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Thread as a Matrix for Biomedical Assays

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ABSTRACT This paper describes the use of thread as a matrix for the fabrication of diagnostic assay systems. The kinds of thread used for this study are inexpensive, broadly available, and lightweight; some of them are already familiar materials in healthcare. Fluids wick along these threads by capillary action; no external power source is necessary for pumping. This paper demonstrates three designs for diagnostic assays that use different characteristics of the thread. The first two designs—the "woven array" and the "branching design"—take advantage of the ease with which thread can be woven on a loom to generate fluidic pathways that enable multiple assays to be performed in parallel. The third design—the "sewn array"—takes advantage of the ease with which thread can be sewn through a hydrophobic polymer sheet to incorporate assays into bandages, diapers and similar systems. These designs lead to microfluidic devices that may be useful in performing simple colorimetric assays that require qualitative results. We demonstrate the function of thread-based microfluidic devices in the context of five different colorimetric assays: detection of ketones, nitrite, protein, and glucose in artificial urine, and detection of alkaline phosphatase in artificial plasma.

KEYWORDS: low-cost • microfluidic devices • diagnosis • thread • paper

INTRODUCTION

This paper demonstrates that thread can be used as an inexpensive and lightweight matrix to fabricate diagnostic microfluidic devices (1). It extends and supports closely related, independent work recently reported by Li et al. on the fabrication of three-dimensional microfluidic devices by sewing cotton thread into polymeric materials (2). The results of Li et al. and those reported here are in agreement where they overlap.

We and others have used hydrophilic paper as the basis for diagnostic assays (3−15). One strategy for using paper for diagnostic devices is to pattern channels into the paper using lines of hydrophobic polymer; the hydrophilic channels thus define the microfluidic paper analytical devices (μ-PADs) (3). In μ-PADs, the flow of fluid is driven by capillarity. Li et al. and we recognized that analytical devices based on thread can follow analogous concepts. The utility of both of these systems—those based on paper, and those based on thread—is in applications in which low cost and portability are paramount concerns (16−18).

Thread has several characteristics that make it a useful matrix for the fabrication of biomedical devices: (i) It is very inexpensive (e.g., the retail price for cotton thread is ~0.008 ¢/cm) and a broadly available material. (ii) It is flexible, lightweight, and difficult to break or tear accidentally. These properties translate into ease of transport and storage (e.g., on lightweight reels or rolls). (iii) It is hydrophilic (or can be made hydrophilic), and no external power source is necessary to move aqueous fluids along the thread. (iv) The capillary flow within a strand of a thread is effectively confined to one dimension, because the aspect ratio (length: diameter) of the thread is high. The lack of lateral transport—which occurs in 2D paper-based assays—limits the required volume of sample (e.g., the volume of blood from a finger prick, ~50 μL, is sufficient). (v) Thread can be functionalized in a wide range of ways, using established chemistry. (vi) It can be manipulated easily by globally used processes such as sewing, knitting, and weaving. These techniques could, in principle, be exploited for the mass production of thread-based devices with ubiquitous machinery (e.g., sewing machines and looms). (vii) The materials of the threads are combustible and can be disposed of (after contamination with biological fluids) by burning.

Li et al. and we utilized similar properties of thread to make functional microfluidic devices. We briefly compare and contrast the two approaches: (i) Li et al. demonstrated that plasma oxidation of hydrophobic cotton thread can promote wicking and enable fluid transport in thread-based devices. Our own studies confirm this result. We also examined threads made from eight different materials (rayon, hemp, nylon, polyester, wool, 50% cotton/50% acrylic, acrylic, and natural silk) as candidates for transporting fluids in thread-based devices. Ultimately, we chose to utilize mercerized cotton—a hydrophilic type of cotton thread—that does not need to undergo plasma oxidation to enable wicking. (ii) Li et al. used sewing techniques to incorporate thread into polymers to create patterns of microfluidic channels. Similarly, we use manual sewing as a method of fabricating microfluidic devices. We also demonstrate that weaving of thread on a loom and encapsulation in tape can be used as an alternative strategy to fabricate thread-based devices. (iii) Li et al. demonstrated that twisting of two hydrophilic threads can promote mixing of fluids in specific locations. We use knots and gel particles to localize reagents in specific locations along the thread, and to

