Microfluidic devices based on textile threads for analytical applications: state of the art and prospects

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Microfluidic devices based on textile threads have interesting advantages when compared to systems made with traditional materials, such as polymers and inorganic substrates (especially silicon and glass). One of these significant advantages is the device fabrication process, made more cheap and simple, with little or no microfabrication apparatus. This review describes the fundamentals, applications, challenges, and prospects of microfluidic devices fabricated with textile threads. A wide range of applications is discussed, integrated with several analysis methods, such as electrochemical, colorimetric, electrophoretic, chromatographic, and fluorescence. Additionally, the integration of these devices with different substrates (e.g., 3D printed components or fabrics), other devices (e.g., smartphones), and microelectronics is described. These combinations have allowed the construction of fully portable devices and consequently the development of point-of-care and wearable analytical systems.

1. Introduction

Microfluidic devices are based on the manipulation and control of fluids physically confined to submillimeter dimensions. The small size of these devices and the interesting physical features that arise when fluids are confined in microscale dimensions allow several chemical processes to be performed using a minute amount of liquid solutions.1 Thus, in these microscale dimensions, reagent consumption and chemical waste are significantly reduced (eventually less than a microliter) when compared to those in the traditional macroscale.

The benefits of microscale dimensions in fluid behavior go beyond the obvious size reduction. A mechanical implication is observed for the low values of the Reynolds number (Re),2 which in microfluidic devices (10^{-3} > Re > 10^{-5}) leads to laminar flow that allows effective control of critical reaction parameters such as temperature, velocity of mixing, and concentration of reagents.3,4 Moreover, when scaling down, the gravity forces are no longer dominant over fluids. Thus, capillary forces have the main effect in porous materials and can be used to drive fluids without the need for external pumps, a principle extensively employed in paper and thread-based microfluidic devices.5,6

When analyzing materials commonly used in microfluidic devices, it is possible to sort them into three categories: silicon-, polymer- and, more recently, fiber-based materials (Fig. 1).

Silicon and glass were the first substrates for the development of microfluidic devices. The first work was reported at the end of the 1970s when Terry et al.18 fabricated a complete gas chromatography system on a silicon wafer (5 cm in diameter) using photolithography processes. Despite this milestone, the research community turned its attention to manipulating substrates for microfluidics only in the 1990s, when Manz et al.11,12 introduced the concept of micro total analysis system (μTAS), where sample pretreatment, separation, and detection would be performed in the same microdevice. Thus, in the 1990s, new material substitutes with suitable chemical, electrical, optical, and mechanical properties were extensively sought for applications in microfluidic devices to overcome the limitations of silicon and glass substrates.

The most commonly employed set of procedures for microfabrication of 3D devices is classified as direct micromachining, usually working on the subtractive mode by removing part of the bulk substrate, creating the microfluidic design (micro-channels, microchambers, etc.). However, subtractive micro machining (e.g., laser ablation assisted13 and photolithography14,15) was laborious, requiring expensive machinery restricted to research center facilities. Flexible polymeric microfluidic devices were introduced by Kim et al.16 as an attractive alternative to overcome these drawbacks. Poly(dimethylsiloxane) (PDMS) monomers were cast onto a relief structure on a mold (produced by photolithographic or other
Fig. 1 Timeline of the type of functional material for microfluidics. μTAS (micro total analysis system); μPADs (microfluidic paper-based analytical devices); μTADs (microfluidic thread-based analytical devices); μCADs (microfluidic cloth-based analytical devices). Adapted and reprinted with permission from ref. 7–9.

processes) and then removed after polymer curing. This process is called soft lithography and allows quick replication of microfluidic devices containing microstructures (e.g., microchannels). Other procedures also involving a reusable mold or master to replicate microstructures are called replicative micromachining methods (e.g., microtransfer moulding, hot embossing, injection, compression moulding, and imprinting), which are simpler, faster, and affordable techniques to produce microfluidic devices using polymeric substrates. In addition to PDMS, other polymers such as polystyrene (PS), poly(methyl methacrylate) (PMMA), cyclic olefin copolymer (COC), polycarbonate (PC), poly(ethylene terephthalate) (PET), and polystyrene (PS) have been the most employed in microfluidic devices because of their low cost and suitable mechanical and electrical properties. In addition, 3D printing methods for the fabrication of microfluidic devices have been receiving much attention in the last 20 years with the arrival of commercially available 3D printers. Rapid prototyping, fast and cheap production, and open-source software are significant advantages for this additive manufacturing technique.

Paper has been applied as a substrate in chemical analysis for more than a century with the development of paper-based pH colorimetric sensors. However, the widespread application of this substrate in analytical microfluidic devices only started in 2007, when Martinez et al. described patterned chromatographic paper using photolithography processes, allowing rapid colorimetric multiplex analysis of glucose and protein (bovine serum albumin – BSA). Paper, as a simple and inexpensive substrate for microfluidics, was a breakthrough in this field. The μPADs show simplicity, low cost, and the capillary effect, which is an attractive characteristic because it provides a spontaneous solution flow without external pumping. In addition to affordable and friendly characteristics, μPADs are robust, allow colorimetric readout with the naked eye, and are easy to discard. Also, various available paper compositions (cellulose, nitrocellulose, covalently or physically modified cellulose, chromatographic paper, etc.) can be used in μPADs. The versatility of the paper substrate has allowed researchers to focus on the functionality of μPADs (2D or planar, 3D or origami, switch, etc.) to enhance analytical performance, which has resulted in several applications such as biosensing, point-of-care (POC) testing, environment monitoring, clinical diagnostics, food safety, and other analytical applications. Although the paper has advantages for developing microfluidic devices, this substrate shows a low mechanical resistance, particularly after aqueous solution wicking.

Although cotton threads are an ancient material in civilization, their use for microfluidics purposes is recent. The fast and simple fabrication of low-cost microfluidic devices using textile threads has been widely exploited being first reported in 2010 when Li et al. described the use of cotton thread for colorimetric detection of biomarkers in urine and artificial plasma samples. Compared to paper, threads are naturally in microchannel form because the solution is confined at the thread surface/air interface, dispensing with the need for hydrophobic barriers. Textile threads are lightweight, have mechanical strength (regardless of dry or wet conditions), have good flexibility, and networks can be easily built up with knots and entanglements, generating microfluidic circuits. Additionally, textile threads can be found with diverse chemical compositions (cotton, silk, polyester, nylon, etc.), are widely commercially available, and are inexpensive.

This paper provides a comprehensive and critical overview of the fundamentals, applications, challenges, and prospects of using textile threads of different compositions in analytical microfluidic devices.

2. Textile threads: fabrication, composition, and physical properties

Fibers are elongated pieces of a given material, roughly rounded in cross-section, while threads are formed by combination and spinning of one or more types of fiber, being widely used in the textile industry to produce clothes, household items, cords, automotive upholstery, etc. Textile fibers can be divided into two main groups: naturally occurring or manufactured (subdivided into synthetic or regenerated), as indicated in Fig. 2. Production of natural fibers is relatively more complex and time-consuming than that of artificial fibers since the harvesting and cleaning process before manufacturing is mandatory. Also, structural differences are remarkable between these two major groups, and play a key role in their performance...
in analytical applications. Natural fibers (e.g., cotton and wool) are complex, presenting irregular surface morphologies, while the surface of synthetics (e.g., nylon and polyester) is more regular and smoother.

The following sections present a brief overview of the general characteristics of production, and the chemical and physical properties of the most relevant natural and synthetic fibers employed in microfluidic devices.

2.1. Cotton fibers

Cotton is the most important natural textile fiber that grows in a boll around the seeds of cotton plants, taxonomically classified in the genus Gossypium in the mallow family Malvaceae. Formed by single and elongated dead and hollow cells, when it reaches biological maturation, the fluffy and staple fibers have a protective function and promote the dispersal of the seeds. The textile industry uses relatively long fibers (above 25 mm) from raw cotton to produce threads via several steps: harvesting, precleaning and cleaning, drying, ginning, and lint cleaning followed by compacting by a bale pressing process for shipping and merchandising. Finally, when in a textile mill, it is manufactured into final products. The chemical composition of raw cotton comprises mostly cellulose content (95%), a linear polymer of β-D-glucopyranose and noncellulosic constituents, including protein, amino acids, polysaccharides, wax pectic compounds, etc. The purity of cotton is increased by removing non-cellulosic components using selective solvents.

Concerning structural characteristics, cotton fibers are composed of a cuticle, primary and secondary cell walls, and the central core or lumen, as shown in Fig. 3. The cuticular layer is wax abundant and considered the “skin” of the cotton fiber and,
along with the primary cell wall, is responsible for most of the fiber properties.\textsuperscript{46} Fiber walls present different degrees of cellulose polymerization, and are composed of amorphous cellulose with a lower molecular weight distribution in the primary cell region in comparison to the secondary cell wall domain.\textsuperscript{49} Additionally, the thickness of the walls depends on the maturity of the fibers, affecting the lumen size, which is the hollow longitudinal microchannel that provides the plant with nutrition during the growth stage.

Morphologically, a cotton fiber has a twisted-ribbon appearance, structured as fiber convolutions, responsible for many unique features. Mechanical strength is attributed to its highly fibrillar and crystalline structure, which can be increased by 25% when wet.\textsuperscript{50} The presence of hydroxyl groups and the core microstructure gives the cotton a high affinity to water and slow evaporation with moisture regain below 10%. Remarkable softness, chemical stability, easy washability, and good heat conductance make cotton clothes comfortable to wear.\textsuperscript{46}

### 2.2. Wool fibers

Wool is a natural protein-based fiber produced by follicles which are small cells located in the skin of sheep.\textsuperscript{45} Until the industrial revolution, wool fibers had served mainly as a protective and clothing material, which was minimized by the arrival of synthetic fibers.\textsuperscript{51}

The labor-intensive processing of wool fibers can be divided into a few stages: the first is the sheep shearing and sorting, in which the woolen fleece is cut off, which is a manual operation. In the sorting stage, the wool is separated into sections of different quality fibers. Next, scouring and carbonizing processes are carried out in warm water, detergent, and/or alkali media to remove residues such as valuable lanolin, sweat residues, sand, dirt, vegetable matter, and grease.\textsuperscript{52} Before the spinning step, which converts wool pieces into threads, the fibers undergo carding to straighten out the fibers and increase softness.

