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Recent advances in thread-based microfluidics for diagnostic applications

Xuan Weng\textsuperscript{a}, Yuejun Kang\textsuperscript{b}, Qian Guo\textsuperscript{a}, Bei Peng\textsuperscript{a}, Hai Jiang\textsuperscript{a}\textsuperscript{*}

\textsuperscript{a} School of Mechanical and Electrical Engineering, University of Electronic Science and Technology of China, Chengdu, Sichuan, 611731, China
\textsuperscript{b} Institute for Clean Energy and Advanced Materials, Faculty of Materials and Energy, Southwest University, Chongqing 400715, China

**Abstract**

Over the past decades, researchers have been seeking attractive substrate materials to keep microfluidics improving to outbalance the drawbacks and issues. Cellulose substrates, including thread, paper and hydrogels are alternatives due to their distinct structural and mechanical properties for a number of applications. Thread have gained considerable attention and become promising powerful tool due to its advantages over paper-based systems thus finds numerous applications in the development of diagnostic systems, smart bandages and tissue engineering. To the best of our knowledge, no comprehensive review articles on the topic of thread-based microfluidics have been published and it is of significance for many scientific communities working on Microfluidics, Biosensors and Lab-on-Chip. This review gives an overview of the advances of thread-based microfluidic diagnostic devices in a variety of applications. It begins with an overall introduction of the fabrication followed by an in-depth review on the detection techniques in such devices and various applications with respect to effort and performance to date. A few perspective directions of thread-based microfluidics in its development are also discussed. Thread-based microfluidics are still at an early development stage and further improvements in terms of fabrication, analytical strategies, and function to become low-cost, low-volume and easy-to-use point-of-care (POC) diagnostic devices that can be adapted or commercialized for real world applications.

**Keywords:** Thread-based microfluidics, Point-of-care, Cotton, Colorimetric, Electrochemical

**1. Introduction**

The miniaturization trend is being driven by portable devices development out of regard for reduction or elimination of reagents and assay time, more efficient analysis with lower energy consumption, and simplified operation. Microfluidics is a powerful tool for downsizing devices, especially the micro-based ones, by providing an integrated platform including sample preparation, delivery and measurement with a high degree of automation, only small amounts of reagents and faster assay time (Chen et al., 2016; Moss et al., 2015; Sackmann et al., 2014; Streets and Huang, 2013). Therefore, microfluidic-based devices have grabbed considerable attention by researchers to produce point-of-care (POC) assays for a broad range of applications. Microfluidic devices are usually characterized by low cost and simple fabrication process, thereby selection of available materials is extremely essential. Conventional microfluidic devices are made of glass, silicon or polymer materials, such as polydimethylsiloxane (PDMS), polystyrene (PS), polycarbonate (PC), polymethyl methacrylate (PMMA), polyethylene terephthalate (PET) and polyvinyl chloride (PVC) (Dauson et al., 2016; Jackson et al., 2016; Kitsara and Ducrée, 2013; Ogończyk et al., 2010; Li et al., 2012; X. Zhang et al., 2017; Y. Zhang et al., 2017). Although these materials are good for the fabrication and prototyping of microfluidic chips, they require sophisticated, expensive and time-consuming fabrication processes without any exception, which limits their use in the development of microfluidic device towards low cost POC testing. In addition, the relative high manufacturing costs due to the materials and cleanliness requirements prevent to some extent the massive use of these devices worldwide.

Paper-based microfluidic analytical devices (µPADs) are emerged as an alternative to overcome the above limitations and was firstly proposed by the Whitesides’ group in 2007 (Martinez et al., 2007). µPADs introduce an innovative platform technology for fluid handling and analysis with wide range of applications including diagnostic biosensing, food safety and environmental monitoring (Tomazelli Coltro et al., 2014; Fernandes et al., 2017; Ghaderinezhad et al., 2017; K. etisen et al., 2013; Ma et al., 2018; Qi et al., 2017; Weng et al., 2018; Weng and Neethirajan, 2018a, 2018b, 2017; Xia et al., 2016; Yamada et al., 2015), due to the advantages of portability, simplicity, economic affordability and minimal sample consumption (Akyazi et al., 2017; Cate et al., 2014; Gong and Sinton, 2017; He et al., 2016; Hu et al., 2014; Yamada et al., 2017). However, the low mechanical strength and surface tension as well as the requirement of hydrophobic barriers still limit their practical applications in making microfluidic analytical devices (Agustini et al., 2016; Akyazi et al., 2017; Dou et al., 2015; X.
Zhang et al., 2017; Y. Zhang et al., 2017).

More recently, thread substrate has been introduced into microfluidic devices, known as thread-based microfluidics, due to its features such as wide use, high availability, low cost, general hydrophilic nature, as well as the lightweight and easy biodegradability (Agustini et al., 2018; Gaines et al., 2018a, 2018b; Kabariya and Ramani, 2018; Ochiai et al., 2017; ChuanaYe, et al., 2016). Thread-based microfluidic devices utilize threads to construct fluidic channels to perform various analytical assays. Thread refers to thin strand of cotton, nylon, or other fibers and the wicking property of which facilitates the fluidic flow without using an external pumping system. In addition, the mechanical strength of thread is higher compared to paper even under wet condition. Therefore, no hydrophobic barriers are required for the formation of microchannels, neither the mechanical pumps for the transportation of solutions. Instead, in thread-based microfluidic devices, these may be achieved solely by the capillary force between fibers of the thread due to the high hydrophilic character (Berthier et al., 2017; Lu et al., 2015). Thread-based platforms are more flexible because they may easily be made in one, two or three-dimensional configuration. Other advantages of threads include the great capacity to be easily modified the properties of their fibers by immobilization or incorporation of chemicals to create reaction and detection regions (Agustini et al., 2018) and the less lost volume of unused volume of solution due to the better confinement of fluids. Thread-based microfluidic devices have been found applications in portable diagnostic systems, smart bandages and even tissue engineering (Berthier et al., 2017). There are a few reports demonstrating the capacity of thread-based microfluidics to be used in electrochemical detection (Agustini et al., 2018; Gaetano et al., 2018; Carneiro et al., 2018; Song et al., 2017), electrochemiluminescence detection (Guan et al., 2016; Liu et al., 2016), chemical sensing and synthesizer (Banerjee et al., 2013; Cabot et al., 2018; Erenas et al., 2016; Li et al., 2017), immunochromatographic assays (Jia et al., 2017; Liu et al., 2017; Meng et al., 2017; Seth et al., 2018; Zhou et al., 2012), capillary electrophoresis electrochemical (CE-EC) detection (Song et al., 2017; Wei et al., 2012), pH sensing (Lyu et al., 2018; Punjiya et al., 2017) and cell culture (Nilghaz et al., 2018). Threads hold a great promising potential for the development of wearable diagnostic devices by simply integrating with wearable materials (Derakhshandeh et al., 2018; Sadeqi et al., 2018).