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promote mixing of reagents in well-defined regions. (iv) Li et al. demonstrated the use of individual stitches of thread for quantitative correlation of concentration of nitrite with color intensity produced in colorimetric assay. They noted that thread by itself can be useful for semiquantitative colorimetric assays; for quantitative colorimetric detection of nitrite, they fabricated an array of six sensors, where each sensor comprised a thread-based microfluidic channel that transports fluid to a detection zone defined by a paper disk. In addition, they used a three-dimensional device composed of thread-based microfluidic channels sewn through tape and detection zones defined by disks of cellulose paper to demonstrate simultaneous detection of two analytes (nitrite and uric acid) (2). The work we report in this paper confirms the ability to perform qualitative colorimetric assays using thread-based microfluidic devices for nitrite and for four additional analytes (protein, ketones, glucose, and alkaline phosphatase). In contrast to Li et al., we did not utilize composite devices composed of both thread and paper, and focused our studies on thread-based devices, and the combination of thread and gel particles. In this paper, we also describe three designs of diagnostic devices that are different from those described by Li et al. Taken together, the results of Li et al., and those we describe, offer a number of complementary strategies with which to design microfluidic thread-based devices.

EXPERIMENTAL DESIGN

Thread-based microfluidic devices require no patterning (e.g., printing, lithography, molding, etc.) for defining channels. Creating thread-based devices for biomedical assays requires consideration of: (i) the rate of transporting fluids by capillary action, (ii) loading of reagents into predetermined locations, (iii) defining easily readable and highly visible detection zones, (iv) encapsulation of microchannels for ease of handling and robustness.

Choice of Designs. We explored designs that use two different characteristics of the thread. The first two designs, which we call the "woven array" (Figure 1A) and the "branching design" (Figure 1B), take advantage of the ease with which thread can be woven on a loom. These two designs (Figure 1A and 1B) rely on similar strategies for loading reagents, defining detection zones, and encapsulating microchannels, yet differ in the arrangement of microchannels in space. These two designs demonstrate the use of hand loom for the fabrication of diagnostic devices. Extension of these procedures to machine looms offers access to very high-speed fabrication. The third design, which we call the "sewn array" (Figure 1C), takes advantage of the ease with which thread can be sewn into various substrates (as also demonstrated by Li et al.) (1, 2). This design relies on different strategies for introducing reagents and defining detection zones than the other two designs.

Choice of Thread. The material of the thread determines the rate of transport of fluids, reagents, and analytes along it, and is a crucial parameter in the design of any thread-based devices. We examined the wicking properties of nine types of thread of different materials: rayon (viscose), hemp, nylon, cotton, polyester, wool, 50% cotton/50% acrylic, acrylic, and natural silk. We also examined the wicking properties of the thread both as purchased, and after treatment with plasma for five min. In these experiments, we applied 10 μL of aqueous solution of food coloring to one end of the thread (length, $l = 1$ cm). Table 1 summarizes the average rate of wicking of the threads before and after surface oxidation using air plasma, and the standard deviation for seven experiments. Several types of thread were hydrophobic, and did not wick any liquid before oxidation with