Wool fibers present a semi-crystalline organization composed of approximately 170 proteins. Keratin, a fibrous protein with high sulphur content from cysteine, present in cross-linking domains, is the most important and abundant. The protein profile of wool fibers comprises more than 20 amino acids which are partially responsible for features such as hydrophobicity, hydrophilicity, cohesion of keratin, structural characteristics, etc.\textsuperscript{54} Moreover, salt bridges between oppositely charged amino acid residues and peptide bridges are another class of crosslinking agents responsible for the molecular structure of fibers. In addition to proteins, superficial fatty acids, mineral salts, nucleic acid residues, and carbohydrates are also present in wool fibers.\textsuperscript{53}

The structure of cross-sectional wool fibers is slightly elliptical (Fig. 4), composed of a complex multi-cell system that varies in composition, shape, and properties, divided into three substructures. The cuticle is the outer layer, composed of flat, slightly overlapping scales. The cortex comprises approximately 60–90% of wool fibers, and it contains cortical cells arranged in a specific helical structure along the fiber axis, which ends up bilaterally, filled with a 5–20 macrofibril matrix. In coarse fibers, air-filled cavities are made up of particular types of cell, forming a central medulla.\textsuperscript{55}

The physical properties of wool fibers include good elasticity, tensile strength, elongation, thermal conductivity, abrasion and crease resistance, excellent moisture absorption, and UV resistance. The strength of wool diminishes by 10–20% under wet conditions.\textsuperscript{48} The existence of hydrophobic scales on the surface of wool fibers makes the wettability and diffusion of molecules (e.g., dyes) difficult.\textsuperscript{55} Modification of the wool surface includes chemical (e.g., alkali, acidic, wet, and oxidation processes),\textsuperscript{53} enzymatic,\textsuperscript{58} and physical treatment,\textsuperscript{57} achieving a more hydrophilic nature. Also, the targets of wool modifications include the improvement of antibacterial,\textsuperscript{59} anti-felting,\textsuperscript{59} and pilling propensity.\textsuperscript{60}

![Fig. 4](image-url) Schematic diagram of a wool fiber and its components.
Although wool fibers present interesting features for microfluidic applications, they have been scarcely used, mainly due to their low wettability. There has been growing interest in producing new materials from natural sources to replace synthetic fibers because of the obvious environmental benefits and the generation of new products with improved mechanical and physicochemical properties.

2.3. Silk fibers

Silk is a protein-based fiber produced as a continuous filament by the larvae of various insects and spiders to build their cocoons and webs. Although many insects produce silk, the filament produced by the domesticated moth *Bombyx mori*, commonly called the mulberry silkworm, is commercially relevant. Additionally, few others in the same genus are used by the commercial silk industry. The manufacture of silk fibers starts with cocoon sorting, followed by a stifling step, in which the aim is to kill the pupa inside the cocoon to avoid its emergence as a moth, thereby preserving the continuity of the filament. Next, the cocoon is cooked to soften the sericin and shell, allowing the filament to unwind smoothly during reeling.

In contrast to cotton and wool, silk does not have a cellular structure, closely resembling a synthetic fiber (Fig. 5A). It is composed of two filaments of structural protein fibroin (75%), which is composed of glycine (43–46%), alanine (25–30%), serine (12%), and tyrosine (5%) amino acids. Two fibroin

![Fig. 5](image_url)  
Fig. 5  Characteristics and applications of silk fibers: (A) structure of a silk fiber. Adapted and reprinted with permission from ref. 62. (B) Scheme of the microstructure of wet-rebuilt spider silk: spider spindle-knots are interwoven by highly random nanofibrils, while joints are composed of aligned nanofibrils. (C) (I) Environmental SEM images of wet-rebuilt spider silk with periodic spindle-knots linking with slender joints. (II) Low-magnification and (III) zoomed-in images. (IV) Low-magnification and (V) high-magnification images of the joint. Adapted and reprinted with permission from ref. 71. (D) Aggregation of water droplets on beads on string fibers with different microstructures. The scale bar is 50 mm. The porous gradient surface has a higher water-collecting ability. Adapted and reprinted with permission from ref. 72. (E) RSF microfluidic applications: (I) flower, cross, and grid shapes, respectively; (II) application as a microfluidic device for pH gradient solutions (left) and color gradient pattern (right); (III) growth of human umbilical vein endothelial cells in a blood vessel-like microfluidic channel. Adapted and reprinted with permission from ref. 74.
strands are enveloped and bonded together by the glue-like protein sericin (25%); waxes (~1.5%) and other components (~1.0%) are also present. The composition, structure, and properties may vary depending on their specific source and function. 

The silk microstructure is uniformly oriented and highly crystalline with extensive hydrogen bonding. Fibrin chains are relatively hydrophobic and oriented parallel to the fiber axis, responsible for strength and stability. Due to various remarkable characteristics such as biocompatibility and degradability, silk fibers have been used in biomedical applications, particularly sutures, wound healing, sensors, drug delivery, and tissue engineering.

The unique properties of silk fibers have inspired research endeavors to develop strategies and devices for artificial spinning. Magnified images of spider silk reveal the special structures responsible for droplet transportation, and they are composed of periodic spindle-knots and joints, with conical spindle-knots. SEM images of wet-rebuilt spider silks show that joints are formed by aligned nanofibrils and spindle-knots, with random nanofibrils generating non-uniform surface wettability (Fig. 5B and C). The difference in surface roughness and Laplace pressure gradient, which is dependent on the geometry of knots, cause the droplet to move directionally. As a result, the droplets on the joints increase their volume.

Fiber fabrication by mimicking bioinspired processes, such as those exhibiting spider and silkworm skills, has attracted attention. Feng et al. fabricated a porous beads-on-a-string polymer and then regulated the size and distribution of pores by controlling the epoxy-resin reaction rate. Among the smooth, homogeneous, and gradient porous structures on beads, the porous gradient surface showed a higher water-collecting ability (Fig. 5D), resembling wetted spider silk. The results can be applied in biosensors, microreactors, and microfluidic devices. Regenerated silk fibroin (RSF)-based microfluidic devices have recently attracted attention due to their excellent biocompatibility and mild processing conditions in aqueous media. After removing the sericin content, the degummed silk fibroin fibers are dissolved in protein denaturants such as LiBr, yielding a fibroin silk solution with random coil-dominated structures. The RSF can be processed to obtain hydrogels, nanospheres, and nanostructured films. Due to its biocompatibility, robust mechanical properties, and relatively slow proteolytic biodegradation, Li et al. developed a colorimetric microfluidic device using RSF as a substrate. To build microchannels, LiBr solution was dropped onto a mask adhesive to etch the substrate in a concentration- and duration-dependent manner. Then, the etched substrate was covered with another RSF, and LiBr was used as glue to assemble a microfluidic device. Applications of the built device were successfully tested for different shapes and purposes (Fig. 5E), such as a gradient concentration generator using different pH solutions and a blood vessel-like channel for the growth of human umbilical vein endothelial cells, indicating that the RSF microfluidic device could be potentially applied in cell analysis and tissue engineering.

### 2.4. Polyester fibers

Polyesters are a class of polymers found naturally in plants and insects, widely known by their synthetic representatives, produced by the condensation reaction of polyols/glycols with di-acids or derivatives (anhydrides). The generic structure of polyesters is \( (ROOR)_n \) where \( R \) represents a carbon chain provided by a polyol (e.g., propylene glycol; diethylene glycol; ethylene glycol; dipropylene glycol; propylene oxide; 1,4-butanediol) and \( R' \) comes from diacids including maleic acid, maleic anhydride, adipic acid, phthalic acid, isophthalic acid, and terephthalic acid. Depending on the nature of reactants, polyesters with a wide range of properties can be produced. In this review, we will focus on polyester fibers, especially PET fibers.

PET, invented in 1941 by J. Rex Whinfield, commonly referred to as a polyester fiber, is the most significant synthetic fiber produced worldwide, obtained by the reaction between dimethyl terephthalate and ethylene glycol. After five hours of oligoester formation followed by five hours of polycondensation, PET fibers with molar mass ranging from 10 to 50 kg mol\(^{-1}\) are produced either in continuous mode, where the as-synthesized molten polymer is extruded under an inert gas to a spinning unit at high temperature (265–290 °C), or the process can be carried out in batch mode, where the polymer is cooled and dried, yielding PET chips which are posteriorly melted and converted into fibers.

The molecular structure of PET fibers comprises a coplanar arrangement between rigid benzene rings and sequences of aliphatic groups (Fig. 6A). The linearity of the backbone and the presence of carboxyl moieties between aliphatic and benzene groups allow dipole–dipole and van der Waals forces with a side-by-side arrangement, which is responsible for the cohesion of PET chains. Benzene rings are partially responsible for the mechanical strength, and the flexibility of PET fibers is related to ethylene groups.

The physical characteristics of PET fibers are low moisture absorbency, good resiliency, weather resistance, good blending with cotton, good spinnability, resistance to microorganisms, physiological inertness, and thermoplastic nature. PET’s versatility enables modifications to produce improvements in fiber properties through modification of the polymer during its preparation (e.g., changing monomers, applying blends, and using additives), adjusting the fiber production procedures (e.g., changing the conditions, texturing, and spinning rate), or modifying commercial fibers (e.g., grafting and plasma etching).

Compared to cellulose, PET fibers have no hydroxyl or amino groups in their backbone, resulting in low reactivity and affinity for water-soluble molecules such as coatings or dyes. To develop functional polyester fibers, several chemical and/or physical procedures have been developed (e.g., sonochemistry, electrical discharge, plasma etching, radiation followed by grafting, UV irradiation, and biocatalysis). Changes in the cross-linking profile, a decrease of crystallinity, and incorporation of hydrophilic functional groups are the main factors responsible for modification in PET fiber properties, improving
the dyeability, absorbency, and wettability since they are extensively used in clothing. Polymers are mostly non-biodegradable, being a significant cause of environmental problems. Reinforcement of cement with PET fibers has been a topic of research extensively studied to create PET-based functional materials increasing recycling and improving properties such as tensile strength, ductility, toughness, and durability.\textsuperscript{85,86}

A class of stationary phases based on polymer fibers, including PET fibers, has received attention in the last few decades. A capillary-channeled polymer (C-CP) has a unique cross-sectional profile, formed by eight- or more segments made with similar or different materials. They can be used to control and split flow, mix solutions, and act as reagent reservoirs or detection zones. Safavieh \textit{et al.}\textsuperscript{42} conducted a systematic study using different types of knot on threads (Fig. 7B) to control flow, and split and mix solutions. The threads were positioned horizontally, and a commercial food dye solution was used for better visualization. The presence of hand-made knots in the thread network showed a surprising behavior, working as an efficient liquid mixer and inflexibility in the molecular chain. Primarily, the properties of the fiber are determined by the molecular structure and organization. For instance, nylon-6,6 and nylon-6 are isomers and share similar physical characteristics (same empirical formula, density, refractive index, and other properties), but they differ in melting point by 40 °C due to structural variances. Additionally, textile characteristics such as style, appearance, comfort, luster, and plushness are often associated with the manufacturing and/or finishing processes.