In this review, we intend to give an overview of thread-based microfluidic platforms for a wide variety of applications. We focus on the primary advances reported in last 5 years with regard to fabrication methods, designs, diagnostic applications and performance of those thread-based microfluidic devices. Finally, a perspective on the future trends for the thread-based microfluidic devices is also given.

2. Thread-based microfluidics

2.1. Fabrication Techniques in Thread-based Microfluidics

As mentioned previously, the distinct characteristics of thread make it a useful material for the fabrication microfluidic devices, such as the simplicity, low cost, lightweight, easy access, functionality as well as suitability for mass fabrication methods like sewing or knitting (Li et al., 2009; Mross et al., 2015). It also facilitates the fabrication of 3D structures and can be easily incorporated into garment due to its higher tensile strength than paper (Nilghaz et al., 2013). Typical thread materials used in making microfluidic devices are cotton (Carneiro et al., 2018; Liu et al., 2017), polyester (Adhikari et al., 2018; Jarajarmus et al., 2018; Nilghaz et al., 2016), nylon (Gaines et al., 2018a, 2018b; Qiu et al., 2018), acrylic (Cabot et al., 2018), wool (Jeon et al., 2015a, 2015b) or silk (Konwarh et al., 2016; Yan et al., 2015). Usually, threads consist of fibers of natural origin such as cotton or cellulose, or of artificial origin such as polymers, aligned or twisted together. The former being hydrophobic in its natural state and thus has to be treated (Li et al., 2009) or mercerized (Reches, 2013; Reches et al., 2010) in order to wick fluids. To obtain wicking of the fibers, wetting properties are necessary, including physical and chemical properties. In addition, the selection of threads for specific applications also depends on the microscale surface morphologies of different fibers (Nilghaz et al., 2014b).

The wicking of the liquid in thread relies on the twisted strands of cellulose fiber and the gap in between. The wicking capability enable the thread to push the liquid along the length without using any auxiliary pump, leading to simplifying the overall set-up significantly. Therefore, wetting properties of the fibers are necessary. Usually, thread needs to be undergone hydrophilically treatment to remove noncellulosic components in order to increase the wettability, including plasma oxidation, chlorination, scouring with specific chemical solution (Erenas et al., 2016; Ulum et al., 2016; Wu and Zhang, 2015; Wu et al., 2016). However, the modified hydrophilicity may reduce gradually with time, typically 24 h, due to the spontaneous reorientation of polar groups (Caschera et al., 2014). To maintain the wettability for a period of time is extremely important, especially for point-of-care testing (POCT) tools. Li et al. (2018) utilized a bio-compatible polymer, chitosan, to functionalize the cotton thread via electrostatic-interaction-mediated adsorption to prolong stable hydrophilicity. The results showed that the hydrophilicity of the chitosan functionalized cotton thread was stable even after storage for 3 months and it was able to increase the wicking rate.

Berthier et al. (2017) investigated the flow patterns in different flow regimes for homogenous and heterogeneous fiber bundles by both theoretical and numerical analysis. They drew a conclusion that a limited number of fibers was sufficient for thread-based capillary flows and various of flow patterns could occur in heterogeneous fiber bundles depending on the compactness and contact angles, allowing numerous of applications. Jeon et al. (2015b) investigated the plasma treatments of wool fiber surface so as to changing the wettability of hydrophobic wool thread herein to control the flow rate of the thread-based microfluidic devices. They reported that plasma treatment was able to improve the wettability due to the removal of the fatty acid layer on the surface. Various gases (oxygen, argon, nitrogen and air), plasma treated wools had different flow rates and oxygen plasma was the most effective to change the wettability. As the treatment time increased, the wicking rate was found to increase, however, increasing time could damage the wool surface. Based on their findings, appropriate plasma treatments may used for different applications to acquire specific flow rate requirement in thread-base microfluidic devices.

In a thread-based microfluidic device, the wicking of fluid depends on the passive capillary of the thread which is hardly controlled precisely due to formation of random orientation of cellulose fibers. To achieve a tunable hydrophobicity in a thread-based microfluidic device, Choi et al. (2018) used twelve-fold diluted polysiloxanes to partially change the hydrophilicity of the cotton thread to obtain desirable fluidic delay and optimum interaction between the antibody-functionalized gold nanoparticle probes and target antigen (Fig. 1). With the modification, more resultant complexes were generated and captured at the test zone thus enhanced the sensitivity (around 10-fold signal enhancement) significantly. They detected Salmonella enterica serotype Enteritidis in PBS, spiked whole milk, juice and lettuce, detection limits of which were 500, 1000, 1000 and 5000 CFU/mL, respectively. The entire sample-to-answer assay needed only 10 min in comparison with several days required for a conventional bacterial plating assay.

