Lab on a Chip

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PAPER

'Fab-Chips': a versatile, fabric-based platform for low-cost, rapid and multiplexed diagnostics†

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Low cost and scalable manufacture of lab-on-chip devices for applications such as point-of-care testing is an urgent need. Weaving is presented as a unified, scalable and low-cost platform for the manufacture of fabric chips that can be used to perform such testing. Silk yarns with different properties are first selected, treated with the appropriate reagent solutions, dried and handloom-woven in one step into an integrated fabric chip. This platform has the unique advantage of scaling up production using existing and low cost physical infrastructure. We have demonstrated the ability to create pre-defined flow paths in fabric by using wetting and non-wetting silk yarns and a Jacquard attachment in the loom. Further, we show that yarn parameters such as the yarn twist frequency and weaving coverage area may be conveniently used to tune both the wicking rate and the absorptive capacity of the fabric. Yarns optimized for their final function were used to create an integrated fabric chip containing reagent-coated yarns. Strips of this fabric were then used to perform a proof-of-concept immunoassay with sample flow taking place by capillary action and detection being performed by a visual readout.

Introduction

The detection of analytes including proteins, DNA/RNA and metabolites from body fluids and other samples of biological origin is essential for a variety of applications including medical testing, toxin detection and forensic analysis. Improved, point-of-care testing of such analytes is an urgent worldwide requirement.[1] Current systems designed for such applications suffer from several drawbacks such as the need for trained personnel, high costs, bulkiness and delayed results. There is therefore a large unmet need for systems that are low-cost, portable, easy to use and provide sensitive detection. These systems should also be capable of rapidly identifying a broad range of analytes from samples of biological origin.

Microfluidic, lab-on-a-chip methods have gained prominence over the past decade as solutions to this problem. Instead of the traditionally used and expensive microfabrication materials like glass and silicon, a number of different materials and processing methods have been explored for the fabrication of microfluidic devices.[2] These materials include plastics such as PDMS (polydimethylsiloxane),[3] PMMA (polymethylmethacrylate)[4] and COC (cyclic olefin copolymer).[5] Plastics are relatively cheap, easy to process and offer the ability to form intricate patterns down to the micron scale. However, they also suffer from some disadvantages such as their natural hydrophobic nature which precludes simple capillary flow, their carbon footprint and the lack of mature manufacturing methods for large scale and low-cost microfluidic plastic chip fabrication. Further, plastic-based microfluidic devices require sophisticated and expensive readers that can direct fluid flow and can provide a read-out from the plastic chip. This can render the entire device and operation unsuitable for very low-cost and robust point-of-care diagnostics.

On the other hand, nitrocellulose membrane-based lateral flow immunoassays (LFIAs) have been hugely successful in the market place. A variety of rapid tests based on this technology, such as home pregnancy tests, are now widely available. In this form of testing, sample deposited on the sample pad flows through the conjugate pad, where antigen elements, if present in the sample, become complexed with loosely held gold-labeled detection antibody. This first complex then continues to flow through the nitrocellulose membrane where a second sandwich complex is formed at the test line. Visual readouts in the form of a color change are used for detection while sample flow occurs automatically through capillary action. In a correctly designed test, a positive control line spotted with antigen always appears next to the test line.

Mature manufacturing processes are already available for such lateral flow devices. However, many LFIAs are limited by certain disadvantages. They are not very reliable and do not provide for the ability to multiplex tests. One of the reasons for the latter is the lack of the ability to define a 'flow-path' in a paper based device. The Whitesides group recently revolutionized the LFIA

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technology by patterning paper into selectively hydrophilic and hydrophobic portions.[6] A patterned flow field was defined allowing for multiplexed testing using urine samples. The controlled transport of reagents within such paper based devices has also been shown.[7] However, such paper-based devices would still require customized manufacturing equipment to both photopattern the paper and to deposit reagents on it.

Cotton and polyester thread have also been explored as a medium for microfluidic chip fabrication.[8–11] Experiments were performed on single cotton threads or cotton threads that had been sewed onto a plastic substrate and color change based readouts were used to detect the presence of a metabolite.[9] The challenge in thread-based approaches is to scale such methods for the reproducible manufacture of point-of-care diagnostic devices using either the cotton fibers or other suitable materials. Further, a variety of different yarns must be used in conjunction to manufacture devices capable of disease detection by means of immunoassay and a unified manufacturing platform to achieve the same is required.

In this paper, we advance textile weaving, especially silk weaving, to manufacture fabric based chips for bio-detection.[12,13] Silk weaving is an art that has developed to a very high degree of skill in India. In such weaving, intricate patterns whose dimensions are limited only to the thickness of an individual thread (~100 μm) are skillfully woven in a highly parallelized manner. We believe that silk weaving can be used to build a low-cost, unified, scalable platform for chip manufacture with the added advantage of low environmental toll and employment of skilled craftsmen.