High elongation, durability, good specific strength, high resilience, low electrical conductivity, and low flammability are essential characteristics for the commercial success of nylon fibers.\textsuperscript{91,92} Despite the textile applications, nylon resins are widely used in the automobile industry, coating,\textsuperscript{93} and food packaging,\textsuperscript{44} and as a matrix for composite reinforcement,\textsuperscript{95} 3D printing,\textsuperscript{96} etc.

3. Fabrication of microfluidic thread-based analytical devices (\(\mu\)TADs)

3.1. Design

The most significant advantage of textile threads in microfluidics is the ease of modifying their flux behavior by creating networks (e.g., braiding, knitting, or tying threads) that develop spatial arrangements, which benefit the gravity force. Additionally, a similar effect can be achieved by simply twisting the threads to modify the dimensions of their microchannels. Since Li \textit{et al.}\textsuperscript{97} and Reches \textit{et al.}\textsuperscript{98} proposed the first \(\mu\)TADs, several designs of these microfluidic devices have been reported. A single thread is held or stretched (tied) on a support (Fig. 7A) in the most used and simple layout. In another design, several threads can be assembled in a parallel configuration to perform multiple assays in the same \(\mu\)TAD.

Knots can be easily made on a thread section or between two or more segments made with similar or different materials. They can be used to control and split flow, mix solutions, and act as reagent reservoirs or detection zones. Safavieh \textit{et al.}\textsuperscript{42} conducted a systematic study using different types of knot on threads (Fig. 7B) to control flow, and split and mix solutions. The threads were positioned horizontally, and a commercial food dye solution was used for better visualization. The presence of hand-made knots in the thread network showed a surprising behavior, working as an efficient liquid mixer and

![Image of chemical structure and SEM image](image-url)
splitter, which strongly contributed to the fiber entanglement and torsion. The authors found that the mixing ratio may be altered depending on knot topology, i.e., when it was rotated 90°, using the two ends of one of the threads as inlets and those of the other thread as outlets, the mixing was almost entirely suppressed. The force used to tighten the threads also had an influence in the flow resistance profile.

Khan et al.61 investigated the influence of wet knitting and braiding techniques to mimic the capillaries in the CE technique. The effects of different thread compositions on their chemistry, textile fabrication technique, and electrolyte ionic strength on the electrophoretic mobility of the test analytes were studied. For polyester threads, the influence of the fabrication techniques on textile-based electrophoresis reveals faster mobility for fluorescein probes in braided structures. The authors have attributed this result to the great number of intra- and interfiber capillaries (12 threads in the braided structure compared to only one thread in the knitted structure), which reduces current values and minimizes the Joule effect at higher voltages. Also, as an effect of a significant number of capillaries, the migration velocity of a moving solute was proportionally increased as a function of the number of threads in a braided structure. The electrophoretic behavior of different textiles was tested for 12 thread braided 3D structures. Overall, cellulosic fibers presented interfibrillar swelling, enlarging the surface area and suppressing the electroosmotic flow (EOF), which is unsuitable for textile-based electrophoresis. Moreover, synthetic fibers with higher hydrophobicity facilitated EOF, resulting in higher solute mobility when compared to natural hydrophilic fibers such as cotton, silk, and wool.

By twisting together two or more threads, µTADS with confluent channels (Fig. 7C) can be obtained to mix different solutions or conduct multiple assays in the same device.99–101 Agustini et al.102 developed a microfluidic electrochemical device (µTED) for the determination of naproxen (NPX) and evaluated parameters related to the flow rate such as gravitational effect, quantity of threads, and the manner in which they are associated (twisted and/or parallel fashion). The electrochemical response was directly affected by the height difference between inlet and outlet reservoirs due to the action of gravity, which facilitates the wicking process. Also, as the number of threads increases, the transient signals become narrower due to the more significant number of inter-thread gaps, resulting in an increased flow rate. Current values showed a strong dependence on the number of twists. As the number of twists increased, the inter-fiber and inter-thread gaps became smaller, increasing the flow resistance and reducing the flow rate. Therefore, the arrangement of threads in parallel (zero twists) was adopted for further studies. After further optimization, the determination of NPX was performed without the use of any mechanical pump system and with low consumption of solutions, little waste generation (~40 µL per analysis), and high analytical frequency.

Fig. 7 (A) ‘On/off’ flow control switches composed of a single thread segment that incorporates a node and a loop with one thread locked by an adhesive. Adapted and reprinted with permission from ref. 113. (B) A web made of yarns and knots operating as a serial diluter. Adapted and reprinted with permission from ref. 42. (C) Chemical synthesis using a Y-geometry reactor with threads as microchannels. Adapted and reprinted with permission from ref. 100. (D) Fabrication of a wearable µTPAD and its application in in situ sweat glucose sensing. Adapted and reprinted with permission from ref. 107.
The good mechanical resistance of the threads allows them to be sewn onto several support materials. Thus, μTADs were fabricated by sewing threads on usually hydrophobic materials, such as polymer tape and ethylene-vinyl acetate. Recently, the so-called microfluidic thread/paper-based analytical device (μTPAD) that combines thread and paper substrates has received attention (Fig. 7D). In this device, threads were usually used for solution (reagent and sample) transportation only by capillary action. Meanwhile, the paper substrate was generally used as an absorbent or detection zone, where reagents for colorimetric detection can be impregnated, or electrodes can be deposited for electrochemical detection. Additionally, the thread and paper combination allows the fabrication of more complex devices, including 3D structures.

The μTADs benefited from the popularization of 3D printing techniques. As discussed in more detail in a subsequent section, supports, solution reservoirs, and platforms could be 3D-printed, allowing fast implementation of μTADs with design from simple to complex.

3.2. Thread surface treatment and chemical modification

The pumping of an aqueous solution by capillary action is one of the essential properties of threads for application in μTADs.

Fig. 8  (A) Wool fiber microfluidic devices treated with oxygen plasma for 5 min (I), 10 min (II), and 15 min (III). (B) Wool fibers treated with plasma using oxygen (I), argon (II), nitrogen gas (III), and air (IV). The 4 lines represent treatment times of 15, 30, 45, and 60 min, respectively. Reprinted with permission from ref. 127. (C) Scheme of the fabrication process of the μTAD for plasma separation from whole blood. (D) Image of the critical zone between red-, yellowish- and white zones on 12 μTAD types on the thread on color-, gray- and negative images. Three different color areas of each critical zone are shown on the colored images: the red-colored area representing the flowed blood cells from the inlet, the yellowish-clear region at the middle representing the separated plasma, and the white-colored dry cotton at the outlet area. Reprinted with permission from ref. 138.
The efficiency of the capillary pump depends on the hydrophilicity of the thread surface. Nevertheless, the most used in microfluidic devices, cotton threads usually have a wax film on their surface that can be natural or added during thread fabrication. This hydrophobic film decreases the wicking properties of the cotton threads. Moreover, threads made of other materials, such as polyester, nylon, and wool, can also show low hydrophilicity. To increase the wicking capacity, surface treatment is conducted to make the threads more hydrophilic. Besides their use in microfluidic devices, derivatization of such moieties has been applied to develop materials with superhydrophilicity,\textsuperscript{113} adsorbents for heavy metals,\textsuperscript{114} antimicrobial fabrics,\textsuperscript{125} flame retardants,\textsuperscript{126} etc.

Plasma (oxygen or air) treatment was one of the first methods used to increase the polarity of the mentioned threads. The exposure to plasma causes oxidation of the thread surface, activating or creating polar oxygen groups. Consequently, the cleaning effect is observed by removing the wax film, activating or creating polar oxygen groups. Jeon \textit{et al.}\textsuperscript{127} used atmospheric plasma treatment to enhance the flux rate of wool fibers in threads commercially available for microfluidic purposes. A simple microfluidic device was fabricated with the treated wool fibers as channels and micro-mixing performed successfully (Fig. 8A). The nature of the gas source (O\textsubscript{2}, N\textsubscript{2}, and Ar) and plasma treatment time were evaluated. The results showed that plasma treatment time increases both the wicking speed and surface damage extent. Among the tested gases, the wicking rate of a solution containing a blue dye solution was remarkably effective (Fig. 8B). FTIR analysis suggests that the formation of cystic acid and the removal of fatty acids on the outermost cuticle are responsible for improving the wettability features of wool threads.

Simpler surface treatment is conducted by washing the threads using water, surfactants, or commercial soap. However, hot (boiling) solutions of NaOH or Na\textsubscript{2}CO\textsubscript{3} have been the most used cleaning solution, particularly for removing waxes from cotton threads.\textsuperscript{90,118} After the washing, the threads are rinsed with water to remove the compounds of the cleaning solution, and finally, they are dried usually at room temperature before being used in the \textmu{}TADs.

Besides the surface treatment to remove impurities and increase the wettability of the threads, additional chemical modifications can be made to make these substrates more suitable for applications in \textmu{}TADs. The highly hydroxylated surface of cellulose fibers can be partially or fully reacted to provide cellulose derivatives. The cotton cellulosic backbone can also be grafted with natural or synthetic polymers to develop fiber/polymer composites\textsuperscript{129} with improved properties. Additionally, biomedical uses of polymer/cotton for drug release such as acrylic monomer\textsuperscript{103,131} and poly(N-isopropylacrylamide)\textsuperscript{122} to improve the thermal responsive behavior of cotton fabrics and chitosan attachment for enhancement of biocompatibility\textsuperscript{132} have been reported.

Li \textit{et al.}\textsuperscript{77} modified cotton threads with the biopolymer chitosan that electrostatically interacted with the negative charges on the cellulose. The authors found that the deposited chitosan film improved the wicking properties of the threads. Moreover, this wettability was maintained for at least three months and was significantly higher when compared with that (one day) for cotton threads only cleaned with Na\textsubscript{2}CO\textsubscript{3} solution. On the other hand, Choi \textit{et al.}\textsuperscript{134} decreased and tuned the fluidic flow on cotton threads by surface modification with polysiloxanes. The wicking speed of the thread was changed according to the concentrations of the hydrophobic polymer solution dispensed. This fluidic flow control was considered essential to improve the sensitivity of immunoassay performed on the developed \textmu{}TAD.