2.2. Thread microfluidics with different detection methods

Threads are a promising alternative material for microfluidic devices. In a thread-based microfluidic device, thread works as the liquid transportation channel and is able to minimal the amount of solution and time due to its small scale. In addition, thread can also be made and manipulated easily since it can be knitted or woven, and twisted to...
network to achieve mixing (Gonzalez et al., 2016). Recently, the use of the thread-based microfluidics has been applied to immunoassays (Jia et al., 2017; Mao et al., 2015a; Song et al., 2017), determination of nucleic acids (Du et al., 2015), proteins (Liu et al., 2017; Mao et al., 2015b; Nilghaz et al., 2014a; Ulum et al., 2016), glucose (Gaines et al., 2013), virus (Weng and Neethirajan, 2018b), small ions (Jarujamrus et al., 2018; Yang et al., 2014a), virus (Weng and Neethirajan, 2018b), small ions (Jarujamrus et al., 2018; Yang et al., 2014a), virus (Weng and Neethirajan, 2018b), small ions (Jarujamrus et al., 2018; Yang et al., 2014a), and metabolite analysis (Cabot et al., 2018). Taken advantage of the thread-based microfluidics, these immunoassays can be performed on a conventional blood sample without the need of specialized equipment and trained professionals. Later, Nilghaz et al. (2014b) utilized a similar nylon thread-based analytical device (µTAD) which was more sensitive (10 and 5000 folds) than previous reported CNT based lateral flow assays. With this method, Meng et al. (2017) successfully detected the human ferritin antigen with a detection limit of 50 ng/mL within 25 min without instrumentation. This sensitivity is nearly 500 folds lower than that of the similar report (Aberra and Choi, 2010).

The technique of thread-based microfluidics has distinct advantages over the colorimetric detection in traditional 96-well plates. It is apparent that small volumes of sample/reagents and short detection time are required due to the small scale of the thread devices and the superior probe absorbent capability with treatments. In addition, the fabrication procedures and required equipment are much simpler and cost effective. Given the cost and efficiency by the thread-based microfluidics, it is a promising alternative to conventional ELISA and may become new diagnostic devices in the growing healthcare industry for probing a wide range of biological interactions.

Nilghaz et al. (2014a) reported a semi-quantitative thread-based analytical device by length measurement of color change on indicator treated threads using a printed ruler. The device was simply made of straight cotton/polyester threads with a knot in the centre. The method utilized a specific colorimetric reaction between analyte molecules and indicator reagents, during which the resultant products underwent chromatographic elution and were driven by fixed volumes of sample as the aqueous carrier phase. Samples of different analyte concentrations could result in specific lengths of color change on the thread, allowing the determination of the analyte concentration. Protein and nitrite assays were successfully performed on the device and linear ranges of 0–1.5 mg/mL and 0–1000 µM were obtained, respectively. Compared to other common used thread-based colorimetric methods, this length measurement method could be easily interpreted without the need of electronic equipment and trained professionals. Later, Nilghaz et al.
| Detection principle | Characteristics | Application | Reported detection limit | References |
|---------------------|-----------------|-------------|--------------------------|------------|
| **Colorimetric**    | Nylon thread    | Goat anti-mouse IgG and rabbit IgG antibodies | 133.8 fmol/zone | (Gonzalez et al., 2018b) |
|                     | Nylon thread    | Acetylcholinesterase (AChE) | 1.74 nM | (Gonzalez et al., 2017) |
|                     | Cotton thread;  | *Salmonella enterica* serotype Enteritidis in phosphate buffered saline, spiked whole milk, juice and lettuce | 500, 1000, 1000 and 5000 CFU/mL respectively | (Choi et al., 2018) |
|                     | Integrating polyoxanes to obtain tunable hydrophobicity; 10-fold signal enhancement; Sample-to-answer process; 10 min for entire assay | | | |
| **Colorimetric**    | Carbon nanotubes | Human ferritin antigen | 50 ng/mL | (Meng et al., 2017) |
|                     | Cotton thread;  | Carcinoembryonic antigen (CEA) | 2.32 ng/mL | (Jia et al., 2017) |
|                     | Immunochromatographic | | | |
| **Colorimetric**    | Cotton thread;  | Squamous cell carcinoma antigen (SCCA) | 3.03 ng/mL | (Liu et al., 2017) |
|                     | Raw cotton thread and synthetic polyester; Length measurement of color change on indicator treated threads | Protein and nitrite | Linear dynamic detection range of 0 – 1.5 mg/mL and 0 – 1000 μM | (Nilghaz et al., 2014a) |
| **Colorimetric**    | Cotton thread and silk fibers | Blood typing analysis | --- | (Nilghaz et al., 2014b) |
|                     | Cotton thread | Potassium in mineral waters ionophore extraction chemistry for the optical recognition | --- | (Reenas et al., 2016) |
| **Colorimetric**    | Cotton thread and paper strip hybrid; Mobile phone detector | Antioxidant | --- | (Sateanchok et al., 2018) |
| **Colorimetric**    | Polyester sewing thread and nitrocellulose membrane hybrid; Immunochromatographic | *H. pylori*, Hepatitis B and IgG antigens | 30 – 300 ng/mL | (Seth et al., 2018) |
| **Colorimetric**    | Nylon thread/paper hybrid | Glucose in artificial urine | Linear dynamic detection range of 0.5 – 15 mM | (Lee et al., 2018) |
|                     | Nylon thread/paper hybrid platform; Simplicity | Glucose | 0.5 mM | (Gonzalez et al., 2016) |
| **Colorimetric**    | EDTA-functionalized μTAD; Whole blood plasma separation | Albumin | 114 mg/L | (Ulman et al., 2016) |
| **Chemiluminescence** | Cloth; wax-screen-printing; Food detection and environmental monitoring | Hydrogen peroxide (H2O2) | 0.46 mM | (Guan et al., 2015) |
| **PicoGreen assay kit** | Cotton/PDMS hybrid; 3D cell culture system | COS-7 cells | --- | (Nilghaz et al., 2018) |
| **Thermal lens**    | Cotton thread with thermal lens detection | Copper and zinc ions | --- | (Yan et al., 2015) |
| **Fluorescent immunosensor** | Cotton thread; Fluorescence resonance energy transfer (FRET)-based MoS2 biosensor; Ease of local manufacture; Small consumption; High sensitive and short time of analysis | Infectious bronchitis virus | 4.6 × 10^2 EID50 per mL | (Weng and Neethirajan, 2018b) |
| **Fluorescence**    | Cotton thread; Gold nanoparticle (AuNP) conjugate modified with adenosine based molecular beacon (ABMB) probe | Human genetic disease related DNA | Linear dynamic detection range of 2.5 – 100 nM | (Du et al., 2015) |
| **Electrochemical** | Cotton thread amperometric detection | Gallic and caffeic acid in wine samples | 1.5 × 10^{-6} M and 8.0 × 10^{-7} M | (Camariera et al., 2018) |
|                     | Cotton thread; Amperometric detection | Phenol in drinking water | 2.91 mmol/L | (Carreira et al., 2018) |
|                     | Cotton thread; Electrodes drawn onto the paper by a mechanical micro pencil | Orthodiphenols | 2 mM | (Dossi et al., 2018) |
| **Electrochemical** | Electrodes made by painting nylon thread with layered silver/ carbon ink, silver/ silver chloride ink and carbon/graphite | Glucose | linear dynamic range of 0 – 25 mM | (Gaines et al., 2018a) |
| **Electrochemical** | Nylon thread; Electrodes painted with conductive inks | Glucose and acetylthiocholine | Linear dynamic detection range of 0 – 15 mM and 0 – 9.84 mg/mL | (Gaines et al., 2018b) |
| Detection principle | Characteristics | Application | Reported detection limit | References |
|---------------------|-----------------|-------------|--------------------------|------------|
| Electrochemical     | Cotton thread; Electrochemical | Strain; gastric and subcutaneous pH in vitro and in vivo | Linear dynamic detection range of 0.1-1.0 mM and 0.1-1.5 mM | (Mostafalu et al., 2016) |
|                     | Electrochemical  | Strain; gastric and subcutaneous pH in vitro and in vivo | Linear dynamic detection range of 0.1-1.5 mM | (Glavan et al., 2016) |
|                     | Electrochemical  | Stable electrode-electrolyte interface leading to a stable current | Linear dynamic detection range of 0.1-1.5 mM | (Song et al., 2017) |
|                     | CE-EC Electrochemical | Gold nanorod | Linear dynamic detection range of 1.28 ng/mL | (Cabot et al., 2018) |
|                     | CE-EC Electrochemical | Polyester; Urea and glucose | Liquid sample | (Yang et al., 2014a) |
|                     | CE-EC Electrochemical | Enzyme doped on thread | Ribonuclease in human urine | (Lin and Lin, 2016) |