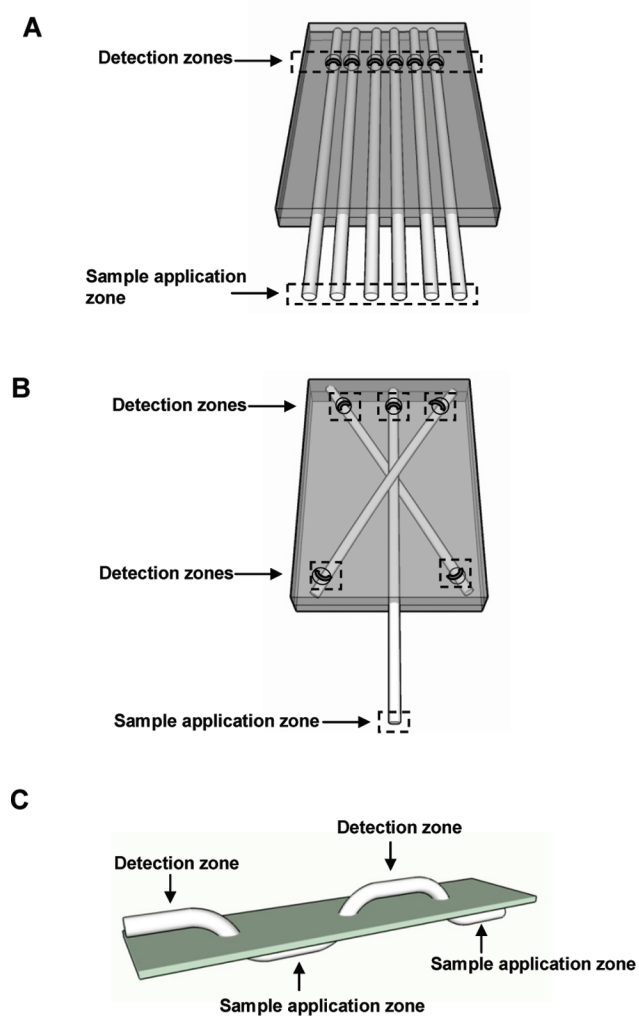

FIGURE 1. Schematic illustrations of the thread-based devices. (A) The woven array device: multiple threads are sandwiched between two layers of transparent tape. (B) The branching design: the thread was organized using a loom to form a system with multiple analytical zones reached by wicking from a common entry point. (C) The sewn array design: the thread was sewn through a sheet of hydrophobic polymer. Each device comprises one sample application zone and one detection zone.

Table 1. Rate of Wicking for Various Types of Thread[a]

thread	R_{wb} (cm/s)	R_{wa} (cm/s)
rayon	0.29 ± 0.06	1.01 ± 0.69
hemp	0.02 ± 0.01	0.55 ± 0.55
nylon	0.03 ± 0.00	0.04 ± 0.01
cotton	0.23 ± 0.04	1.89 ± 0.52
polyester	0.13 ± 0.03	1.98 ± 0.79
wool	did not wick	2.20 ± 0.40
50% cotton, 50% acrylic	did not wick	2.11 ± 0.30
acrylic	did not wick	1.91 ± 0.42
natural silk	did not wick	0.60 ± 0.21

[a] R_{wb} is the average rate of wicking before oxidation (cm/s), R_{wa} is the average rate of wicking after oxidation (cm/s) from seven experiments. The length of the thread was 1 cm, and the volume of solution applied was 10 μL. The standard deviation was calculated on the basis of seven experiments.

plasma. Plasma oxidation increased the rate of wicking. In fabricating the devices, we used threads that demonstrated the most rapid rate of wicking without requiring plasma oxidation: the most suitable threads included rayon, cotton, and polyester. The thread made of hemp was the most absorbent and durable material, but it wicked fluids very slowly. In addition, the color of hemp (brown) interfered with the detection of color changes in assays.

Examination of several types of thread affirmed the choice of cotton by Li et al., as a suitable material for bioassays (2). We also chose to use cotton thread for our devices for these reasons: (i) it is durable and easy to manipulate, and (ii) it adheres well to inexpensive adhesive tapes (e.g., polyolefin tape with acrylic adhesive, ScotchMultiTask Tape, and vinyl tape with rubber adhesive, 3M Vinyl Tape 471). Adherence to tape is an important feature since in both the "woven" and "branching" designs an adhesive-backed tape encapsulates the thread. For example, we found that weak adhesion between the rubber or acrylic-based adhesive of the tape and polyester or nylon threads complicates the fabrication of the devices using these threads. Nylon and polyester threads, however, may be useful for fabricating devices that require rapid wicking but do not require the tight encapsulation of the thread by the adhesive of the tape.

We used commercially available cotton thread (see the Supporting Information). This thread has a diameter of 0.3 mm, and is, therefore, sufficiently wide to be seen by eye and imaged without a microscope, but is not so wide that the analyte—which would otherwise contribute to the intensity of the signal of the assay—is lost in the interior of the thread (this diameter is comparable with the diameter of the thread used by Li et al. (2)). This type of cotton thread is manufactured by a process that includes mercerization (exposure of a thread under tension to a NaOH bath, followed by neutralization with acid). Mercerization increases both the strength of the thread and its ability to absorb water. We, therefore, did not need to use a vacuum plasma reactor to obtain a hydrophilic thread, as previously reported (2).

Choice of Tape. The new designs that we present here (the "woven array" and "branching design") require two pieces of transparent, water-impermeable, polymer tape to encapsulate the thread. The tape: (i) acts as a mechanical support on which the thread can be arranged; (ii) serves as a "handle" to aid in manipulating, labeling, storing, and dispensing the device. (iii) protects the assays from the environment; and (iv) minimizes evaporation along the thread—which could alter the concentration of analyte or rate of wicking of the fluid.

For these studies, the important difference between the transparent tapes that we used is that the vinyl tape makes complete conformal contact with its substrate (in this case, the thread), while the Scotch tape combines optical transparency, strength, and moisture resistance, but does not adhere conformally to the entire surface of the thread.

We used a heat and pressure laminator to promote contact between the tape and the thread (see the Supporting Information). We ran the thread-based devices through the laminator three times to ensure contact between the tape and the thread.