Swiner \textit{et al.}\textsuperscript{135} developed a thread-based blood microsampling system (manipulating small sample volumes and maintaining analyte integrity) for diazepam and cocaine analysis in the dry state at room temperature. Silanization of cotton threads and subsequent encapsulation in glass capillaries were performed to increase hydrophobicity and reduce the absorption of aqueous-based components. The mass spectrometry analysis of dried blood samples stored for 42 days indicated that cotton threads could be used as an all-in-one substrate for sample collection, storage, and direct analysis of blood components without dilution or any pretreatment.

Some thread surface modifications aimed to allow colorimetric detection. Erenas \textit{et al.}\textsuperscript{136} covered cotton threads with a modified PVC (polyvinyl chloride) that worked as an ion-exchange film, selective for colorimetric detection of potassium ions. This selectivity was obtained by modifying the PVC with dibenzo-18-crown-6-ether that formed a complex with potassium. Additionally, potassium tetrakis (4-chlorophenyl) borate and Nile Blue were also added to PVC as a lipophilic salt reagent and colorimetric indicator, respectively. Wu \textit{et al.}\textsuperscript{137} used wax with a low melting point (19 °C) to pattern the detection zone in cotton threads that could show hydrophobic or hydrophilic behavior depending on the temperature. Below 19 °C, the wax was solid and made the detection zone hydrophobic. When the temperature was increased to 23 °C, the wax became liquid and wick along with the aqueous solution. The authors exploited this dependence of hydrophilicity on temperature to improve the sensitivity of a colorimetric assay for DNA detection.

Ulum \textit{et al.}\textsuperscript{138} evaluated the cotton thread treatment using an ethylenediaminetetraacetic acid (EDTA) anticoagulant solution to wick the whole blood sample and separate its plasma. A simple \textmu{}TAD was built by stretching a cleaned cotton thread in the frame of a glass slide with a paper support, and a volume of whole blood or whole blood–EDTA mixtures was then dropped onto the \textmu{}TAD (Fig. 8C) using six different methods. The wicking and plasma separation were captured using a digital cell phone camera. The EDTA-thread provides a good separation of plasma from whole blood, especially for threads that have had EDTA deposited on them and been dried (Fig. 8D), presenting performance comparable with that of conventional separation methods.

Nanomaterials have also been used for the modification of threads for analytical applications. Adamo \textit{et al.}\textsuperscript{139} used the microfluidic properties of cotton threads for conducting \textit{in situ} Turkewich synthesis of gold nanoparticles (AuNPs) using small...
quantities of reagents. The AuNP-containing threads were evaluated as SERS substrates showing good performance for detecting crystal violet and nicotine. Molybdenum disulfide (MoS₂) nanosheets modified with antibodies for infectious bronchitis virus (IBV), an avian coronavirus, were immobilized on cotton threads by electrostatic and hydrophobic interactions. This µTAD was able to detect IBV using fluorescence resonance energy transfer (FRET).

Wang et al. developed direct formate membraneless microfluidic fuel cells (MMFCs) containing a black palladium catalyst and multi-walled carbon nanotubes deposited on cotton threads, which act not only as the anode and cathode but also as flow microchannels. Cross-sectional SEM images of modified cotton threads reveal a highly loaded surface and the absence of catalysts inside the threads. Although the characteristics of the cotton surface are drastically modified, the internal hydrophilic fibers and the inter-fiber gaps could provide microchannels for the transport of solutes, which is maintained by the sum of the capillary and gravity forces. Comparative results showed a high maximum power density in comparison to other pump-free MMFCs. The proposed device shows enormous potential as a micropower source for portable devices.

4. Applications of microfluidic systems based on textile threads

4.1. Electrochemical detection

Electrochemical detection systems are composed of a set of electrodes that measure the electrical properties of a given solution (e.g., electrical current, potential, conductivity, or resistivity). Potentiometry, voltammetry (linear, cyclic, differential pulse, and square wave), amperometry, and conductometry are the most popular and versatile techniques to perform such tasks. The practical features of electrochemical detection, such as simplicity, easy operation, portability, and analytical advantages (high sensitivity and analytical frequency), provide multi-detection and low limits of detection (LODs).

Thread-based devices can be perfectly combined with electrochemical detection by adjusting the threads in physical contact with electrodes or modifying the thread surface by coating or printing methods to create integrated electrodes, decreasing the number of procedure steps. In the following, some examples of µTEDs are described, showing how simple, rapid, efficient, and cost-effective they can be.

Various methods are used to produce electrodes for µTEDs (e.g., stencil and screen printing, dip coating), or a simple array of commercial and low-cost materials such as pencil lead or pins can be used as electrochemical sensors. Table 1 lists the performance of µTEDs and microfluidic cloth-based analytical devices (µCADs) based on electrochemical or electroluminescence (ECL) detection and their corresponding sensing application.

The use of low-cost materials such as pins and cotton threads was described by Glavan et al. Carbon ink (graphite and MWCNTs) was deposited on a clean stainless-steel pin to produce a working electrode (WE), and both reference (RE) and auxiliary (AE) electrodes were used without any coating treatment. Electrodes were nailed in a plastic substrate with constant interspacing. The cotton thread was sequentially wrapped around each electrode (Fig. 9A). The electrochemical response was evaluated by combining linear arrays of electrodes and the effectiveness of hydrophobic barriers along the thread, creating separating zones, which allowed simultaneous analyses in the same thread. The proposed device was applied to lactate detection with a sensitivity of 0.06 µA L mmol⁻¹.

Agustini et al. demonstrated the use of cotton threads with low cost and accessible materials such as pencil lead as electrodes, glass plates as supports, and polymeric materials as reservoirs, with an estimated cost per device of $0.39 (Fig. 9B). Simultaneous determination of acetaminophen (ACT) and diclofenac (DCF) was performed by multiple pulse amperometry (MPA). The analytical performance evaluation of the proposed device revealed LODs of 1.4 and 2.5 µmol L⁻¹ for ACT and DCF, respectively, with an analytical frequency of 45 injections per hour.

The combination of the enzymatic biosensor and cotton threads was proposed by Caetano et al. Tyrosinase (Tyr) was deposited on nanocomposite modified screen-printed carbon electrodes (SPCEs) to detect phenol in water samples. The reduction of the so-formed o-quinones by enzymatic catalysis of Tyr on a phenol substrate was monitored by chronoamperometry, and the analytical performance evaluation of the proposed device revealed a LOD of 2.94 nmol L⁻¹, analytical frequency of 54 injections per hour, and phenol recovery rates between 90% and 110% for spiked tap water samples.

Carbon paste electrodes (CPEs) are widely employed due to their versatility, as well as simple and rapid construction. Kalinke et al. introduced a µTED using a 3D printed platform with an orifice for carbon paste accommodation and electrical contact through a copper plate. To detect glucose, chronoamperometry measurements for a CPE modified with biochar and anchoring Ni showed a LOD of 0.137 µmol L⁻¹ and recovery rates varying between 100 and 113% for saliva and serum samples. The authors also presented an Eco-scale calculation (which considers parameters such as nature of reagents, energy consumption, and waste generation) for the proposed assay, reaching a score of 81 points, indicating a green procedure.

The combination of cotton thread and paper electrodes was used to draw a paper-based electrochemical detector. Hydrophobic barriers and a hydrophilic region were patterned onto filter paper with a permanent marker, which was used to draw the working electrode (W1), AE, and RE with an additional working electrode (W2) on the rear position to the first one (Fig. 9C) with the aim of a pencil. Cotton threads were coupled to the paper-based dual electrodes, and different potentials were applied to W1 and W2 electrodes to selectively detect ortho-diphenols (o-DP) with an LOD of 2 µM, without suffering from the possible presence of monophenols. The proposed method showed good agreement (±14%) with the standard method for o-DP in oil samples.
Table 1  A summary of microfluidic thread- and cloth-based electrochemical devices