Experimental methods

Characterizing the wetting behaviour of yarn

All yarn wetting experiments were conducted by fastening equal segments of yarn tightly on a transparent substrate. An aqueous solution of food coloring dye (50 μl) was loaded on one end of the wound yarn and wetting behavior was observed. Two types of silk yarn, boiled silk and brass coated silk were obtained from Silk Touch (Bangalore, India). Prior to testing, boiled silk yarn was made more hydrophilic to ensure optimal flow by immersing in blocking solution (1% BSA + 0.5% Tween-20 in 1× PBS) for 1 hour followed by complete drying at 40 °C. Both were then tested and compared for wetting ability. Second, to study the effect of the number of Twists Per Inch (TPI) on a wicking rate of yarn, 3 different yarns of low (~3 TPI), medium (~20 TPI) and high (~50 TPI) twists per inch were similarly treated and tested for wicking behavior. In the second case, the time taken with every centimetre wetted was noted.

Characterizing the wetting behaviour of fabric

Plain-woven fabrics of three varieties, crape, tabby and a third silk fabric (Silk Touch) with known specifications (Table 1) were tested for wicking using an aqueous solution of green food-dye. The fabric samples were first treated with blocking solution (as described earlier). Since the width of the fabric strip also influences the wicking, all experiments were carried out on 5 mm (width) × 60 mm (length) strips, the dimensions of a standard lateral flow test strip.

A strip was fastened to a glass slide and placed on a horizontal surface. Stretching the fabric beyond what is needed to smoothen out the creases was avoided. The dye solution (50 μl) was loaded onto one end of the strip and the time required to wet each successive centimetre of the fabric was noted. All experiments were done keeping the manner of use of the final product in mind.

Therefore, a predefined volume of liquid was wicked on the strip, unlike the standard practice in textile characterization which involves immersing one end of the fabric strip in a beaker of liquid.

The absorptive capacity of fabric samples was measured using a previously described method.[14] One square inch of fabric was dried in a hot air oven and weighed. Each sample of fabric was then immersed in water for an hour before allowing the excess water to drip away for 5 minutes, and the wet piece of fabric weighed again. The percentage weight of water held to weight of dry fabric quantifies the absorbency.

Synthesis of immunoassay reagents

Colloidal gold. Colloidal gold particles with a mean diameter of 40 nm were synthesized by the chloroauric acid reduction method.[15] Briefly, an aqueous solution of chloroauric acid (20 ml of 0.04% w/v $HAuCl_4 \cdot 3H_2O$, Sigma) was heated to boiling and 1.2 ml of 1% w/v sodium citrate solution was then added to it at a stretch. The reaction mixture was allowed to boil gently for 20 min, stirring all the while, until the color of the solution changed from straw yellow to black and eventually to deep red. A deep red solution is indicative of monodisperse gold nanospheres of ~40 nm size. The colloidal solution obtained was used for conjugation with antibody.

Gold nanoparticle–antibody conjugate. The detection antibody, Goat Anti-Rabbit (GAR) IgG, was conjugated to the 40 nm size gold particles according to a published protocol, with minor modification.[16] Briefly, a preliminary titration was performed to determine the minimum amount of antibody concentration needed to stabilize colloidal gold particles. The pH of colloidal gold suspension was adjusted to 9.0 using 100 mM K_2CO_3 solution and dispensed into a series of test tubes (1 ml per tube). GAR antibody (0.1 mg ml^{-1}) was added to each tube in series from 0–150 μl. Following that, 0.1 ml of 10% NaCl was added to each tube and mixed before observing a color change after 10 minutes. In the experiment, 0.02 mg ml^{-1} of GAR antibody was found to be the minimum amount for stabilization of colloidal gold sol. Therefore, 1 ml of antibody (0.02 mg ml^{-1} prepared in pH 9.2, Borax 2 mM) was added drop wise to 5 ml of gold sol. The solution was stored for 1 hour at room temperature to allow for conjugation to take place, and was then centrifuged to remove un-conjugated antibody. The centrifugation was done at 9500 rpm at 4 °C followed by re-dispersion of the pellet in 5 ml of pH 8.2 Tris–Buffered Saline. The conjugate is stored at 4 °C for further use.

