flow resistance of the yarn. The increase in volumetric flow resistance of 25 mm long yarns with a single knot of different types and tightened with different forces was measured using the setup described.
below, Fig. 4. We observed that once single stranded knots are fastened with more than 3 N tension, they added a significant resistance to the circuit; however, when the double stranded knots were fastened with the same tightening force, the resistance took much smaller values. We believe that this is due to the gap between two knotted strands, which acts as a short circuit and significantly decreases the resistance of the knot compared to that of a single stranded knot. Even with a force of 10 N, the resistance of the double stranded knots corresponded to only 3 mm length of the yarn.

A web-based microfluidic network

Yarns may be knotted into webs to form microfluidic circuits with predictable fluidic properties. Fig. 5A shows a web of yarns connected with overhand knots. Based on the mixing produced by overhand knots, and on symmetry argument, it is apparent that two liquids applied to inlets 1 and 2 will be combined, mixed, and split at each node, overall reproducing the architecture of the classical microfluidic serial dilutor widely used as part of microfluidic gradient generators.26 Is it possible to predict the exact mixing ratio of this circuit? Eqn (5) mirrors Ohm's law for electrical current \( U = R \times I \). Furthermore, based on the complete mixing achieved by overhand knots, the flow ratios in the output yarns solely depend on the flow rates of the liquids,23,26 and not on which yarn the liquid originates from. In addition, the results shown in Fig. 4 indicate that double-stranded overhand knots fastened with 3 N only generate negligible fluidic resistance, thus satisfying the characteristic of a "node", which is widely used to model electrical circuits. Based on these similarities, a fluidic web is equivalent to an electrical circuit made of nodes and resistors, whereas each resistor represents the volumetric flow resistance of the branch. It should thus be possible to calculate the fluidic properties of the circuit using the electrical circuit analysis methodologies. For the sake of simplicity, the length and flow resistance of each branch of the web were assumed to be equal.
and to be \( r \). The pressure that drives the flow is generated by applying a paper wick with a pressure \( P_1 \) on each yarn. The distance from the outlet node is the same for all yarns, and is \( n \) times the length of a branch, and thus produces a resistance \( nr \) to the flow. The inlets have a resistance \( nr \), but it has no influence on the flow distribution if the circuit is symmetric, Fig. 5B. The mixing ratios of inlet fluids 1 and 2 in each of the outlets can be deduced readily for outlets 3 and 8, because no mixing occurs, as well as for outlets 5 and 6 that carry 50 : 50 of both inlet fluids because of symmetry. The ratios in outlets 4 and 7 however are not evident and were calculated. Again, because of symmetry, it is sufficient to determine the mixing ratio in outlet 4, and outlet 7 can then be deduced as the complementary to outlet 4. The right half of the circuit used for analysis is shown in Fig. 5C. Kirchhoff’s law and Nodal analysis, 27 commonly used for the analysis of electrical circuits, were applied to the half circuit and used to calculate the potentials \( P_A \) and \( P_B \) defined at the two nodes (Fig. 5C). The flow ratio in each outlet is determined by the flow rates \( Q_1 \) and \( Q_2 \), whereas \( Q_4 \) is a 50 : 50 mixture of both fluids (see Fig. 5A and B). The detailed calculation is provided in the ESI†. The flow ratio \( k = Q_2/Q_1 \) is found to be:

\[
\begin{align*}
q = \frac{Q_2}{Q_1} &= \frac{3n^2 + n}{5n^2 + 5n + 1} \\
\end{align*}
\]

and the concentration \( C_4 \) for outlet 4 can be expressed as:

\[
C_4 = \frac{0.5}{K + 1}
\]

and for outlet 7, based on the rule of complementarity \( C_4 + C_7 = 1 \), we find:

\[
C_7 = \frac{K + 0.5}{K + 1}
\]

The outlet flow ratio of the two fluids thus depends on the resistance ratio \( n \). For \( n = 0 \), \( C_4 = 50 : 50 \), but rapidly increases asymptotically towards \( C_4 = 68.75\% \) of the ipsilateral fluid as \( n \) becomes larger than 1, see Fig. S4 in the ESI†.

The circuit was tested with a blue and a yellow dye delivered to inlets 1 and 2, respectively, Fig. 5. The outlet mixing ratios show a good match with the expected values of \( 0 : 100, \sim 69 : 31, 50 : 50, \sim 31 : 69, \) and \( 100 : 0 \), Fig. 5B. The discrepancies between the theoretical and the experimental values can be attributed to unequal lengths of the different branches and inhomogeneity of the yarn itself. If all outlet yarns were aligned side by side, a dilution ladder would be formed that could produce a continuous gradient upon diffusion. 26 By using knots with unequal mixing ratios or by adding more knots, intermediate mixing ratios could be produced as well. The predictability and reproducibility of such networks is not reliable when they are made by hand using natural cotton yarn, and will need to be further improved for making more complex circuits. If better defined yarns can be made, and if knitting or sewing machines could be adapted and used to...
fabricate such kinds of patterns with high accuracy, more complex fluidic circuits may be designed and built.

**Conclusion**

Here we showed methods to characterize and modify basic fluidic properties of the yarns, and then demonstrated examples of using cotton yarns for constructing passive microfluidic systems. We modified the surface property of the cotton to improve its hydrophilicity, and then measured the flow resistance of the yarns. Subsequently we employed knots for both controlling the flow resistance, and making various microfluidic elements such as passive splitters, blenders and mixers out of yarns. We also quantified the mixing ratios of the proposed knotted mixers, and found that topologically different knots can make different mixing ratios. Finally, we made a web of knots (Fig. 6), which can be used as a serial dilutor.

In all these examples, we used yarns made of cotton, but there is a wide range of natural and synthetic yarns which may be useful for different fluidic functions. We believe that the “microfluidic fibres” introduced here may be combined with some of the above mentioned approaches to further enhance the functionality of smart textiles. To fulfill the potential of yarn-based microfluidics, it will be necessary to adopt and adapt the technologies commonly used in the textile industry towards building advanced yarn-based microfluidic circuits by weaving and knitting yarns with different functions. Yarn-based microfluidics may further be enhanced by developing tailored surface treatments of the yarn and using yarns with different diameters to increase flow rates while minimizing the effects of evaporation. We thus believe that the combination of textiles and fluidic yarns represents an exciting new paradigm that will expand the scope of both areas of research.

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