<table>
<thead>
<tr>
<th>Threadcloth/ref.</th>
<th>Analyte (sample)</th>
<th>Electrode</th>
<th>Technique</th>
<th>Linear range</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO116</td>
<td>Glucose (blood plasma)</td>
<td>SPCE/CNT/Ni</td>
<td>CA</td>
<td>25–1000 μmol L⁻¹</td>
</tr>
<tr>
<td>PE144</td>
<td>Na⁺ (perspiration)</td>
<td>SPCE/PEDOT:PP/PVC/DOS ionophore</td>
<td>PM</td>
<td>10–160 mmol L⁻¹</td>
</tr>
<tr>
<td>CO145</td>
<td>UAA-EP and HCZ (SS)</td>
<td>Pencil lead (graphite)</td>
<td>CA</td>
<td>5.0–750 μmol L⁻¹ for HCZ</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>100–2500 μmol L⁻¹ for both AA and EP</td>
</tr>
<tr>
<td>CO102</td>
<td>NPX (SS)</td>
<td>Pencil lead (graphite)</td>
<td>CA</td>
<td>1.0–1000 μmol L⁻¹</td>
</tr>
<tr>
<td>CO146</td>
<td>Glucose (saliva and blood serum)</td>
<td>SPCE/CNTs/Ni</td>
<td>CA</td>
<td>5.0–100 μmol L⁻¹</td>
</tr>
<tr>
<td>CO147</td>
<td>Cortisol (saliva)</td>
<td>SPCE</td>
<td>CA</td>
<td>0.25–25.0 μmol L⁻¹</td>
</tr>
<tr>
<td>CO148</td>
<td>Estriol (CS)</td>
<td>SPCE</td>
<td>CA</td>
<td>1.0–1000 μmol L⁻¹</td>
</tr>
<tr>
<td>CO149</td>
<td>AA (SS)</td>
<td>PED cells</td>
<td>CA</td>
<td>20–800 μmol L⁻¹ for AA</td>
</tr>
<tr>
<td></td>
<td>O-DP (olive oil)</td>
<td>PED cells</td>
<td>CA</td>
<td>20–400 μmol L⁻¹ for O-DP</td>
</tr>
<tr>
<td>CO-PE145</td>
<td>[Fe(CN)]₃⁻ / [Fe(CN)]₆⁻ (SS)</td>
<td>CO-PE/Py/AuNPs</td>
<td>CA</td>
<td>0.5–10 mmol L⁻¹</td>
</tr>
<tr>
<td>CO152</td>
<td>AA (orange juice)</td>
<td>Graphite lead</td>
<td>CA</td>
<td>0.05–1.0 mmol L⁻¹</td>
</tr>
<tr>
<td>CO153</td>
<td>Acetaminophen and diclofenac (SS)</td>
<td>Graphite lead</td>
<td>MPA</td>
<td>10–120 μmol L⁻¹ for both species</td>
</tr>
<tr>
<td>CO154</td>
<td>pH (SS)</td>
<td>Thread coated with CI and PANi ink</td>
<td>PM</td>
<td>pH 3–8</td>
</tr>
<tr>
<td>CO155</td>
<td>Lactate (SS)</td>
<td>Pin coated with CI/MWCNTs</td>
<td>CA</td>
<td>2–15 mmol L⁻¹</td>
</tr>
<tr>
<td>CO156</td>
<td>Phenol (tap water)</td>
<td>SPCE/CNTs/Tyr</td>
<td>CA</td>
<td>1.0–200 mmol L⁻¹</td>
</tr>
<tr>
<td>CO-PTB157</td>
<td>Glucose (tear drop)</td>
<td>Pencil lead</td>
<td>CA</td>
<td>0.075–7.5 mmol L⁻¹</td>
</tr>
<tr>
<td>CO158</td>
<td>Vibrio parahaemolyticus (seafood)</td>
<td>NY/carbon</td>
<td>DPV</td>
<td>10–106 CFU mL⁻¹</td>
</tr>
<tr>
<td>CO159</td>
<td>Human ferritin (serum samples)</td>
<td>CPE</td>
<td>ASV</td>
<td>5–5000 ng mL⁻¹</td>
</tr>
<tr>
<td>CO160</td>
<td>Glucose (urine and serum)</td>
<td>Screen printing/CI Ru(bpy)₃²⁺ and luminol as mediators</td>
<td>ECL</td>
<td>0.01–1.0 mmol L⁻¹ for milk</td>
</tr>
<tr>
<td></td>
<td>H₂O₂ (milk)</td>
<td>Screen printing/CI Ru(bpy)₃²⁺ and luminol as mediators</td>
<td>ECL</td>
<td>0.025–10 mmol L⁻¹ for glucose</td>
</tr>
<tr>
<td>NY161</td>
<td>Glucose and acetylthiocholine (SS)</td>
<td>NY/CI (carbon and Ag/AgCl)</td>
<td>LSV</td>
<td>2.5–15 mmol L⁻¹ for glucose</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.64–9.84 mmol L⁻¹ for acetylthiocholine</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>20 mmol L⁻¹ to 5 nmol L⁻¹</td>
</tr>
<tr>
<td>CO-cloth162</td>
<td>Listeria monocytogenes (milk)</td>
<td>SPCE/CdTe QDs/MWCNTs/ DNA probe</td>
<td>DPV</td>
<td>80–600 ng dL⁻¹ for glucose</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2.3–14 g dL⁻¹ for hemoglobin</td>
</tr>
<tr>
<td>SK-cloth163</td>
<td>Glucose and hemoglobin (blood)</td>
<td>Carbon/K₃Fe(CN)₆/GOx</td>
<td>CA and DPV</td>
<td>0.25–8 mmol L⁻¹</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2–10 mmol L⁻¹</td>
</tr>
<tr>
<td>CO-cloth164</td>
<td>Glucose</td>
<td>SPT/CI/PB</td>
<td>CA</td>
<td>0.25–8 mmol L⁻¹</td>
</tr>
<tr>
<td>CO-cloth165</td>
<td>Glucose (SS)</td>
<td>SPT/CI</td>
<td>CA</td>
<td>0.25–20 mmol L⁻¹</td>
</tr>
<tr>
<td>CO-cloth166</td>
<td>Glucose (urine)</td>
<td>CI (carbon), Ru(bpy)₃²⁺ and luminol as mediators</td>
<td>ECL</td>
<td>0–5 mmol L⁻¹ for glucose</td>
</tr>
<tr>
<td></td>
<td>H₂O₂ (milk)</td>
<td>SPE, Ru(bpy)₃²⁺/TPA and luminol as mediators</td>
<td>ECL</td>
<td>0.025–2.5 mmol L⁻¹ for H₂O₂</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.5–10 mmol L⁻¹</td>
</tr>
<tr>
<td>CO-cloth167</td>
<td>Glucose (artificial urine)</td>
<td>SPE, Ru(bpy)₃²⁺/TPA and luminol as mediators</td>
<td>ECL</td>
<td>0.025–10 mmol L⁻¹ for both species</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.05–2.5 mmol L⁻¹</td>
</tr>
<tr>
<td>CO-cloth168</td>
<td>H₂O₂ (milk)</td>
<td>SPE, Ru(bpy)₃²⁺ and luminol as mediators</td>
<td>ECL</td>
<td>1.0–5000 μmol L⁻¹</td>
</tr>
<tr>
<td></td>
<td>Glucose (serum)</td>
<td>SPE/luminol as a mediator</td>
<td>ECL</td>
<td>0.05–2.5 mmol L⁻¹</td>
</tr>
<tr>
<td>CO-cloth169</td>
<td>Lactate-Saliva</td>
<td>SPCE</td>
<td>ECL</td>
<td>0.05–2.5 mmol L⁻¹</td>
</tr>
</tbody>
</table>


As mentioned above, most of the μTEDs are composed of a three-electrode system in intimate contact with threads. The versatility of such an approach is the easy electrode exchange, avoiding contamination and fouling issues. Other systems are focused on the integration of electrodes with threads.

Farajikhah et al.²⁷ demonstrated the incorporation of pyrolyte (PPy) modified stainless-steel threads with AuNPs as the WE and AE and silver-plated nylon as the pseudo-RE, which were sewn onto the 3D tubular structure (polyester threads) using a knitting machine (Fig. 9D). The results showed good electrochemical stability and response for the [Fe(CN)₆]³⁻⁻ probe, indicating that similar approaches can be potentially used to develop wearable sensors.

Due to high hydrophilicity, most electrochemical detection has been carried out in combination with cotton threads, and few examples with alternative fibers are found. Gaines et al.²⁷₆
developed a μTED using nylon to detect glucose. Nylon threads were painted with conductive inks (carbon for the WE and AE and silver/silver chloride for the RE) and then wrapped with a piece of nylon thread (Fig. 9E). Then, nylon threads were tested as a glucose sensor to demonstrate their analytical performance, using GOx and K₃[Fe(CN)]₆ as redox mediators.
second microfluidic system was produced by dropping a solution of acetylcholinesterase (AChE) to detect acetylthiocholine (ATC) and thiocholine. The proposed device showed good analytical performance for both systems and the advantage of reusing electrodes after washing.

µCADs can be regarded as a specific group of µTEDs. In electrochemical detection, electrodes are directly designed on substrates by printing techniques, most of which are based on screen printing which is suitable for fabric patterning. Wax transfer, stencil printing, or weaving processes are also employed for µCAD construction.

Exploiting the properties of wicking and flexibility, Mostafalu et al. reported the use of a thread-based microfluidic system integrated with hydrophobic fabric to produce physical and chemical sensors and applied them as a tool for biological tissue monitoring. Cotton thread was modified in an automated experimental setup composed of two consecutive baths of conductive CNT and PANI (polyaniline) inks (for both the WE and AE) and Ag/AgCl ink for the RE interspersed in the drying stage to cure the coating layer. A microfluidic device was built using the modified threads sewn into hydrophobic woven fabric with a patterned splitter and microchannels (Fig. 9F). The pH was measured using a wireless system, and non-treated thread was passed through chicken skin to mimic the sensor behavior in subcutaneous measurements. A Nernst response was found (slope of potential vs. pH: \(-59.6 \text{ mV per pH}\)), and the stability of the potential signal was measured in a 7.4 pH solution for 4 hours, presenting a satisfactory 2.5 mV h\(^{-1}\) drift, without leakage of biological fluids onto the chicken fat.

In addition to sensing applications of µCADs, the creation of barriers and regions with controlled flux rates is also a point of interest. Downs et al. developed an integrated wax valve for fluid control in a fabric-based device. A three-electrode system and wax valve integrated with an on-device heating element were patterned on cotton fabric (Fig. 9G) using a wax transfer printing process. To demonstrate the utility of the proposed valve, glucose detection was performed, where an incubation zone was created, which allows the rehydration of the GOx enzyme, reducing the burden on the analyst in the form of multiple pipetting when compared to an off-device incubation. After a specific time, a heating element is activated, and the barrier is removed, allowing the solution to reach the detection zone. The LOD was estimated to be 0.11 mmol L\(^{-1}\), compatible with the µPAD’s performance.

Ma et al. developed a system based on the capillary effect to collect and transport perspiration through a wearable microfluidic device for continuous Na\(^+\) monitoring. Selective electrodes used for potentiometric analysis were prepared using carbon and Ag/AgCl screen-printing on PET film followed by polymerization of poly(3,4-ethylenedioxythiophene) (PEDOT) and drop-casting of a Na\(^+\) ionophore on the indicator electrode. A microfluidic channel was obtained by simply sandwiching the patterned double-sided pressure-sensitive adhesive tape with two PET films, where the bottom PET film was also used as the substrate for screen-printed electrodes. The upper PET film was punched to form the outlet, and polyester threads (300 µm in diameter) were used as microfluidic channels (Fig. 9H). The detection of Na\(^+\) was carried out using a portable potentiostat with electrodes positioned on the arm and forehead regions. The results showed a stable potential response with a low noise signal for both bending and on-skin test monitoring of Na\(^+\) levels in perspiration. In contrast, conventional skin sensors must tightly adhere to the skin, and the mechanical friction between the sensing surface and the skin during body movement may damage the sensing component, which may also challenge the long-term use. Therefore, wearable microfluidic devices hold great promise to develop accurate and safe metabolite monitoring for health and point-of-care applications.

When a potential is applied to the electrodes, electronically excited states of chemical intermediates can be created, and then relaxation to a lower-level state results in light emission. Systems that present such a detection principle are called electrochemiluminescent, and they present high sensitivity and a wide dynamic range. As a disadvantage, a luminophore (e.g., Ru(bpy)\(_3\)) or luminol) is required. To act as an ECL sensor, threads need to be functionalized to increase their electrical conductivity. A capillary microchannel (CMC) assisted thread-based microfluidic analytical device (CMCA-µTAD) using ECL detection was proposed by Liu et al.