Fabric device assembly

A customized sample handloom was specifically built for the purpose of the fabric chip weaving and located in a clean,

Table 1 Coverage and TPI specifications of fabric selection. The range of twist frequencies is as follows: 'low' twist = ~3 TPI, 'medium' twist = ~25 TPI, and 'high' twist = ~50 TPI

Fabric	Coverage (yarn intersections per square inch in warp × weft notation)	Yarn TPI (warp × weft)	Yarn denier (g per 9000 m^{-1})
Crape silk	328 × 99	Low × high	22 D in all fabrics
(Other) plain weave silk	160 × 89	Medium × low	
Tabby 1	100 × 132	Low × low	
Tabby 2	134 × 109	Low × low	
Tabby 3	137 × 127	Low × low	

air-conditioned atmosphere maintained at 30 °C. This temperature was found to be suitable to prevent re-uptake of ambient moisture by the treated yarns. A Jacquard attachment (ESI†) in the loom enabled us to achieve pattern versatility and intricacy. Patterned punched cards were generated from a CAD file of the chip design using a computer program (CadVantage Win, Teckmen Systems, India). Two kinds of fabric devices were woven on the sample handloom, one to demonstrate defined flow paths of water soluble green dye along the hydrophilic portion of the fabric chip and another to perform a direct immunoassay.

For the first device, boiled silk yarn was treated with blocking solution as described earlier. This treated and dried hydrophilic yarn was then woven in a serpentine pattern along with readymade hydrophobic brass coated silk yarn set as the hydrophobic background. Wetting was observed using green food-dye solution.

The second assembly aims to emulate a simpler, straight lateral flow strip. Four types of silk yarn (varying in twist frequency) were used on different regions of this fabric device. The warp yarn is common to the entire strip and is of ~3 twists per inch (TPI). Weft yarns were varied as follows: for the main wetting region of the fabric, ~20 TPI weft yarn; for the conjugate pad region, ~3 TPI weft yarn; for the analytical region, ~50 TPI weft yarn. All yarns are treated with blocking solution to avoid non-specific binding. The yarn for the detection conjugate was pre-blocked (with 1% BSA, 0.5% Tween-20, 1% polyethylene glycol 20 000 M_w, 20% sucrose in 1× PBS) prior to coating with 1 μl of detection conjugate per cm of yarn. Yarns for the analytical region were coated with 1 μl of 0.25 mg ml^{-1} Rabbit IgG (Test) or 1 μl of 0.25 mg ml^{-1} Mouse Anti-Goat IgG (Control) per cm of yarn.

Assay procedure

A parent chip of 125 mm width was fabricated, from which 5 mm wide strips were cut. Strips for testing were sampled from across the width of the parent chip. Unused strips are stored in a sealed pouch containing desiccant packs at room temperature (20–30 °C). The direct immunoassay test was carried out at room temperature, by flushing a strip with PBS from the sampling region located upstream of the detection antibody zone. The backflow of detection conjugate is prevented by saturating the sampling region first. Flow and accumulation of gold-labeled antibody over test and control areas leads to the appearance of two red lines visible to the naked eye, indicating the successful binding of proteins.

Results and discussion

The construction of a fabric chip begins with the selection of yarns to constitute different sections of the chip. For the sake of repeatability, it is important to assemble yarns having well-defined properties such as Denier and Twist frequency (TPI) (Fig. 1a). The 'Denier' is the linear density (in grams per 9000 m) of the bundle of native silk filaments that have been reeled into an individual strand. The 'Twists Per Inch' (TPI) is the number of twists imparted per inch of an individual strand. Pre-boiled and readily available yarn with known Denier and TPI are treated with the reagents that are required to functionalize the fabric using a wet process. The yarns are then dried and assembled on a loom before being woven into an integrated fabric chip (Fig. 1a). Individual devices can then simply be cut from the integrated fabric (Fig. 1b). Fabric was always cut along the warp direction as reagents were woven in along the weft (Fig. 4a).

The singular advantage of weaving as a process to make such chips is that the chip is fabricated in one weaving step and does not require any other operations such as alignment, assembly or reagent spotting. This lowers capital costs and introduces parallelization of operations that can lead to manufacturing efficiencies. In contrast, both plastic[17,18] and patterned paper-based microfluidic devices[19] must typically be first fabricated before being loaded with reagents. In the customized sample loom that we were using which has a weft dimension of about 0.2 m, the significant parallelization that is natural to the weaving process enables 20 to 25 'strips' to be woven simultaneously. This can be scaled up by using regular sized looms. Depending upon the complexity of the design, a single weaver is able to make between 10 and 100 fabric chips per hour, aside from the set-up time for the loom which is 2–3 hours and is required to be done only at the start. Further, 16 man hours (one day's time of 2 semi-skilled workers) are required for the reagent coating and drying of the yarns required for 2000 fabric chips. Chips with serpentine patterns (Fig. 1b) could be fabricated at a rate of 10 per hour per weaver while the non-patterned chips (Fig. 4) could be woven at rates exceeding 100 per hour per weaver.