Erenas et al. (2016) demonstrated a cotton thread microdevice to quantitatively detect human genetic disease related DNA at room temperature. The device (Fig. 3(b)) was consisted of a single cotton thread strip positioned on two parallel double faced adhesive tapes and an absorbent pad at the downstream end. The glass fiber loading gold nanoparticle (AuNP) conjugates modified with adenosine based molecular beacon (ABMB) probes was attached to the other end of the thread. In an assay, a mixture solution of sample containing desired complementary and single base mismatched DNA sequences and AuNP-ABMB conjugates was added to the sample pad. In the presence of target DNA sequences, the hairpin structure of ABMB sequences and AuNP-ABMB conjugates was added to the sample pad. The device was able to discriminate the single based DNA related the disease hereditary tyrosinemia type I with a linear dynamic range of 2.5-100 nM. The distinguished advantage of this device is the room-temperature detection capability with high sensitivity, which is much simpler and superior than those previous reports (Lin and Tseng, 2012).

2.2.2. Electrochemical detection

Fluid handling in microfluidic devices with colorimetric detection used cotton materials as substrates, and treatment processes are usually required to cleanse the impurities on surface for acquiring wicking capabilities. These thread-based microfluidic devices with colorimetric detection either obtained qualitative results by naked eyes or semi-quantitative measurement by a scanner or digital camera followed by software analysis of the color intensity. However, accurate color change is hardly to achieve due to the errors generated by different models of electronic devices or lightness, saturation and optical background noise. These may affect significantly the interpretations of the same colors resulting in reducing the sensitivity of the system.
making microchannels (Li et al., 2009; Nilghaz et al., 2013). Agustini et al. (2016, 2017) made some efforts in making low-cost thread-based microfluidic devices for electroanalytical applications, such as a thread-based device for microflow injection analysis based on electrochemical technique. They exclaimed that thread microchannels presented high polarity because no wax or other non-cellulosic substance coated on the threads, thus no need for any treatment before use. A relative high flow rate of 2.2 µL/s could be achieved. This presented that thread-based microfluidic channels could provide high polarity and flow rate without using external components, and showed good performance when used in an electroanalytical device for microflow injection analysis. They utilized a similar microfluidic thread-based electroanalytical system to perform chromatographic separations (Agustini et al., 2018) which was based on an ion exchange mechanism and detected by gold electrodes manufactured directly on the cotton threads. Carneiro et al. (2018) presented a 3D printed microfluidic electroanalytical device boning with cotton threads for amperometric detection of antioxidant in wine samples. The structure of the thread-based electroanalytical device (µTED) is shown in Fig. 4(a). The SPEs (working electrode, counter electrode and reference electrode) were manufactured by the micro DEK 1760RS printer. The device consisted of an inlet, outlet and the SPEs attached near to the outlet. Nine parallel threads without twisting were positioned throughout the inlet and outlet reservoirs to form the microchannels. The detection zone on the SPEs was filled with cotton thread to keep the entire electrode immersed in the flowing solution during amperometric measurements. The detection limits of the biosensor for gallic and caffeic acid were 1.5 × 10⁻⁶ M and 8.0 × 10⁻⁷ M, respectively. The results obtained were highly in agreement with those by the Folin-Ciocalteu Method. Using the device, their group (Cañano et al., 2018) detected phenol in drinking water. It utilized both the capillary force and gravity along the thread to transport the fluid and electrochemical detection to analyze the results. This method achieved a low limit of detection of 2.91 nmol/L and superior sensitivity compared to other phenol sensors.