Liu et al. presented bipolar electrodes that were screen printed on cotton fabric (Fig. 9I), and the performance was evaluated for glucose and H\(_2\)O\(_2\) determination. Also, remarkable bending properties without loss of signal and long-term stability of the proposed sensor were found, attributed to the mechanical characteristics of cotton fabric in association with screen printing methods. Wang et al. presented an enhancement of sensitivity through the decoration of a µCAD BPE with chitosan/multi-walled carbon nanotubes/graphene quantum dots–gold nanoparticles. The BPE was produced by screen printing, where carbon ink was deposited on cotton cloth and then modified with the nanocomposite. Under optimized conditions, glucose presented a LOD of 64 nmol L\(^{-1}\), which is 1000 times lower than that of the bare BPE.

Since the recent emergence of µTEDs, several devices have been proposed, most of which are based on chronoamperometric detection, which provides an almost real-time response. Although the 3D printing technique is a useful technique to build suitable platforms, the device construction is still quite rudimentary, with manual adjustment of electrodes and thread replacement as well. In the coming years, improvements in the fully automated production of threads and coupled electrodes will be expected to drive the growth of the µTED field.

### 4.2 Colorimetric assays

Colorimetric assays have been the broadest application of the µTAD for the detection and determination of several analytes, as summarized in Table 2. Cotton thread has been the most used in colorimetric assays because of its good wicking properties (hydrophilicity) and the high capacity of adsorption and absorption of compounds for surface modification and functionalization of the threads. Although less frequent, colorimetric assays using nylon, polyester, and silk threads have also been reported.
<table>
<thead>
<tr>
<th>Thread material</th>
<th>Colorimetric detection approach</th>
<th>Application</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
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<td>Nylon</td>
<td>Hybrid thread/paper-based microfluidic device with color detection on paper</td>
<td>Detection of glucose and BSA protein in artificial urine</td>
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<tr>
<td>Cotton</td>
<td>Hybrid thread/paper-based microfluidic device and detection using a smartphone</td>
<td>Determination of total phenolic antioxidants in green tea</td>
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<tr>
<td>Cotton</td>
<td>Hybrid thread/paper-based microfluidic device and detection using a smartphone</td>
<td>Glucose in sweat</td>
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<td>Cotton</td>
<td>Measurement of the length of a colored section of the thread using a ruler</td>
<td>Acid–base titration of a drinking mineral water sample, glass cleaner,</td>
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<tr>
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<td></td>
<td>bathroom cleaning liquid, vinegar, and aspirin</td>
<td></td>
</tr>
<tr>
<td>Cotton</td>
<td>Cotton nanotube/gold nanoparticle nanocomposite reporter probe</td>
<td>Immunochromatographic assay for carcinoembryonic antigen (CEA)</td>
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<td>blood plasma</td>
<td></td>
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<tr>
<td>Cotton</td>
<td>The thread was modified with chitosan to improve the fluidic properties and detection sensitivity</td>
<td>Detection of glucose in artificial urine</td>
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<tr>
<td>Cotton</td>
<td>Chitosan was used for modifying the thread and immobilizing the colorimetric reagent 4-(2-</td>
<td>Determination of cobalt(s) in water</td>
<td>187</td>
</tr>
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<td></td>
<td>pyridylazo) resorcinol (PAR)</td>
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</tr>
<tr>
<td>Cotton</td>
<td>Measurement of the length of a colored section of the thread using a ruler</td>
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<td>Cotton</td>
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<td>Cotton</td>
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<td>Enzyme-linked immunosorbent assays (ELISAs) for quantitative detection of the biotinylated goat anti-mouse IgG and rabbit IgG antibodies</td>
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Colorimetric detection in a μTAD shows many advantages over other detection methods because of its low cost and simplicity. Colorimetric readouts are easy to interpret, and they can be obtained using naked human eyes, scanners, professional digital cameras, or ubiquitous and inexpensive optical systems, such as a digital camera integrated into a smartphone. When using digital images, software for image processing can obtain more sensitive and precise color intensities. Another approach for colorimetric detection is to measure the length of the colored section produced by the assay along the thread. This length measurement can be performed using imaging software or a simple ruler.

In the most straightforward colorimetric assay procedure, few microliters of aqueous solutions of sample and reagents are sequentially added to a specific region of the thread, producing a colored segment. Using this approach, acid–base (Fig. 10A),

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complexometric, and argentometric titrations were conducted, as well as detection and determination of albumin, glucose, cobalt(II), carbaryl, nickel, nitrite, creatinine, acetylcholinesterase, amino acids, and iron. In some μTADS using colorimetric detection, the reagents are physically or chemically immobilized (Fig. 10B) in a region (detection zone) or throughout the thread. As described elsewhere, Erenas et al. deposited a chemically modified PVC film in a short (1.0 cm) cotton thread that worked as an ionophore that selectively changed its color when in contact with an aqueous solution containing potassium ions. Colorimetric detection zones were used in μTADS designed for immunoassays, such as enzyme-linked immunosorbent assay (ELISA) and immunochromatography (Fig. 10C). This detection zone was created on the thread by deposition and drying of a few microliters of solutions of the immunoreagents. As depicted in Table 2, immunoassays performed on threads were able to determine genetic disease and blood type, and detect carcinoembryonic antigen, biotinylated goat anti-mouse IgG and rabbit IgG antibodies, squamous cell carcinoma antigen, salmonella enterica serotype enteritidis, ferritin, and pathogen microorganisms (Candida albicans and Escherichia coli).

In hybrid thread/paper analytical devices (μTPADs), generally one or more threads transport the sample into one or multiple paper pads impregnated with colorimetric reagents, where the color develops. Xiao et al. described a wearable μTPAD (Fig. 10D) using cotton thread and filter paper for in situ monitoring the concentration of human sweat glucose. A smartphone was used to acquire and process the digital images to obtain color intensity associated with glucose concentration. Other μTPADs were also reported to diagnose infectious diseases (H. pylori and hepatitis B), perform immunoassays by ELISA and detect antioxidants (in green tea), and glucose and albumin in artificial urine.

Although there are sensitivity limitations of colorimetric assays using μTPADs, they have been widely applied for several analytical applications. This successful application of the μTPADs can be due to the simplicity of obtaining analytical readouts measuring color intensity. Moreover, the tendency to use smartphone cameras for image acquisition and running software apps for data analysis improves the precision and provides smaller LODs than visual detection (naked human eye). For these reasons, we believe that colorimetric assays in μTPADs will continue to be performed in the following years.

4.3. Separations

Textile threads seem interesting for conducting separation mainly because of their microfluidic properties, requiring small quantities of sample and reagent. The possibility of using different materials or performing surface chemical modification can make a thread interesting for chromatography separation, for example. Moreover, visual or instrumental detection can be adopted with few modifications to a μTAD for

Fig. 10  (A) Acid–base titration using a μTAD. Adapted and reprinted with permission from ref. 178. (B) The cotton thread-based colorimetric sensor for the simultaneous detection of glucose and urea. Adapted and reprinted with permission from ref. 185. (C) Thread/paper and fabric enzyme–linked immunosorbent assay (ELISA). Adapted and reprinted with permission from ref. 105. (D) Wearable μTPAD hybrid thread/paper for in situ monitoring the concentration of human sweat glucose. Adapted and reprinted with permission from ref. 107.
separations. Nevertheless, separation based on chromatography mechanisms has hardly been explored. Ballerini et al.\textsuperscript{200} described a chromatography separation of agglutinated red blood cells (RBC) from plasma when a whole blood sample wicked through polyester threads. Antibody blood-typing reagents were immobilized on the thread, and solutions of printer inks (cyan and magenta) were also added to improve the visual identification of the aggregated RBC. This µTAD allowed the determination of the ABO and Rh groups using only 2 mL of whole blood. However, only in 2018 did Agustini et al.\textsuperscript{201} demonstrate that green chromatographic separation using a µTAD was feasible. Cotton threads were treated with a 0.5 mol L\textsuperscript{-1} citric acid solution for 24 h under heating to incorporate carboxyl groups by an esterification reaction with the hydroxyl groups on the cellulose surface. As a result of this modification, the thread acquired an ion exchange capacity to separate ascorbic acid and dopamine (Fig. 11A) using a µTAD with integrated electrodes for electrochemical detection. Using this thread-based device, the studied analytes were determined in tear samples.

Recently, Wang et al.\textsuperscript{202} reported the use of PET C-CP fiber columns to isolate exosomes from a human plasma sample in two different configurations: as an SPE sorbent for ELISA application and stationary phases for hydrophobic interaction chromatography (HIC). Exosomes are extracellular vesicles (30–150 nm) secreted by cells, working as biomarkers for the diagnosis and prognosis of several diseases.\textsuperscript{203} High purity isolation of exosomes is a highly challenging task since blood plasma is a complex matrix. In this context, low-density lipoproteins (LDLs) are a source of contamination of exosomes due to similarities in size, density, and hydrophobicity.\textsuperscript{188} The isolation of exosome vesicles from human plasma using PET C-CP with two-step gradient elution allowed a well-defined band in HIC and a good response as an SPE sorbent for ELISA. Although mass spectrometry-based proteomics analysis revealed 20% of exosomal material associated with the LDL fraction, the combination of bioassays and proteomics analysis showed the versatility of C-CP platforms for a simple chromatographic resolution of exosomes in the presence of LDLs.

Capillary electrophoresis (CE), a separation technique based on the differential migration of ionic or ionizable species under an electric field, can be implemented using threads. The first use of threads to fabricate a microfluidic CE system was reported by Wei et al.\textsuperscript{204} Polyester threads, with 200 and 500 μm diameter, were stretched using a typical cross design over a PMMA substrate containing electrodes for application of the

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**Fig. 11** (A) Chromatographic separation using a µTAD with cotton threads. Adapted and reprinted with permission from ref. 201. (B) µTAD for capillary electrophoresis with C\textsuperscript{4}D detection. Adapted and reprinted with permission from ref. 122. (C) Application of the thread-based electrofluidic analytical device and desorption electrospray ionization mass spectrometry (DESI-MS) for the separation and concentration of proteins. Adapted and reprinted with permission from ref. 114. (D) Polyester threads to form 3D tubular structures to perform capillary zone electrophoresis and isotachophoresis. Adapted and reprinted with permission from ref. 61.
separation voltage and electrochemical detection. Separation and detection of mixtures of ions (Cl\(^-\), Br\(^-\), and I\(^-\)) and compounds of biological interest (dopamine and catechol) were achieved, demonstrating that CE could be conducted on threads. In another study,\(^{205}\) the same \(\mu\)TAD was applied to determine blood urea nitrogen (BUN) in whole blood. The authors emphasized that improved sensitivity was attained because of the 3D electrodes (produced by the hot embossing method) used in the electrochemical detection and the variable volume injection of the sample. The same research group used a similar \(\mu\)TAD, but the polyester thread was doped with enzymes urease and glucose oxidase and then covered with a PVC film.\(^{206,207}\) Thus, glucose and urea in whole human blood could be indirectly determined by electrophoretic separation and electrochemical detection of the products of the reaction catalyzed by the trapped enzymes in the thread. Another version of this \(\mu\)TAD was coupled to an electrospray ionization (ESI) mass spectrometer using a single polyester thread with 200 \(\mu\)m diameter.\(^{208}\) The liquid sample was directly applied on the thread, where the electrophoresis separation was conducted. An end of the thread was used as a tip for ESI, where a second voltage was applied. This \(\mu\)TAD for ESI-MS detected several compounds in energy drinks and sulfamethoxazole in eye drops, with a limit of detection of 10 ppb.