Yarns characterized by different values of the parameters mentioned above show differences in their ability to wick and absorb liquid. It is therefore anticipated that any integrated fabric chip will consist of yarns with wicking and absorption properties suited to the functionality required from each section of the chip. Using lateral flow assays as a comparison, different yarns can be used to serve as a blood or urine (sample) entry portion (sampling region), hold reagents (conjugate region, test and control lines), function as the long wicking channel which promotes the reaction (main channel) and as a capillary pump (absorption pad). For fluid manipulation on a fabric chip, the primary requirement is to introduce the ability to pattern flows in desired geometries. Guiding flow along a certain path needs hydrophilic yarn to set the flow path, hydrophobic yarn to set the

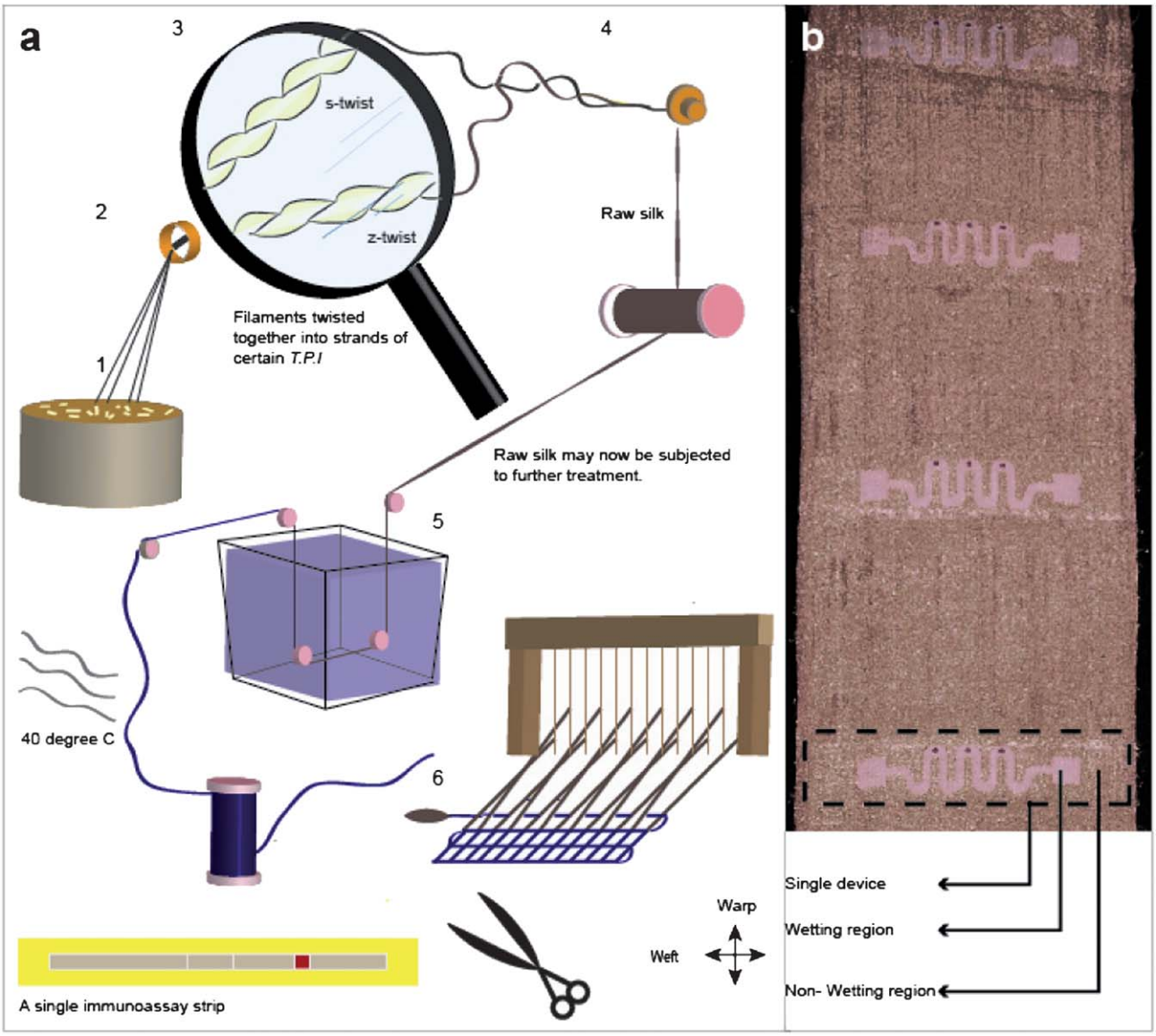

Fig. 1 (a) Schematic showing the process of silk fabric chip manufacture from start to finish. Cocoons are immersed in a boiling water bath to soften the sericin and permit unwinding of silk filaments (1). Multiple silk cocoon filaments are reeled to form a single strand of yarn (2). The single strand can then be twisted in different orientations before being combined with other strands to multi-'ply' the yarn (3) and (4). The yarn may then be subjected to a variety of treatments or combinations of treatments including boiling, coating with reagents or blocking (5). The coated yarn is dried before being woven. The yarn could be placed in the warp or weft way (see axes in bottom right). Multiple chips are simultaneously woven before being cut to form individual test strips (6). (b) Multiple chips woven onto a stretch of fabric. Hydrophilic threads (off-white) form the wetting, serpentine path with the metal coated golden threads forming the hydrophobic background. The length of the entire stretch is ~50 cm and the width is ~10 cm.