Dossi et al. (2018) developed a cotton thread fluidic device coupled with paper-based wall-jet electrochemical detector. Hydrophobic barriers were firstly patterned onto the filter paper by a permanent Lu-mocolar marker. Then, a mechanical micro pencil was used to draw the working, counter and reference electrodes onto the paper under a pressure of 1.91 N at a writing speed of 3 cm/s. Afterwards, 7 cm long pieces of cotton thread with hydrophilicity improvement by NaOH were positioned horizontally on a Teflon support. One end of the thread was immersed in the electrolyte solution while the other was in contact with the working electrode. To obtain good alignment, an X, Y, Z mechanical manipulator was used. A filter paper was attached to the rear face of the PED-PDE to drain the running buffer. The device allowed the rapid and selective detection of hydrophilic orthodiphenols with a low enough detection limit of 2 mM.

Gaines et al. (2018a) reported a thread-based electrochemical sensor for the detection of glucose. The device is shown in Fig. 4(b), three thread electrodes were made by painting nylon thread with layered silver/ carbon ink, silver/ silver chloride ink and carbon/graphite ink to serve as working electrode (WE), reference electrode (RE) and the counter electrode (CE), respectively. The chip was made on a piece of fabric attached to the laminated adhesive plastic. Laser cutter was used to make holes for fixing the thread electrodes. A paraffin film was merged onto the back of the chip by a heat gun to prevent the leakage. The overhanging thread outside the chip connected with the potentionstat for measurement. Bee wax was finally painted layer by layer onto the edges of the chip to form the barrier. PBS solution containing glucose oxidase (GOD) (10 mg/mL), potassium ferricyanide (K₃[Fe(CN)₆]) (10 mg/mL) served as the mediator, and glucose of concentrations ranging from 0 m to 25 mM were tested in this electrochemical sensor. The results of which presented good suitability. By using thread-based electrodes and nylon thread, Gaines et al. (2018b) made another electrochemical sensor to detect glucose and acetylthiocholine. As seen in Fig. 4(c), the device consisted of three nylon-based electrodes painted with conductive inks and a piece of nylon thread wrapped around to hold the solution and connect the electrodes. Cyclic voltammetry measurement was conducted by this device on the detection of glucose (0–15 mM) and acetylthiocholine (ATC) (0–9.84 mg/mL), good linear regression line with R² values of 0.985 and 0.995 were achieved. This nylon-based electrochemical sensor is very simple and will find intensive applications in the development of POCT diagnostic devices for the resource-limited regions.

Implantable diagnostic devices and smart wearable systems provide significant and powerful tools for diagnostic analysis. Selection of proper materials for fabricating these devices are extremely important as the risks of mismatch or incompatibility between the properties of the biological tissues and the semiconductor-based electronics. For instance, Polyimide13 and Parylene14 are two of the extensively used as the substrates for making such devices. However, the microfabrication cost is high due to the complicated microfabrication procedures and the requirement of clean room facilities when using these materials. As mentioned previously, the degradation and mechanical properties of threads can be easily modified by selected various material composition, hence enable threads to be an excellent candidate for the development of implantable diagnostic devices and smart wearable systems.
Mostafalu et al. (2016) reported a thread-based microfluidic device for cotton thread carbon nanotube/gold nanoparticles (CNT/GNPs) nanocomposite reporter probes (Jia et al., 2017). (b) The schematic of cotton thread device for room temperature nucleic acid rapid detection based on adenosine-based molecular beacon probe (Du et al., 2015).

Mostafalu et al. (2016) reported a thread-based microfluidic sensor for 3D tissue embedding to measure strain, gastric and subcutaneous pH in vitro and in vivo. As shown in Fig. 4(d), microfluidic channels were formed by the hydrophilic threads embedded into the hydrophilic woven fabric to transport fluid to the testing zone. Conductive threads functionalized with nanomaterials served as electrodes for the in vitro and in vivo measurement of physiological properties. A smartphone was then connected the sensor via signal processing electronics and wireless communication. They evaluated the performance of this integrated system and the results suggested that it was able to act as a part of human skin or clothing and even be implanted. This method presents a promising pathway to make smart wearable equipment, such as smart bandages, smart sutures for point-of-care diagnostics, surgical implants or other applications.

Glavan et al. (2016) developed an electroanalytical device by using stainless steel pins as the electrodes and cotton thread and paper as the liquid support and substrate. A stainless steel pin coated with a graphite and carbon nanotube ink served as the working electrode (WE) while bare stainless steel pins served as the reference and counter electrodes (RE and CE) and with a distance of ~2.53 mm away from one another. In an assay, a droplet of liquid was pipetted to the thread, allowing for wicking to the electrodes and followed by the cyclic voltammograms recorded at a scan rate of 100 mV/s in a 500 μM solution of FeCo3H in 1 × PBS (pH 7.6). Fig. 5 shows the variation in the CVs of the FeCo3H solution for seven different devices. They found the results did not vary with the tension of the thread hence enable to form a stable electrode-electrolyte interface and stable current. Their method provides a promising way for the development of portable, versatile, easily storable and low-cost electroanalytical devices for various applications, though the variation in terms of the relative standard deviation (RSD) need to be improved.