Strategies for Defining Detection Zones. One of the challenges in the fabrication of diagnostic devices for point-of-care is to have well-defined detection zones that enable the users to interpret the results readily. For the fabrication of low-cost devices, it would be ideal to define these detection zones without the aid of laboratory equipment. To meet this requirement, we explored two strategies for introducing reagents and defining detection zones within thread-based microfluidic devices: the use of (i) knots, and (ii) gel particles.

"Woven Array" Design. The objective of the woven array design is to perform multiple assays with a minimal amount of analyte solution (i.e., a minimal fluidic "foot print"). We, therefore, sought methods of arranging the thread to have a simple (ideally single) input that uses wicking to distribute analytes to multiple detection zones. Each detection zone contains reagents for an assay. Different zones may perform different assays, or duplicate assays, or controls and standards.

For the fabrication of the "woven array" device illustrated in Figure 1A, we used a loom to arrange several threads in parallel. To generate the detection zones, we immersed a piece of thread into a solution containing the reagents for the assay, and dried it under ambient conditions for ~1 h. We then used this piece of thread to create knots on the threads that we aligned on the loom. We reasoned that the knots would serve as a "reservoir" for the reagents, and promote mixing of reagents with analytes in well-defined regions. To make these knots reproducibly, we used an inexpensive tool, Tie-Fast Knot Combo Tool (see the Supporting Information). This tool usually makes tight knots for fishing.

We assumed that enhanced evaporation of the solution in the detection zones will concentrate the analytes to create well-defined detection zones. We, therefore, used a laser cutter to prepattern a piece of tape with equally spaced holes onto which the knots (detection zones) could be placed (see Supporting Information). When a laser cutter is not available, a hole puncher can create the holes in the tape.

We inserted the piece of tape patterned with holes underneath the aligned threads on the loom, pressed the threads against the tape to make intimate contact between the tape, knots, and thread, and pressed a second piece of tape on top of the threads to encapsulate them between the two pieces of tape (see Figure S1 in the Supporting Information for a sequence of digital images that show the procedure). To disconnect the thread from the loom, we cut the thread where it contacted the pins of the loom and used a laminator to ensure contact between the thread and the tape. We cut the threads of the device such that one end of each piece of thread (i.e., "branch") protruded from the tape portion of the device and served as the sample application zone (i.e., inlet). To facilitate the application of the sample, we rolled the device such that the ends of the treads converged to a single point (Figure 2A,B). The length of each thread that protruded from the device had to be long enough to allow their convergence.

The inlet of the device is designed to absorb small volumes of fluid. This could be, in principle, useful for the analysis of a drop of blood from a finger prick, a small amount of urine (including the small amount of urine available from a newborn or a small animal such as a mouse), tears, gingival crevicular fluid, etc.

"Branching" Design. The ability to weave the thread using a loom allowed us to fabricate devices with various designs. One example, shown in Figure 1B is the "branching design". The threads in this design cross each other at a common point. One thread, which protrudes from the device, acts as the sample application zone and wicks the sample toward the common point, where it distributes to each branch. Figure 1B shows a device with six branches. It would be simple to increase the number of branches by placing additional threads that will intersect with one another. This particular orientation of the threads results in a narrow "foot print" of the device (i.e., the surface area of the device is small because the threads are not aligned perpendicular to each other). The advantage of the "branching design" in Figure 1B compared to the "woven array" design in Figure 1A is that it requires a small volume of sample, and is less prone to user error, because it has only one inlet. We created the detection zones for this device in the same manner as we did for the woven array device.

"Sewn Array" Design. The ability to sew thread into different substrates to make diagnostic devices was demonstrated by Li el al (2). Using similar principles, we fabricated a third device—