background and weaving to create the complex architectures. Silk fibroin is a naturally hydrophilic material[20] whose hydrophilic properties can be enhanced with surfactant treatment. In contrast, the brass coated yarn which is widely used and commonly available in India is extremely hydrophobic (Fig. 2a). Wetting tests were performed by simply winding the yarn around a plastic substrate in order to demonstrate that the metal coated yarn is non-wetting while the boiled silk is wetting. In order to demonstrate the potential for creating complex flow paths, we wove a chip that has a serpentine flow path (white) set on a hydrophobic background (golden). A water soluble green dye was used to test the wetting properties of the fabricated device. The dye is seen to selectively wet the flow path without wetting the background (Fig. 2b).

As a platform, fabric chips also offer a great deal of flexibility to manipulate such fabric properties as absorptive capacity and wicking rate by means of varying both weaving parameters and yarn properties. Weaving parameters include the coverage area density (a product of the number of yarns in the warp and the number of yarns in the weft per square inch of fabric) and weaving style (*e.g.* plain, twill or satin). Our initial studies were performed using three different kinds of pre-woven silk fabric

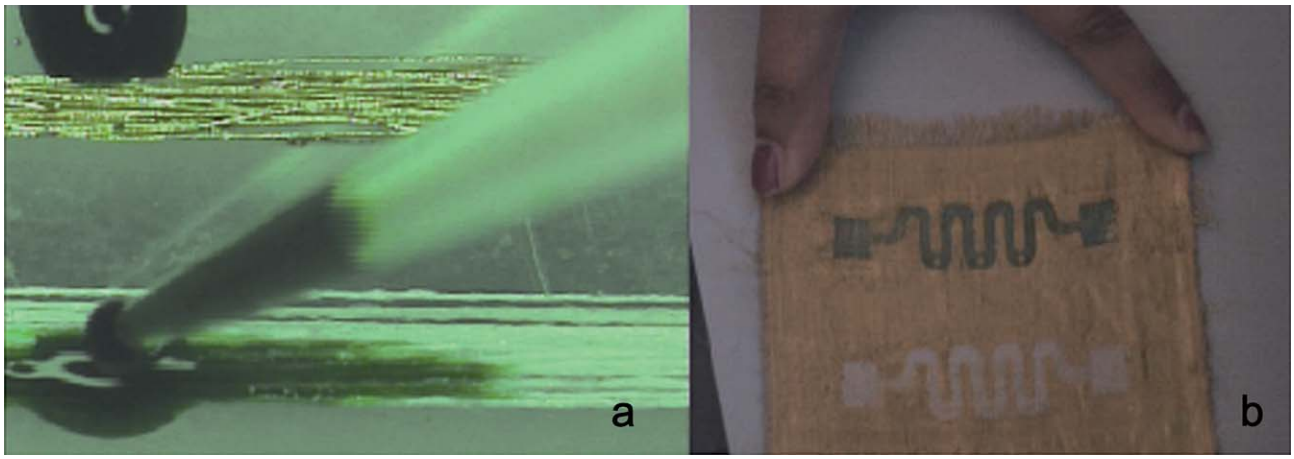

Fig. 2 (a) Brass coated yarn (upper) rejects a drop of aqueous dye deposited on it, while degummed, surfactant treated silk yarn (lower) allows the fluid to wick along its length. (b) A fabric chip showing proof of concept of selective wetting. Aqueous green dye solution deposited at one end flowed only along the hydrophilic yarns. An unused chip (white) is shown for contrast. Note that the water-soluble dye has not spread to the hydrophobic portions.

where multiple weaving and yarn parameters were simultaneously varied (Table 1). The fabrics selected were each a different combination of denier, TPI and coverage area. The three samples of fabric were tested for suitability as a wicking strip. The results (Fig. 3a) were found to obey the simple Washburn–Lucas equation (eqn (1))