Quero et al.\(^{122}\) demonstrated that capacitively coupled contactless conductivity detection (C\(^4\)D) could be successfully applied in a \(\mu\)TAD for CE separation. Polyester threads with about 315 \(\mu\)m diameter were stretched on a 3D-printed platform (Fig. 11B) containing solution reservoirs and the electrodes used to apply the separation voltage and conduct C\(^4\)D detection. Separation of potassium, sodium, and lithium ions was achieved with LODs of around 1 \(\mu\)mol L\(^{-1}\), and commercial samples of diet soft drinks were successfully analyzed.

Xu et al.\(^{209}\) reported a simple and easy to fabricate \(\mu\)TAD for demonstration of CE separation for undergraduate students in universities and colleges. The microfluidic device was built using cotton threads and solution reservoirs made by cutting plastic micropipette tips. No detection system was required because the qualitative separation of a mixture of the food dyes carmine and sunset yellow was visually observed.

CE separations followed by fluorescence detection have also been conducted on textile threads.\(^{118,210}\) For these \(\mu\)TADs, the thread is usually assembled on a 3D-printed platform containing solution reservoirs (run electrolyte and sample) and electrodes for voltage application. Generally, a fluorescence microscope is used to acquire images, and the excitation radiation is provided by a light-emitting diode (LED). Cabot et al.\(^{118}\) used fluorescence images and commercial threads of cotton, nylon, and silk to fabricate microfluidic devices to perform electrophoresis experiments. Using these thread-based devices, the authors demonstrated electrophoretic migration of a protein (R-phycoerythrin). Also, using electrophoresis migration, intact cells of the bacteria \(E.\) coli (spiked in urine) were trapped and focused on a cotton thread for posterior counting of colonies that could be a proof of concept for applications in the diagnosis of urinary tract infection. All threads showed significant electroosmotic flow (EOF) towards the negative electrode (cathodic), demonstrating that negative charges were on the surface of the thread. Among the studied threads, the one made of nylon was demonstrated to be the most adequate for CE analysis, particularly involving biological samples. As a proof of concept of this potential application, riboflavin was separated, detected (fluorescence), and quantified in human urine in about 2 min.

Isoelectric focusing of norfloxacin, quinidine, ofloxacin, insulin, and proteins, such as bovine serum albumin, R-phycoerythrin, myoglobin, and cytochrome C, was performed on nylon and acrylic threads assembled on a 3D printed platform (Fig. 11C).\(^{114}\) The thread was wetted with a solution containing an ampholyte, and a voltage of 100 V was applied to form the pH gradient and facilitate electrophoretic migration of the analytes. When these compounds reach the thread region where the pH corresponds to their isoelectric point (zero liquid charge), the analytes stop migrating (focused). Thus, separation and preconcentration of the analytes were achieved on the thread within 30 min. The \(\mu\)TAD was coupled to a desorption electrospray ionization mass spectrometry (DESI-MS) system, allowing desorption, ionization, and transport of the analytes to the MS system. The preconcentration obtained with the isoelectric focusing for insulin allowed a LOD of 1 \(\mu\)g mL\(^{-1}\) to be achieved.

Khan et al.\(^{140}\) braided and knitted polyester threads to form 3D tubular structures that simulated CE capillaries (Fig. 11D). These textile-based structures were evaluated to perform capillary zone electrophoresis and isotachophoresis (ITP) of fluorescein and rhodamine-B. Moreover, it was demonstrated that solute preconcentration could be conducted using ITP focusing.

We believe that chemical separations performed on textile threads have a high potential for several applications. Despite the studies reported here, this field is almost unexploited, mainly related to the possible chemical interactions involving the functional groups (native or incorporated) on the thread surface. Thus, several separations by chromatography and electrochromatography could still be developed using textile threads.

4.4. Luminescence assays

Luminescence detections, such as fluorescence and bioluminescence, have also been used in \(\mu\)TADs, particularly for bioassays. Weng et al.\(^{140}\) described a cotton-thread fluorescent immunosensor to detect infectious bronchitis virus (IBV). The detection method is based on fluorescence quenching caused by a fluorescence resonance energy transfer (FRET) after the immunoreaction between the IBV antigen and molybdenum disulfide (MoS\(_2\)) nanosheets modified with IBV antibodies and a fluorescent dye. Using this immunosensor, the authors detected IBV in spiked chicken blood, with high specificity and sensitivity (limit of detection of 4.6 \(\times\) 10\(^{-5}\) EID\(_{50}\) per mL).

A hybrid thread/paper analytical device for detection of antibodies in a single drop of whole blood using bioluminescence resonance energy transfer (BRET) was described by Tomimuro et al.\(^{113}\) Luminescent antibody sensing proteins (luciferase) and their luciferin substrate (furimazine) were
deposited on cotton threads. The interaction of the target antibodies with the luciferase decreased the efficiency of the BRET, and the color of the emitted light shifts from green to blue according to the antibody concentration. The bioluminescence signal was acquired using a standard digital camera or a built-in camera of a smartphone. This µTAD with bioluminescence detection provided a limit of detection in the range of 2.1 to 14.9 nmol L\(^{-1}\) for different antibodies.

As one can note, luminescence detection in µTADs is still in the early stage, and there is plenty of room for new applications. Because of the high sensitivity and selectivity of these detection methods, µTADs with luminescence detection can be attractive for several applications, especially involving environmental and biological samples.

4.5. Other applications

This section discusses other applications and detection methods for the µTAD that were considered less frequent or still show few reports in the literature. For instance, chemical synthesis conducted on textile threads can be interesting for in situ modification of the thread surface. Adamo et al.\(^{[19]}\) synthesized gold nanoparticles (AuNPs) on cotton threads using the Turkevich method. The cotton threads were assembled on a 3D-printed platform, and tens of microliters of reagents flowed by capillarity through the thread to form AuNPs. According to the synthesis conditions, AuNPs with diameters ranging from 20 to 40 nm were obtained. In this work, the cotton thread also worked as a physical support for the AuNPs to be used as a substrate for surface-enhanced Raman scattering (SERS) detection. Using this approach, nicotine was detected with a limit of detection of 0.18 mg L\(^{-1}\).

The coupling of a µTAD with a mass spectrometry (MS) system has been a promising application. Jackson et al.\(^{[21]}\) described a spray ionization method by introducing thread segments (35 mm long) into a glass tube (1.63 mm OD and 30 mm long). A high voltage (~5 kV) was then applied along the wetted thread to produce an ionization spray similar to that obtained by a conventional nano-spray method. This simple setup was easily adapted to a mass spectrometry system, and threads made of cotton, polyester, and nylon were demonstrated to be suitable for the thread spray MS. Direct and fast detection of capsaicinoids from different pepper samples was achieved, and a LOD of 1.54 ng mL\(^{-1}\) was obtained for capsaicin.

Yan et al.\(^{[20]}\) demonstrated that thermal lens spectroscopy (TLS) detection could be adapted to a µTAD for the determination of copper and zinc ions. A three-channel device was obtained by twisting cotton, polyester, and silk threads together (“Y” geometry). Solutions of the metal ions (Cu and Zn), buffer, and metal chelating agent 2-carboxy-20-hydroxy-50-sulfoformazylbenzene (zincon) were transported through the thread channels by gravity and capillary forces. When these solutions are mixed, Cu-zincon and Zn-zincon complexes are formed at the junction of the channels placed into a micro-cell for TLS detection. This µTAD provided LODs of 0.20 and 0.25 mg mL\(^{-1}\) for Cu\(^{[2]}\) and Zn\(^{[2]}\), and these ions were successfully determined in hair samples.

The versatility of the composition and hence the physico-chemical features of threads allow several applications in different fields, offering chemical support to anchor particles and provide special functionalities. The low cost, biocompatibility, and stability of threads are advantageous for pre-analysis, such as cleanup pre-concentration steps, which are expected to be discussed in more detail in related studies in the near future.

5. Future prospects

5.1. Point-of-care systems

The construction and application of POC systems bring many benefits to the population. These systems allow the realization of diagnoses in real time and close to the patient, such as at home, at the workplace, or during emergencies (e.g., accident places, ambulances, and emergency care hospitals).\(^{[21]}\) In addition, POCs allow tests to be carried out with little sample manipulation, more quickly and usually at a lower cost when compared to traditional laboratory tests, which is especially interesting in remote locations or with few resources.\(^{[21]}\)

The importance of POC tests has been evidenced during the COVID-19 pandemic, when a large number of people need to be tested and have their diagnosis completed in the shortest possible time, which is essential to assess the situation of the pandemic in a given population, assist government officials in decision-making and define the necessary sanitary measures to be implemented to reduce the spread of the virus.