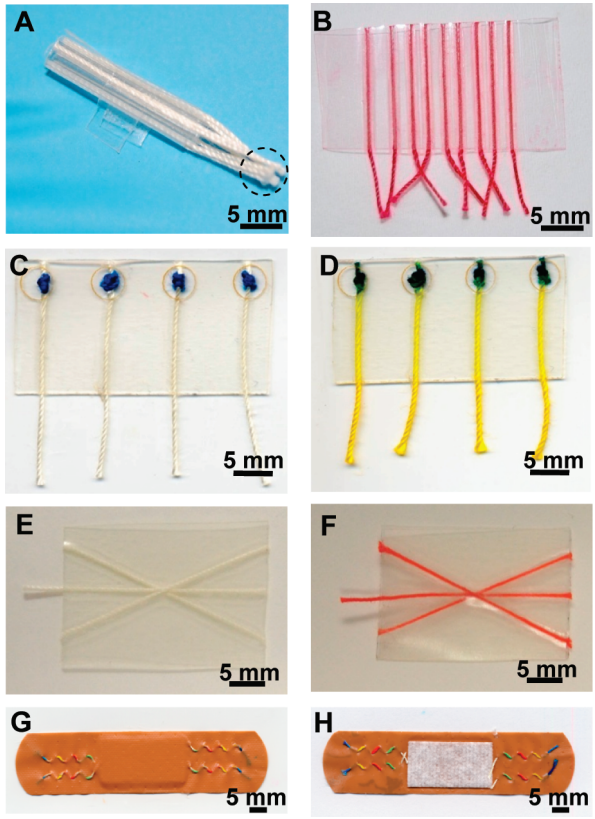

FIGURE 2. Wicking properties of the devices. (A) A photograph of the "woven array" device fabricated with a transparent Scotch tape. The device was rolled to facilitate application of the sample; the inlet zone is indicated by a dotted line. (B) The device was unrolled after 30 μL of red ink were introduced to the inlet and wicked into the device. (C) A photograph of the "woven array" where the knots (detection zones) contain blue dye. (D) A photograph of the woven array after wicking a yellow dye. The knots changed color from blue to green. (E) A photograph of the branching device. (F) A drop of 15 μL of red ink was introduced to the inlet of the device. (G) An image of the top side of a sewn array device fabricated by sewing the thread through a bandage. The stitches were spotted with different colored inks. (H) An image of the bottom side of the device.

the "sewn array" design (Figure 1C)—in which the sample travels only a short distance until it reaches the detection zone.

To fabricate this design, we sewed a thread into a plastic substrate. The stitches on the bottom and the upper side of the substrate defined zones for the sample application (i.e., inlet) and the detection, respectively (Figure 1C). To define these two stitches as one unit and isolate them from the other stitches, we applied a 3 μL drop of nail polish to block the holes (formed by sewing) in the substrate, and the thread located at these holes. The design has three characteristics that are well-suited for assays: (i) Every pair of stitches (i.e., a portion of the thread that spans both sides of the substrate, Figure 1C) could, in principle, serve as a different assay. (ii) The analyte travels a short distance, and the response of the device is, therefore, fast. (iii) The device can be incorporated into clothing, bandages, or diapers. The stitches close to the skin would serve as the sample application zone, whereas the stitches on the exterior side of the device would serve as the detection zone, where it would be easy to observe.

RESULTS

Wicking Properties. We used aqueous solutions of colored dyes to examine wicking in the different devices. We created the woven device, rolled it, and dipped the protruding ends of the threads (i.e., the inlets) into different volumes of red ink (Figure 2A,B). To fill a device composed of nine threads, we used 30 μL of ink. We tested the wicking properties of the branching design by dipping the protruding end of the thread in different volumes of red ink. The solution of 15 μL wicked along the threads and filled the device within five minutes (Figure 2E,F).

We also tested the ability to use knots for localizing and mixing reagents at specific regions along the thread. We first immersed a piece of cotton thread in blue dye and dried it for an hour in ambient conditions. We then made knots onto the aligned array of threads using the Tie-Fast Knot tool. We encapsulated this array in tape, and dipped the ends of the threads into a solution of yellow dye. The solution wicked along the threads at a rate of 2.5 cm/min. Upon reaching the knot (detection zone), the yellow ink mixed with the blue dye at the knot to produce a green color (Figure 2C,D).

The "sewn array" design is well-suited for assays embedded in bandages, because one side of the device can absorb and wick biological fluids (such as sweat or wound exudates), and transport them to a read-out region on the other side of the device. We demonstrated this concept by sewing thread into a bandage. Figure 2G shows the top side of a device with a thread sewn into a latex-free bandage, while Figure 2H shows the bottom side of the device. We used 0.5 μL of aqueous solutions of blue, yellow, red, and green dyes to show that the individual assay segments do not "bleed" together by capillarity (Figure 2G,H). This volume of solution provided the thread with excess solution and allowed us to observe the colors of the dyes easily. It is possible to wick a sample with a smaller volume (0.1 μL for 6 mm of thread), as was demonstrated by Li et al. (2).