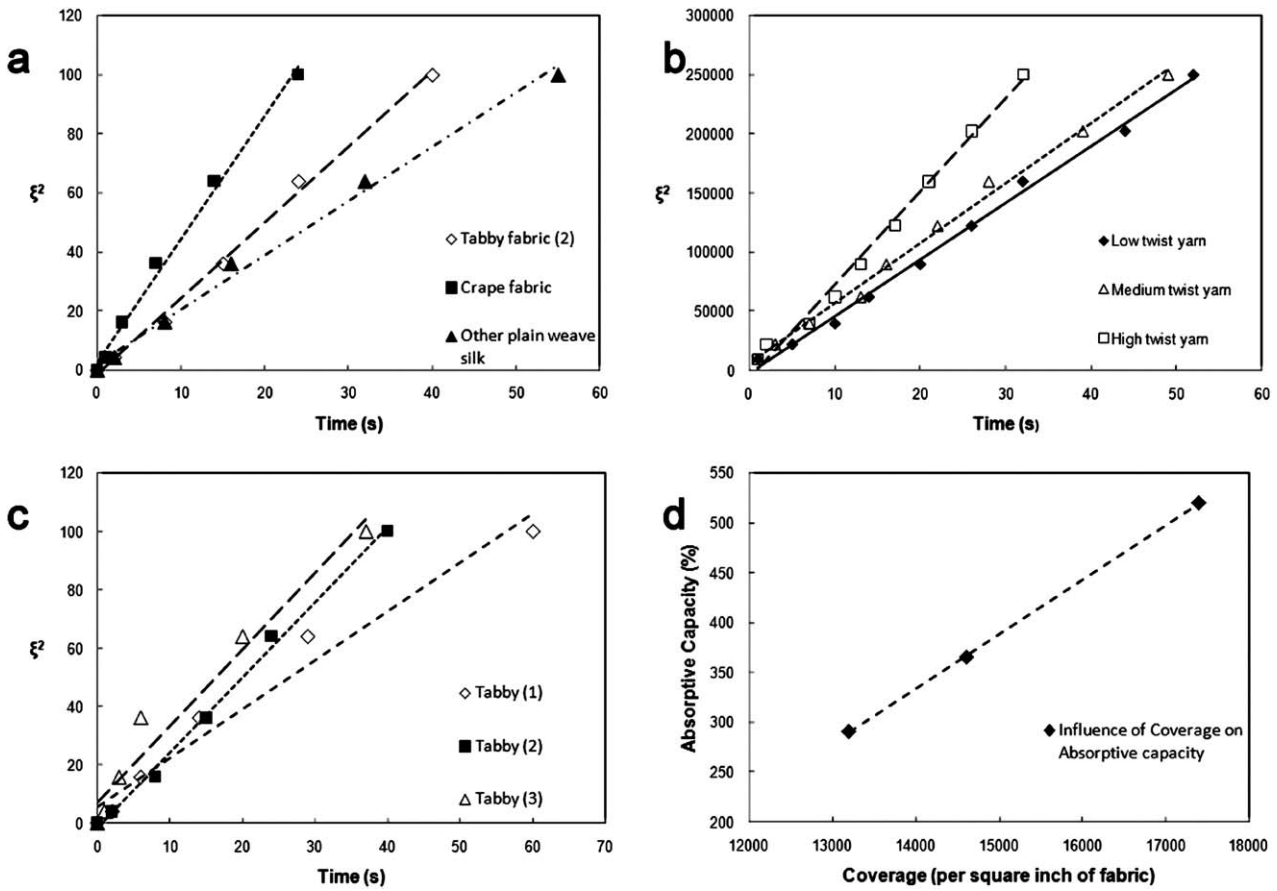

Fig. 3 Comparison of the tuning parameters on some common silk fabrics. (a) Wicking rate is shown as a plot of square of dimensionless length, ξ ($=l/W$) *vs.* time for fabric strips of dimensions 5 mm × 60 mm. Crape shows the highest wicking rate. (b) Plots of the wicking rate of three yarns of low, medium and high TPI respectively, showing an increase in wicking rate with twist. (c) Wicking rate shown as a plot of ξ *vs.* time for three varieties of tabby fabric with increasing coverage area densities, 13 200, 14 606 and 17 399 per square inch, respectively (and identical twist in yarn). (d) Plot showing the increase in absorptive capacity with coverage among the three tabby fabric varieties. The error bars are within the limits of the symbols shown in all the figures.

$$L^2 = \frac{\gamma D t \cos\theta}{4\nu} \quad (1)$$

where L is length of the fabric strip, D is average pore diameter of the fabric, ν and γ are viscosity and surface tension respectively for capillary flow through a porous material. The square of the cumulative distance wicked, ξ ($= l/W$), was found to be proportional to the time taken for wicking. Here, l is the instantaneous wicked length and W is the width of the strip. We also estimated a pseudo-pore diameter (of the interstices in the fabric) using these data. The estimated values were crape: 5.5 μm; tabby: 3.3 μm and plain silk: 2.8 μm.