Song et al. (2017) demonstrated an electrochemical immunoassay device using natural cotton thread for human ferritin determination. An enhanced sensitivity of 1.58 ng/mL ferritin was achieved by using the electroactive report probe gold nanorod (GNR) compared with the gold nanoparticle report probe. The principle and procedures of the nature cotton thread based immunoassay device was shown in Fig. 6. The GNR-antibody-Ferritin complex was added onto the sample pad to wick along the cotton thread to the test zone and then reacted with the pre-immobilized capture antibody (dAb). The resultant sandwich GNR-dAb-Ferritin-dAb complexes would present a purple band on the thread due to the accumulation of the GNR. To achieve quantitative results, the test zone was transferred to an electrochemical cell containing 750 μL HBr–Br2 (6.0 mol/L HBr–0.6 mol/L Br2) solutions to dissolve the captured GNR to perform ASV detection. The ASV detection had a dynamic range of 5–5000 ng/mL with a detection limit of 1.58 ng/mL and a total assay time of 30 min. This method offers a great way for bioassay applications that have high sensitivity requirement.

Thread-based electrochemical devices are of distinct advantages compared to those coupled with surface-plasmon resonance, fluorescence or chemiluminescence, including simple instrumentation and signal quantification, low cost of the entire assay, and potential for on-site detection. Although high sensitivity may be obtained in comparison with thread-based devices with colorimetric detection, the fabrication of electrodes increases the complexity of the device.

2.2.3. Other detection methods

There are a few thread-based microfluidic devices coupled with some other detection methods. Jarujamrus et al. (2018) described a complexometric and argentometric titrations using microfluidic thread-based analytical device for Mg(II) determination in water, rubber latex samples and chloride ion in water and food seasoning samples by length measurement. The fabrication of the microfluidic thread-based analytical device for the complexometric titration is shown in Fig. 7(a). The analytical device was made from two 15 cm cotton pretreated thread tied together with a central knot and then immobilized onto a support to facilitate the sample loading with indicator solution. After the reaction between the target and deposited indicator at the test zone, a color change occurred along the thread with different lengths depending on the concentration of the analyte. The working concentration range of this device on Mg(II) determination and chloride ion were 25–1000 mg/L and 75–600 mg/L, respectively. The test results were in agreement with those obtained by classical titrations. This length measurement method on thread is very simple and results can even be read by naked eye, which offers a cost-effective and convenient alternative tool for the conventional analytical chemistry or biochemistry.

In addition, thread can also used for the applications of electrophoresis (Cabot et al., 2018; Yang et al., 2018, 2014a, 2014b, 2014c). Usually, thread-based electrophoresis devices usually require plasma-treated threads to obtain greater currents than that on native threads (Wei et al., 2012). Cabot et al. (2018) developed a thread-based
microfluidic electrophoresis system to evaluate the zone electrophoresis upon 8 commercially available threads including 100% nylon bundle, 100% silk, 100% cotton, 100% polyester, 100% acrylic, 50% acrylic 50% nylon, 100% pure Merino wool and the waxed dental tape. It was found that the synthetic threads presented higher EOF, with acrylic (cyanide based) provided the highest value, while nylon bundle presented higher resolution and lower solute dispersion and, whilst also helped to minimize the contribution of Joule heating. They quantified of low abundance metabolites, riboflavin in human urine, on the system. Results showed that the separation could be achieved in less than a minute and a linear working range of 0.1–15 mg/mL was obtained.

Yang et al. (2014a) demonstrated an enzyme-doped thread-based device (Fig. 7(b)) on which liquid transportation, bio-reaction and capillary electrophoresis (CE) and electrochemical detection of urea and glucose could be simply achieved. When used in urea and glucose samples detection, linear dynamic range of 0.1 mM~10.0 mM ($R^2 = 0.9850$) and 0.1 mM~13.0 mM ($R^2 = 0.9668$) were obtained, respectively, which were sufficient for blood urea nitrogen and serum glucose determination. By using thread-based microfluidic devices for CE-EC detection, micromachining procedure can be avoid to fabricate the microfluidic channels. Therefore, flexible microfluidic configuration design can be easily achieved for a variety of bioanalysis.

Lin and Lin (2016) developed a single polyester thread microfluidic
Fig. 5. Schematic (A) and picture (B) of the electro-analytical device made by stainless steel pins and cotton thread and paper and the fabrication procedure. (C) Cyclic voltammograms recorded in a 500 μM solution of FeCO₂H in 1 × PBS, pH 7.6 at a scan rate of 100 mV/s using seven independent thread-and-pin arrays (Glavan et al., 2016).

Fig. 6. Schematic showing structure and procedures of the nature cotton thread based immunoassay device for electrochemical detection of human ferritin (Song et al., 2017).
system for rapid mass spectrometry detection of liquid food and medical samples. The system was capable of sample loading and separation, pinch focusing and electrospray ionization. It consisted of a two electrodes and a single polyester thread. The thread was fixed on a PMMA chip with one end immersed in a buffer well to keep it wet through capillary force. Electric fields were applied on the electrodes to achieve the CE separation and electrospray ionization during which two neighboring buffer drops were applied to pinch the sample band into smaller width. The other end of the thread was put 4–10 mm in front of the mass spectrometry inlet for analyzing. This method is much simpler than conventional microfluidic electrophoresis chips.

2.3. Thread/paper (PDMS) hybrid microfluidics

As porous materials for fabrication of microfluidic devices, threads have many advantages over paper including stronger mechanical strength, surface tension, easy biodegradability and no requirement of hydrophobic barriers. Introduction of threads in paper-based microfluidic devices simplify the fabrication and enhances the performance (Neris et al., 2018).