Modifying the Wicking Properties of the Thread. To find the optimal set of conditions for fabricating thread-based diagnostic devices, we examined several additional strategies for controlling the wicking properties of the thread. We examined the effect of the tape that we used, for encapsulation of the threads, on the wicking properties of the device. We used both vinyl tape and clear Scotch tape, and a combination of the two tapes to fabricate the woven device. When we used vinyl tape to fabricate the device, the tape softened upon lamination and formed a conformal seal around the threads. In contrast, the Scotch tape did not form a conformal seal, and thus a small gap formed between the tape and the thread at its edge (see Figure S2 in the Supporting Information).

We did not observe any difference between the wicking behavior of the two tapes when we dipped only the tips of the threads and not the edge of the two pieces of tape into a solution of yellow ink. For both devices, the threads wick the solution of ink (see Figure S2 in the Supporting Information). This difference between the tapes, however, affected the wicking properties of the device and became apparent when the entire bottom of the device (thread and tape) was submerged in fluid (see Figure S3 in the Supporting Information). The vinyl tape formed a tight seal with the tread; this seal ensured that the solution wicks only through the thread

(an observation confirmed by examining the wicking under an optical microscope). The Scotch tape did not form a conformal seal around the threads, and the solution wicked both through the threads and though a small gap between the tape and the thread (see Figure S3 in the Supporting Information); this gap effectively forms a fluidic channel. The wicking through the gap was faster (6 cm/min) than the wicking through the thread (2.5 cm/min).

When we laminated the threads between one piece of Scotch tape and one piece of vinyl tape and submerged the bottom of the device in the ink solution, the ink wicked through a gap between the tape and the thread. This gap was significantly smaller than the gap formed with the threads laminated between two pieces of Scotch tape (see Figure S3 in the Supporting Information).

Both types of contacts made by the tapes are useful: (i) the conformal seal formed by the Vinyl tape ensures that wicking occurs only within the thread such that individual diagnostic assays remain separated and individually encapsulated. (ii) The small gap created by the Scotch tape is useful as a capillary to allow rapid wicking through what is effectively an open microfluidic channel with one hydrophobic wall.

We also explored simple ways to modify the wicking properties of the thread. When we applied a hydrophobic coating (petroleum jelly or wax) on the outer side of the hydrophilic thread and laminated it between two pieces of Scotch tape, we observed wicking only within the threads (see Figure S3 in the Supporting Information). The use of petroleum jelly also allows us to easily pull the thread out of the encapsulating tape (see Figure S4 in the Supporting Information). We believe that the ability to separate the thread from the tape may be useful for further analysis of the analytes on the thread.

Colorimetric Assays for Detecting Protein, Nitrite, and Ketones. We demonstrated the ability to perform colorimetric assays using the three devices by performing three colorimetric assays for analytes in urine: (i) protein, the presence of protein in urine may indicate kidney dysfunction; (ii) nitrite, for the detection of a urinary tract infection (UTI); and (iii) ketones, high concentration of ketones in urine may indicate presence of diabetic ketoacidosis. To detect protein, we immersed the thread in a solution containing 250 mM citric acid (pH 1.8) and 3.3 mM tetrabromophenol blue (TBPB) in 95% ethanol. To detect nitrite, we immersed the thread in a solution of 2 mg/mL sulfanilamide, 1.7 mg/mL 3-hydroxy-1,2,3,4-tetrahydrobenzo(h)quinoline, and 25 mg/mL tartaric acid in methanol. To detect ketones, we immersed the thread in a solution of 20 mg/mL sodium phosphate, 20 mg/mL sodium borate, 10 mg/mL glycine, a 0.5 μL solution of 20 mg/mL nitroprusside, 30 mg/mL polyethylenglycol (PEG, $M_w = 2000$), and 2 mg/mL poly(acrylic acid) (PAA, $M_w = 2000$). The reagents dried in ambient conditions for ~1 h.

Figure 3 shows the woven-array device 6 min after the addition of a 10 μL sample of artificial urine that contained 1 μM Bovine Serum Albumin (BSA), 1 mg/mL lithium acetate,

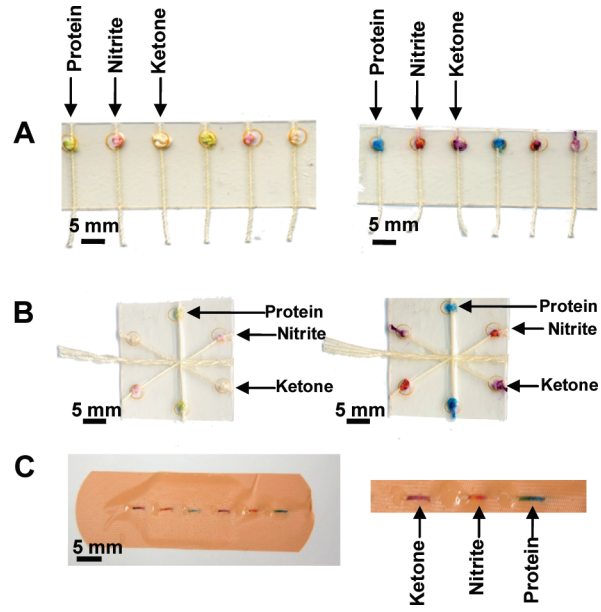

FIGURE 3. Colorimetric assays performed using the (A) woven array device, (B) branching device, (C) sewn array design.