As multiple parameters were changed in moving from one fabric to the next, we next performed a set of experiments to isolate the effect of selected input variables. As an initial demonstration of the kind of tuning that is possible, we have looked at one yarn parameter, TPI, and one weaving-dependent parameter, coverage area. These can significantly influence the use of the fabric chips in performing diagnostic assays by directly influencing the wicking rate and absorptive capacity.

The wicking rate in individual yarns was found to increase with the TPI (Fig. 3b). The increase in the wicking rate with increased TPI can be attributed to the increased surface area per unit length as the twists are increased.[21] However, increasing yarn twist beyond a certain value can lead to dimensional instability of the final fabric.

The wicking rate and absorptive capacity of fabrics can also be influenced by the weaving parameter, coverage area. To vary coverage area, we selected 3 varieties of tabby fabric made of identical yarn combinations but with an increasing number of yarns per unit area. The wicking rate was found to increase linearly with coverage (Fig. 3c) with all the fabrics following the Washburn–Lucas equation. The coverage area may be increased, up to a certain limit, by increasing the density of yarns placed on the loom. This matches earlier studies that prove that the wicking rate increases with the coverage area because of greater contact between individual yarn strands.[14] The wicking profile can influence assay time and line darkness. The ability to tune the wicking rate (by changing yarn twist and coverage area) can be advantageously used while designing an integrated fabric chip which requires different zones with different wicking rates.

Absorptive capacity is a measure of the amount of liquid the fabric can hold. It occurs both when the hydrophilic fibers imbibe water and when water enters the interstitial spaces between fibers. The presence of interstitial spaces therefore aids in absorption.[14] An increase in the fabric coverage constitutes an increase in the number of water-imbibing fibers per unit area, and was also found to increase fabric absorptive capacity (Fig. 3d).

The immunoassay fabric chip

As a proof-of-concept of the utility of the fabric chip, we performed a direct immunoassay using a polyclonal Goat Anti-Rabbit-Rabbit IgG system. With a view to emulate the wicking and absorptivity requirement in the different regions of a lateral flow strip (Sampling, Detection Conjugate, Main Channel, Capture and Absorption), the optimal yarn and fabric parameters were chosen.

The flow was intended along the warp axis on the woven patch (Fig. 4a). The warp yarn has a twist frequency of ~3 TPI (low). Weft threads were varied in each zone to define the properties of the zone in question. Thus, the sampling zone, the main channel region and the absorption zones are fabrics of 3 TPI × 20 TPI (warp × weft) yarns with a coverage area density of 160 × 90 (warp × weft) per square inch and have a cumulative wetting rate of 0.5 mm s^{-1}. This imparts an assay run-time of 2–3 minutes, required for line formation.

The conjugate pad region is a fabric of 3 TPI × 3 TPI (warp × weft) yarns with a lower coverage area density of 160 × 45 (warp × weft). This fabric is 'loosely' woven and can therefore easily release the detection conjugate when wetted. Further, the yarn