Gonzalez et al. (2016) utilized thread/paper hybrid analytical device for glucose detection through a colorimetric assay. In the device, nylon thread was trifurcated three branches to serve as channels to dispense the glucose oxidase, horseradish peroxidase and potassium iodide solution to the analysis sites comprised of circular shaped chromatography paper. The analysis sites were spotted with glucose of standard concentrations. After loading sample, the yellowbrown color at the analysis sites could indicate the oxidation of iodide to iodine. The intensities were then scanned with a Desktop Scanner to quantitate the glucose in sample. The results showed good correlation in the detection of glucose in artificial urine. More recently, Gonzalez et al. (2018a) made another microfluidic thread/paper-based analytical device (µTPAD) for ELISA in detection of biotinylated goat anti-mouse IgG antibody. The µTPAD provided good R² value for the linear range and sensitivity.

Sateanchok et al. (2018) developed a simple cotton thread/paper based microfluidic device with mobile phone detector for antioxidant. As shown in Fig. 8(a), a bunch of cotton thread were stuck on a plastic sheet to serve as microchannels to deliver solution to the paper band functionalized with reagents. After the reaction, the results could be observed on the cotton thread (total phenolic content assay) or paper strip (DPPH assay). Pictures of resultant cotton thread and paper strip were taken for further measurement by a mobile phone camera. The platform was used in green tea sample detection and the results obtained were found to highly agree with that of the standard methods.

Zhang et al. (2018) developed a PDMS/paper/cotton thread hybrid
microfluidic device on a piezoelectric substrate. A PDMS channel of 1 mm in diameter was filled with cotton thread and the terminal part of which was distributed on the PDMS surface equally. A filter paper of 6 mm in diameter with dried reaction solution was then mounted on the cotton thread. This channel was located on a piezoelectric substrate which served as an interdigital transducer. Surface acoustic wave was used in this study to transport solution to the target inlet zone of the device and then flowed to the reaction zone via capillary force. A linear rate of fluidic transportation of 4.558 mm/s was achieved in the cotton thread. This method combines both advantages of paper and thread, no external driving force for loading sample solution into target testing zone is required but solely by controlling the electric signal applied to interdigital transducers.

Nilghaz et al. (2018) developed a three-dimensional cell culture system by stacking multiple layers of polydimethylsiloxane (PDMS) connected through thread (Fig. 8(b)). The stacking layers of PDMS were embedded with the functionalized hydroxypropyl cellulose methacrylate (HPC-MA) scaffold as the cell adhesion structure while the cotton thread served as a readymade hydrophilic channel for liquid transportation. The cotton thread was sewn into the four layers of PDMS and crossed the scaffolds. The thread between the PDMS layers were well positioned into the channels to wick the cell culture media from the top to the growing cells into the scaffold hence avoided the requirement of either pump or external pressure. An average thickness of 300 µm of the thread provided was tested to be able to provide sufficient rate to continuously transport he media. COS-7 cells could proliferate over a period of 3–6 days with the present number present identical with that grown in the tissue culture dish and scaffold in a microwell plate.

To achieve high throughput detection, Seth et al. (2018) made a multi-channel immunochromatographic diagnostic device by using combination of polyester sewing thread and nitrocellulose membrane. As shown in Fig. 8(c), the device had a plain paper disc in the centre as the sample pad and connected with nitrocellulose membrane strips of test zone. Thread was sewn to connect the sample pad and the test zone strips. Absorbtent pads were placed at the end of the membrane strips to facilitate the wicking and draining liquid. Gold conjugates were added on the glass fiber pad and dried out at room temperature for assembly. The test and control antibodies were dispensed onto the nitrocellulose membrane to make lines by a Lateral Flow Reagent Dispenser with a syringe pump. To demonstrate the performance of the device, *H. pylori*, *Hepatitis B* and IgG antigens were detected. A test could be completed within 15 min with lowest detection limit within the range of 30–300 ng/mL which was comparable to other lateral flow tests (Jimenez et al., 2017). The device was capable of multiplexed testing, however, more efforts are required to further improve the sensitivity and make it work in practical diagnostic solution.

Lee et al. (2018) designed a microfluidic thread/paper based analytical device (µTPAD) combined with artificial neural networks to quantify glucose in artificial urine. The schematic of the configuration is shown in Fig. 9. The device was made of five major layers: top layer single-sided tape with centre hole, topside double-sided tape and blank chromatography paper with centre and nine circle holes, bottom side double-sided tape with nine holes and bottom layer single-sided tape with nine circles hole. Three pieces of nylon thread were trifurcated on the bottom layer with single-sided tape. Increasing glucose concentrations or artificial urine with known glucose was pre-spotted on the chromatography paper circles to determine the target glucose. A dynamic detection range of 0.5–15 mM was achieved. They used an artificial neural networks trained on the four-channel CMYK color data from 54 µTPAD and the ANN to correctly classify 94.4% µTPAD analysis sites.

Fluorescence-based biosensors are the most popular category because they offer superior sensitivity in analysis (Li et al., 2015). Our group (Weng and Neethirajan, 2018b) utilized a cotton thread device in the application of avian coronavirus detection. Usually, the concentration of the target virus is very low, therefore, the sensitivity of the biosensor is extremely important. To achieve that, a nanomaterial, molybdenum disulfide (MoS₂) was used due to its distinct optical property. In the work, we developed a single-step immunosensor on cotton thread with antibody-functionalized MoS₂ based on a homogeneous fluorescence resonance energy transfer (FRET) immunoassay. The thread-based network interconnected by knots to achieve the fluid

![Fig. 8](image)
mixing and separation. Two individual thread for sample and probe reagent dispensing were split to two streams. One of the two streams was then recombined with knot for mixing and the other two streams were used for negative control and background testing. Spiked chicken blood sample were successfully detected with high specificity, ease of local manufacture, small consumption and high sensitive.