0.2 mM sodium nitrite. When we applied the sample, the fluid wicked along the thread. We noticed color changes in the thread ~10 s after spotting the sample; the colors reached their maximum intensity after 3 min for nitrite and protein, and 6 min for ketones; these response times are similar to those of a conventional dipstick. We observed similar color intensity for some of the detectable concentrations, and therefore, could not determine accurately the quantity of the analyte in the sample (see Figure S5 in the Supporting Information). We also demonstrated the performance of colorimetric assays with the "branching design" (Figure 3). The threads comprising this device had to be long enough to prevent cross-contamination between the assays through the common point. The thread in the middle of our device, which acts as the sample application zone, is a combination of three cotton threads braided together to create a central cord. We inserted the threads that constitute the branches of the device through the central cord in order to increase the surface of contact and promote faster wicking. We defined the detections zones to ensure that no cross-contamination will occur, and that there is a separation of the sample application zone from the detection zone. In both devices—the branching design and the woven array—the multiple threads laminated together in one device enable the performance of different assays or replications of the same assay.

We also demonstrated the ability to perform colorimetric assays using the "Sewn array" device. After sewing the thread into the bandage, we defined each of the two stitches as one microfluidic channel with the application of a clear nail polish. We then applied the reagents for the assays (using a pipet) on the stitches located on the upper part of the bandage (Figure 3C). Next, we introduced a sample containing the analytes (Figure 3C,D). Colorimetric changes occurred at the same rate as in other designs.

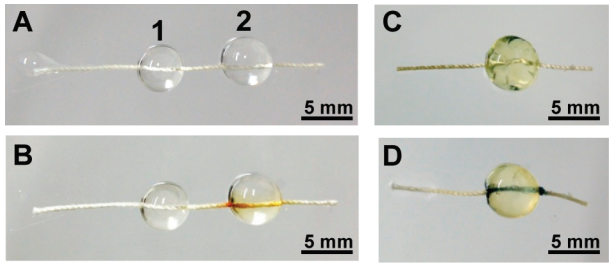

FIGURE 4. Enzymatic reactions performed on a cotton thread. Spheres of polyacrylamide gel contain the reagents and enzymes for the reactions. (A) The sphere labeled 1 contains potassium iodide, the sphere labeled 2 contains horseradish peroxidase/glucose oxidase. (B) Colorless iodide is oxidized to brown iodine on the thread in the presence of glucose. Change in color is noticeable 15 min after applying artificial urine containing glucose. (C) The sphere contains nitrotetrazolium blue chloride and 5-bromo-4-chloro-3-indolyl phosphate. (D) Fifteen minutes after the application of 140 units/L alkaline phosphatase, a portion of the thread in contact with the sphere of polyacrylamide gel changed its color to purple.

Enzymatic Colorimetric Assays for Detecting Glucose and Alkaline Phosphatase. Many biomedical assays are based on enzymatic reactions. We, and others, have demonstrated the ability to perform enzymatic colorimetric assays on paper (3, 19−21). Enzymatic assays on paper include a number of challenges, such as potential reduction in activity of enzymatic reagents stored on paper over prolonged period of time, and rapid evaporation of fluid during the assay that terminates the enzymatic reaction. We sought to overcome these challenges by combining thread with spherical particles of polyacrylamide gel containing reagents and enzymes needed for the assay. We reasoned that polyacrylamide gel should promote the hydration of enzymes and reagents during the enzymatic reaction, and enable the device to store these reagents in a hydrated state, if necessary. We chose to examine two clinically relevant enzymatic assays: detection of glucose in artificial urine using potassium iodide and glucose oxidase/horseradish peroxidase, and detection of alkaline phosphatase in artificial plasma using 5-bromo-4-chloro-3-indolyl phosphate and nitrotetrazolium blue chloride.