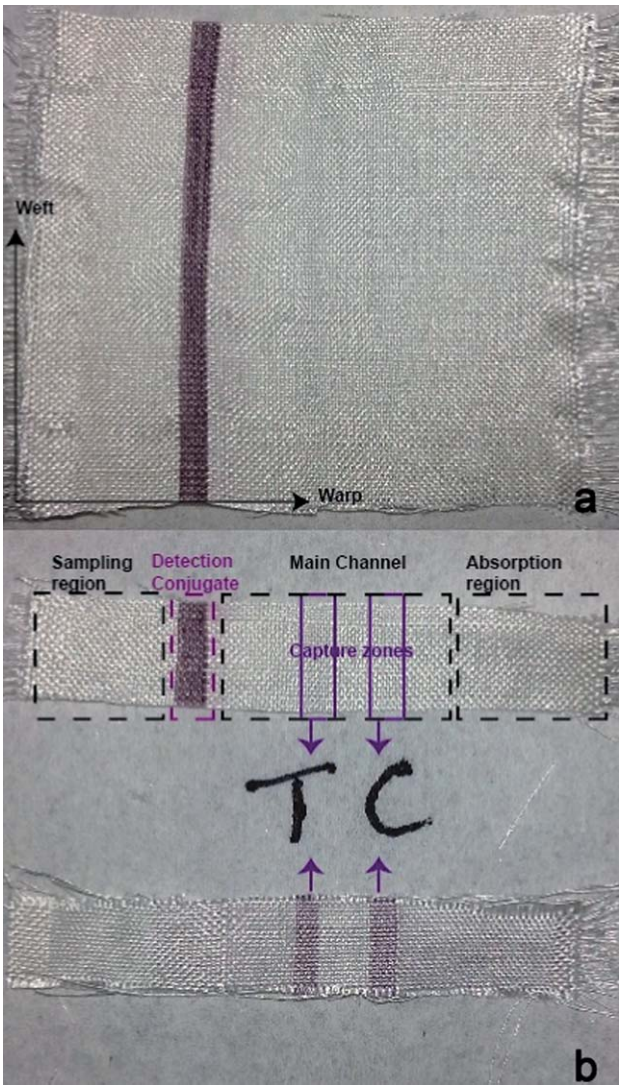

Fig. 4 Direct immunoassay on the fabric chip. (a) The parent-chip which may be cut into individual test strips such as the one in (b). (b) The upper part is a cut strip before testing. The pink region houses the gold labeled detection antibody. Test and control lines are labeled as (T) and (C). The assay is performed by wicking sample/buffer along the strip. The lower is a test strip after performing the assay. The detection antibody has leached out of the conjugate zone and lines are formed at both the test (T) and control (C) regions, signaling a positive result for 500 ng of antibody at each of the capture zones.

used in the conjugate has been pre-treated with blocking solution containing surfactant and PEG before being treated with the gold–antibody conjugate.

The high (~50) TPI weft yarn was used in the analytical region for optimum uptake of capture antibody. Twisting increases the surface area per unit length of yarn available for coating of capture antibody. Further, the yarn was treated with capture antibody before being treated with blocking solution so that the yarn is able to permanently absorb the antibody. Uniform wicking is required for the even flow of detection conjugate across the capture region. High TPI weft yarns facilitate the lateral distribution of liquid (Fig. 3b demonstrates the increase in the wicking rate with twist). Thus, when paired with a lower TPI warp yarn, high TPI weft yarns also ensure a uniform wicking front.

A chip of the aforesaid composition and 125 mm across was fabricated on a sample loom (Fig. 4a). Preliminary results on a 5 mm section of it demonstrate a positive test (Fig. 4b) with the appearance of both test (T) and control (C) lines. It was noteworthy that additional washing was not required to produce the clear contrast between the lines and the chip background seen in Fig. 4b. We have noticed that, in comparison with current LFIAs, the silk fabric shows much better resistance to non-specific binding of gold conjugate. This was observed even 1 hour after the test result whereas a typical LFIA shows a faint pink background throughout the length of the strip at the same time. Strips sampled from across the parent chip (one of which is shown in Fig. 4b) yielded identical results, showing that the coating of reagents onto the yarn was uniform. With this system, we have demonstrated detection of 500 ng of Rabbit IgG per test strip. We have also used the fabric for the detection of prolactin in human serum using a sandwich immunoassay format, where 100 ng ml^{-1} of prolactin antigen yielded a positive result. Line formation in the latter was faint (ESI†) and is in the process of optimization. The physical and biochemical properties of the fabric chip were stable over a period of 2 weeks when stored in a desiccant containing plastic pouch at room temperature (20–30 °C). Longer term studies of shelf life and stability of the devices are being performed currently.

Conclusion

We have demonstrated that weaving can be used as a simple, scalable platform for the manufacture of fabric-based microfluidic chips. Using only pre-coated silk yarns as a starting material, complex patterns containing reagents at specific locations can be fabricated in just one step. By using handlooms and readily available low-cost coating equipment, fabric chips can be manufactured with out making a significant capital investment. The other benefits of using silk yarn for this purpose include the low environmental toll because of easy disposability and the generation of employment for skilled weavers to make a value-added product. Manufacturing rates achieved can be near 1000 non-patterned chip per weaver per day. These rates are large enough to contemplate a manufacturing facility using only handlooms. For increased throughput and reliability, automated coating equipment and power looms can also be considered. The throughput of a single power loom could easily exceed 10 000 fabric chips per day. Further, because of the widespread presence of textile fabrication expertise throughout the developing world, we anticipate that such a technology could be easily transferred to other geographies. While the demonstrated application of the fabric chip is an immunoassay for which tuning parameters have been optimized and direct immunoassay demonstrated, we envisage that a wide variety of tests, including those for metabolites, could be performed on such a platform. The subsequent channeling of flow on this strip by the introduction of the hydrophobic frame will allow patterning of flow along multiple 'strips' with a common sampling region. This will facilitate the detection of more than one analyte molecule in the same sample.

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