2.4. Other applications

Although thread-based microfluidics offer a promising alternative to conventional microfluidic systems, it also has limitations (Chen et al., 2016). By making thread network, it is able to achieve the complicated fluidic manipulation of transportation, separation, mixing and generating concentration gradients. However, it depends on the passive capillary of the thread which is hardly controlled precisely due to the formation of random orientation of cellulose fibers. To overcome this, Ramesan et al. (2016) utilized high frequency sound waves convective flow generator to realize rapid, precise and uniform flow control in the thread network as well as a continuous and stable concentration gradient of dilution. The method could also do the dynamically regulation which was a distinct advantage over the passive capillary driving mode. As shown in Fig. 10(a), the time series images show that in the presence of SAW, it takes significantly shorter time for the dyes to go through the thread network and the mixing is much more uniform. They embedded this thread concentration gradient generator into a cell-laden 3D hydrogel component. Results presented that it was able to better mimic the in vivo tissue microenvironment than that by conventional 2D microchannel structure.

Electrophoretic separation is another important application of thread-based microfluidics in chemistry and biology. Xu et al. (2018) reported a cross channel thread-based microfluidic device to conduct electrophoretic separation of mixed food dyes. The device consisted of four micropipette tips fixed onto a glass slide as the reservoirs and a cross channel by threads sticking to the reservoirs (Fig. 10(b)). Four platinum electrodes were inserted the reservoirs to provide electrical potential. After wetting the thread with buffer solution, food dye was added and followed by applying potentials. Desired voltages of different values were applied on the reservoirs at the steps of sample loading, injection, and separation. By controlling and adjusting the potentials on the inlets and outlets, the sample could be loaded, injected, and separated within 3.5 min.

Yan et al. (2015) reported a thread-based microfluidic device combining with thermal lens to determine Cu (II) and Zn (II) sequentially. The device consisted of a U-shaped supporter and “Y” geometrics thread channels. As shown in Fig. 10(c), sample and reagent solution were loaded into the two inlet threads (A, B) and flew into the “Y” geometrics channel via micropipettes while the buffer thread channel (E) was immersed into the buffer solution inlet (D). The flow in “Y” geometrics traveled faster than that in the buffer solution channel at a certain inclination angle, thus when solutions mixed, the Cu-zinc complex could be formed and determined first. With the buffer solution wicked into the “Y” geometrics channel, the Cu-zinc and Zn-zinc complex would be detected, thus the copper and zinc ions. Using this device, they determined Cu(II) and Zn(II) in hair samples and the results showed good agreement with those by the AAS method. This study shows that thread is a good substrate for fluidic transportation without any external power. In addition, the transportation rate can be changed by adjusting the inclination angle of the thread channel network, which is crucial in many applications of chemistry, biochemistry, and biological samples detection.

Ulum et al. (2016) reported an ethylenediaminetetraacetic acid (EDTA)-functionalized µTAD for whole blood plasma separation assay. The thread-based device was simply fabricated by stretching the scouring treated cotton thread in a frame of glass slide with a paper support. Critical zone where significant color change occurs was selected to take images to perform the analysis. They tested several cotton thread treatments and found that the cotton-thread without any treatment preventing the blood from wicking while that with EDTA functionalization followed by 60 min drying at refrigerated temperature presented excellent results in terms of the clearest separation boundary and longest wicking distance. This cotton thread microfluidic device on albumin assay had a limit of detection of 114 mg/L.−1 which was superior than conventional blood analysis and provided a promising strategy for combining separation and assay in a single device.

3. Concluding remarks

We have described thread-based microfluidic diagnostic devices in terms of fabrication and various applications over the past few years. As an alternative porous material for microfluidic devices, thread-based microfluidics overcome the disadvantages of paper-based microfluidics, including the form of paper and the one-dimensional methods like litmus paper and lateral flow immunoassays (He et al., 2015).

Thread-based microfluidics, used in the development of POC

Fig. 9. (A) Schematic of the different layers and materials of the µTPAD multiplex. Left hand side, exploded view of the chip. (B) Representative pictures of a control chip with glucose standard solution and an example of sample chip (Lee et al., 2018).
diagnostic devices, meets the ASSURED (affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free, and deliverable to end-users) criterion for a number of reasons: light weight, available in various forms with diversity of properties and easily obtained, compatible with many biological samples, low-cost, and easy handling after use. While the field of thread-based microfluidics has expanded rapidly, there are many challenges that still need to be addressed. For example, to obtain controllable flow rate on the thread so as to accurately manipulate the flow transportation. To develop complicated 3D thread devices that carry out multiplexed diagnostics in a simple-to-use format without any external reading equipment. In terms of the performance of current thread-based microfluidic devices, the sensitivity is relatively lower or comparable to conventional analytical techniques (Malon et al., 2017) due to the evaporation and water retention factor occurs during the fluid transportation. In addition, future efforts may also focus on the integration functional components, including electronic and optical elements on the threads, in order to realize the fully miniaturization, maneuverability and flexibility. Seeking biocompatible
materials, i.e., flexible and stretchable fibers, for thread creation or functionalization and flexible electronic circuits is necessary and crucial if thread-based microfluidics want to find its applications in wearable or long-term implantable devices. The requirement of the mass production of thread-based microfluidic devices necessitates the improvement in fabrication techniques.

Currently, thread-based microfluidics is still in the laboratory research stage of development although many of thread-based microfluidic platforms demonstrated in this review present their potential to provide portable, low-cost and reliable diagnosis real life. It should broaden the scope of biological systems expand its applications outside the academic research laboratories. Overall, improvements in terms of platform fabrication, analytical and detection techniques are required to enable them to be adapted and commercialized in practical applications in the real world. Particularly in the fields where analytical measurements have traditionally been a cost limiting factor and the underdeveloped and resource limited regions due to the low cost and ease of use of threads.

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Credit author statement

H. Jiang and X. Weng conceptualized the article. X. Weng and H. Jiang wrote the original draft. All the authors evaluated and reviewed the manuscript.

Conflicts of interest

The authors have declared no conflict of interest.

Declaration of interests

None.

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