To detect glucose in artificial urine, we incubated dry spherical particles of polyacrylamide (see Supporting Information) in ∼50 μL of 0.6 M aqueous solution of potassium iodide or 1:5 horseradish peroxidase/glucose oxidase aqueous solution (15 units of protein per 1 mL, Sigma G3660) for 1 hour. After the beads expanded in the solution to a diameter of ∼5 mm, we threaded one sphere containing potassium iodide and one containing horseradish peroxidase/glucose oxidase onto a cotton thread. We determined experimentally that a distance of ∼1 cm between the two spheres will allow the transfer of solution without cross-contamination. In the presence of oxygen, glucose oxidase catalyzes the oxidation of glucose to gluconic acid and hydrogen peroxide. The horseradish peroxidase then catalyzes the reaction of hydrogen peroxide with potassium iodide. The colorless iodide is oxidized to brown iodine: this reaction induces a color change from colorless to yellowish-brown in the presence of glucose (Figure 4). After the assembly of the device, we wicked a solution containing glucose through the thread. For solution containing 50 mM glucose, we detected a change in color of the thread after 6 min, and full development of color after 15 min. We noticed color change for lower concentrations (2−10 mM) after 2 h. The gel particles preserved the reagents for the assays for at least 5 days when incubated in a Petri dish covered with aluminum foil at 22 ± 2 °C and relative humidity (RH) of 20−30%.

To detect alkaline phosphatase in artificial blood plasma (22), we suspended spherical particles of polyacrylamide in ∼50 μL of nitrotetrazolium blue chloride (1.5 mg/mL) and 5-bromo-4-chloro-3-indolyl phosphate (1 mg/mL) dissolved in 100 mM Tris buffer pH 9.5 for an hour.

Alkaline phosphatase catalyzes the cleavage of 5-bromo-4-chloro-indolyl phosphate (BCIP) to metaphosphoric acid and the corresponding indolyl moiety. The indolyl group then rearranges and dimerizes to form either 5,5′-dibromo-4,4′-dichloro-indigo under acidic conditions, or 5,5′-dibromo-4,4′-dichloro-indigo white under alkaline conditions. In the process of dimerization at any pH, the indolyl moiety releases hydrogen ions; these ions reduce the nitro blue tetrazolium, and cause the formation of a blue-colored precipitate (23, 24).

After the assembly of the device, we applied 10 μL of artificial blood plasma containing 140 units/liter of alkaline phosphatase to the inlet of the thread (23). We detected color change on the thread after 10 min (Figure 4).

CONCLUSIONS

This paper describes the use of thread as a matrix for the fabrication of diagnostic assays, a concept that Li et al. have also demonstrated (2). Some of our conclusions are similar to those reached by Li et al (2). We conclude that: (i) Cotton thread is well-suited as a matrix for diagnostic assays. (ii) Thread-based devices can be fabricated using established techniques that rely on common household tools for manipulating threads (e.g., sewing machines and looms). The fabrication schemes could potentially be adapted for large-scale manufacturing of these devices. (iii) Intersections of threads in the form of twisting, as demonstrated by Li et al. (2), and weaving (1) (as we demonstrate in the "woven array" and "branching design) can be used to control the transport and distribution of fluids along the thread. (iv) Knots of thread and gel particles (as demonstrated here) and paper disks (as demonstrated by Li et al.) integrated into thread-based devices can be used to introduce reagents into specific regions along the thread and define the detection zones. (v) A variety of designs (e.g., "woven array", "branching", and "sewn" as shown here, as well as 3D thread-paper composite devices demonstrated by Li et al.) can be used for diagnostic assays. (vi) The resulting systems wick fluids by capillary action, require no external power to operate, and are capable of testing small volumes of sample for relevant physiological concentrations of multiple biomarkers of disease (e.g., protein, nitrite, ketones, glucose and alkaline phosphatase); the results of the assays can be interpreted by the unaided eye. The ability to test for multiple analytes using a small volume (15−30 μL) of sample is especially

important when analyzing urine samples from premature babies or infants, or when drawing blood from a finger prick in remote locations. In addition, we confirmed the results obtained by Li et al., that cotton thread enables detection of relevant concentration of analytes; however, in our experiments, the detection is done in a less quantitative manner than paper-based devices (1, 2).

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Supporting Information Available: Experimental procedures, a sequence of images showing the assembly of the "woven array" device, schematic illustrations demonstrating the effect of the width of a gap between two threads encapsulated between two pieces of Scotch tape, images of the woven-array device fabricated with different types of tapes after dipping the bottom of the device into a solution of red ink, an image of two threads coated with petroleum jelly, and an image of a device for the detection of protein in artificial urine (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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