The development of microfluidic devices based on fully portable textile threads has allowed expanding the possibilities of applications of these systems outside the laboratory and constructing simple and low-cost POC systems. The main factors that led to the development of POC systems based on textile threads were the integration with smartphones, which offer a series of facilities and connectivity options,\(^{[21]}\) and miniaturized universal electronic modules, which are generic programmable units of open source and advanced technology (e.g., integrated circuits, prototyping boards, microcontrollers, and programmable port arrangements).\(^{[21]}\)

POC systems based on textile threads meet the World Health Organization (WHO) recommended requirements to be classified as ASSURED (affordable, sensitive, specific, user-friendly, rapid and robust, equipment-free, and deliverable to end-users).\(^{[21]}\) This is due to the characteristics of the textile threads such as diversity in the available shapes and properties, low cost, simple construction of the devices, compatibility with biological samples, and ease of disposal after use.\(^{[21]}\)

Disposable microfluidic devices based on cotton threads for POC assays were built by Mao et al.\(^{[16]}\) (Fig. 12A) to detect proteins and nucleic acids in human serum.\(^{[21]}\) For the construction of the devices, two pieces of double-sided adhesive tape were glued (separated by a space of 4 cm) on a clean plastic card, followed by the fixing of cotton thread and absorbent pads at each end. To demonstrate the proof of concept of the devices, the authors developed two immunoassays: (1) detection of squamous cell carcinoma antigen (SCCA), a lung cancer-related biomarker, and (2) detection of the fumarylacetoacetate hydratase gene mutation for the human genetic disease
hereditary tyrosinemia type I. Prior to sample injection, the device used to detect SCCA was treated with a capture antibody against SCCA (in the cotton thread), gold nanoparticles modified with a detection antibody and DNA1 (Au NP-dAb–DNA1), and gold nanoparticles functionalized with streptavidin (AuNP-SA) (in the absorbent pads). Likewise, the device used to detect hereditary tyrosinemia type I was previously treated with streptavidin (in the cotton thread) and gold nanoparticles modified with an adenosine-based molecular beacon probe (AuNP-ABMB) (in the absorbent pad). Each analysis required the injection of 8 μL of the sample, with identification and quantification done through colorimetric detection. For SCCA (after 20 min of testing), a linear response of 1–500 ng mL⁻¹ and a LOD of 1 ng mL⁻¹ were obtained, and for hereditary tyrosinemia type I (after 15 min of testing), the useful analytical range of the device was 75 to 3000 fmol.

Tomimuro et al.111 (Fig. 12B) developed another analytical microfluidic device based on textile threads for POC assays, detecting antibodies in blood samples using bioluminescence resonance energy transfer (BRET). The device consisted of overlapping plastic film layers containing a membrane for separating blood and cotton threads treated with LUMABS (luciferase) or with its bioluminescent substrate furimazine (luciferin) for antibody detection. For each analysis, the device used 5 μL of a blood sample, and after 5 min, the emission of bioluminescence was captured by a digital camera or a smartphone camera. The color changed with the concentration of the specific antibody, varying from green (absence) to blue (high concentration). Using blood enriched with the respective antibodies against HIV1-p17 (HIV), hemagglutinin (HA), or dengue virus type I (DEN), the device was tested for the detection of HIV, influenza, and dengue, with the LOD estimated at 4.0, 2.1 and 14.9 nM for anti-HIV, anti-HA, and anti-DEN, respectively.

A POC device with electrochemical detection based on textile threads was developed by Agustini et al.145 (Fig. 12C), where the microfluidic channels were composed of cotton threads, and the base of the device consisted of adaptable and replaceable parts (built in a 3D printer with an ABS polymer), that allowed the device to be configured in different layouts. The authors also developed a miniaturized electronic system responsible for electrochemical measurements and data transmission via USB or Bluetooth and a smartphone application to allow reception, visualization, and data storage. The analyses were performed using the chronoamperometry technique, requiring 2 μL of sample to determine the analytes. Comparisons of measurements made between the miniaturized system and a commercial potentiostat indicated no significant differences. Three different configurations of the measurement system were evaluated, each with different electrodes and analytes: graphite electrodes for uric acid (UA), screen printed electrodes for hydrochlorothiazide (HCZ), and gold film electrodes sputtered directly into the microfluidic channel to detect mixtures of ascorbic acid (AA) and epinephrine (EP) (previously separated on the device channel through the ion exchange mechanism). The limits of detection obtained were 1.1 μM for UA, 1.1 μM for HCZ, 6.8 μM for AA, and 5.3 μM for EP.
5.2. Wearable systems

Another promising application that has attracted much interest in portable microfluidic systems based on textile threads is their integration into the body, forming the so-called wearable systems. The main characteristic of these systems is the possibility of in situ and continuous measurement and quantification of chemical or physical signals from the human body, and thus monitoring of ions or biological molecules in fluids such as sweat, saliva, tears, and interstitial fluid. In this way, it is possible to monitor people’s health conditions, allowing rapid, early, and accurate diagnosis of diseases, in addition to improving the treatment of health problems and being extremely useful in other areas, such as in sports, where they improve performance or help prevent injury to athletes.

To allow integration and measurement in situ on the human body, wearable systems must be built on flexible substrates, such as fabrics or polymeric films and, subsequently, be integrated into routine garments such as shirts, bandages, gloves, and belts or be fixed directly to a specific region of the human body (skin, eyes, teeth, or hair). Other components of wearable systems (sensors, microchannels, and electronic components) must also be malleable and miniaturized, a fact that highlights the potential of textile threads, both traditional (cotton, polyester, wool, silk, etc.) and conductive threads, which are generally constructed using conductive polymers, micro/nanomaterials based on conductive carbon or metallic nanostructures.

Several wearable systems based on textile threads have been proposed in recent years (Fig. 13A–J). Regarding the detection techniques, the wearable sensors based on the textile threads developed can be grouped into two classes: (1) optical, which explores the colorimetric analysis techniques, with the identification of the compounds and quantification being performed through the alteration and intensity of the colors, respectively, with the analyses performed through visual comparison or capturing images with smartphones and (2) electrochemical, with examples of analyses based on potentiometric, amperometric, impedance or conductometric techniques, with measurements taken by miniaturized electronic systems and/or transmitted to mobile devices, especially smartphones. The predominance of these two techniques (colorimetric and electrochemical) in the proposed wearable devices can be explained by the essential characteristics that these techniques have for use in analyses and in situ monitoring of compounds in the human body, such

Fig. 13 Wearable microfluidic systems based on textile threads for sensing and/or monitoring human biomarkers. Optical devices for sweat analysis: (A) cotton thread/paper-based device for measurements of glucose. Reprinted with permission from ref. 107. (B) Cotton thread/fabric-based device for pH, chloride, and glucose sensing. Reprinted with permission from ref. 221. (C) Silk thread/paper-based device for detection of lactate and pH. Reprinted with permission from ref. 222. (D) Cotton microfluidic platform for pH analysis. Reprinted with permission from ref. 223. (E) Polymer-modified cotton textile/paper-based sensor for the detection of glucose. Reprinted with permission from ref. 224. Electrochemical devices for analysis of body biomarkers: (F) cotton thread-based device for gastric and subcutaneous pH and glucose sensing. Reprinted with permission from ref. 154. (G) Polyester and stainless steel thread-based multiplexed sensor for sodium, ammonium, lactate, and pH detection in sweat. Reprinted with permission from ref. 226. (H) Smart bandage biosensor for determination of uric acid in wounds. Reprinted with permission from ref. 227. (I) Cotton textile sensor for blood leakage monitoring. Reprinted with permission from ref. 228. (J) Gas sensor based on a polyaniline-coated polyester thread for ammonia monitoring. Reprinted with permission from ref. 229.
as rapid measurements (seconds or minutes), small sample volume, high sensitivity, better integration with the body and/or textile materials, simplicity of their components, possibility of miniaturization and ease of integration with portable platforms for receiving, viewing and processing data.

The developed wearable systems (Fig. 13A–J) have a diversity of textile threads and substrates used, with the possibility of monitoring body fluids through the analysis of several biomarkers. The wearable systems are based on microfluidic channels built mainly with cotton threads,116,134,212,213,224,227,228 in addition to silk222 or polyester threads.238,239 The microchannels were integrated with substrates of cloth/fabric107,114,212,213,224,227,229 or smart/adhesive bandages,236,237 or fixed directly on the body with the aid of adhesive tape.233,238 Sweat has been the body fluid most widely studied, with examples of devices applied to determine glucose,167,211,214 chloride,231 lactate,233,236 sodium,236 ammonium,256 or in pH measurements.231,232,233 Other fluids and compounds have also been studied, including blood to evaluate blood leakage,238 air for ammonia,239 gastric and subcutaneous fluids for pH154 and wound fluids for uric acid.237

5.3. Smartphones and 3D printers
Smartphones with integrated digital cameras have been widely used with analytical microfluidic devices for detection purposes. Currently, smartphones are portable, ubiquitous, easy to use, and allow quick digital image acquisition and analysis for colorimetric detection. Naturally, the coupling of the µTADS with smartphones was a matter of time, and it has been increasing. The smartphone-based colorimetric detection in µTADS is performed similarly to that in paper-based microfluidic devices. First, a digital image of the colored detection zone (on the thread or in another coupled substrate) is acquired by the built-in camera of the smartphone. In the following, the images are usually analyzed by appropriate software to obtain the color intensities of the detection zones that can be associated with the concentration of the analytes. As already described, smartphones have been used with µTADS for colorimetric detection of volatile compounds,230 urea,231 creatinine,138 glucose,230 carbaryl in food samples,222 phenolic antioxidants,108 albumin,138 antibodies,111 and microbes.197

The popularization of 3D printers tended to couple 3D printed parts in the µTADS, allowing more complex designs and improving the versatility and functionalities. The 3D printers based on the Fused Deposition Modeling (FDM) technology have been the most used because of their low cost and ease of use. Usually, 3D printing has been used to fabricate platforms61,114–116,118–120,122,124,211 to assemble (stretching and holding) the threads for conducting assays. Moreover, some platforms can also integrate solution reservoirs and holders for electrodes used to apply voltage in electrophoresis61,114,118,122,210 or electrochemical detection.116,120,237 3D printing was also used to fabricate a lens adapter for smartphone-based colorimetric detection in a µTAD.111

5.4 Remarks on future prospects
The future prospects of µTADS are very promising, especially for systems integrating 3D printed platforms and smartphones, which allow the construction of portable analysis systems and, consequently, expansion in the development of devices for in situ and in vivo analysis such as point-of-care and wearable systems. The good prospects regarding the analytical applications of threads can be explained by the possibility of combining their interesting characteristics (e.g., ease of acquisition, integration with detection techniques, transport of solutions by capillarity, and disposal) with the advantages of microfluidics, allowing the creation of simple, cheap, accessible, and versatile analytical microfluidic systems.

However, some challenges and gaps need to be overcome to effectively materialize the prospects mentioned earlier. An important issue concerns the oscillation in the analytical signals over time or for different devices, which is largely due to changes in the transport of solutions and samples along the threads. To solve this drawback, it is necessary to understand better how solutions and samples with different characteristics and/or properties (e.g., hydrophilic/hydrophobic character, viscosity, pH, presence of particles) behave when in contact with the threads. Moreover, these interactions can also be affected by the difference in the thread properties, formats, and composition, consequently influencing the solution flow and performance of the µTADS.214 Additionally, enhancements in the robustness, stability (mechanical and chemical), and evaporation and/or retention (reducing) of solutions and samples in the threads can make it possible to produce more accurate µTADS for long-term analysis.

Another challenge to be considered is the great diversity of available threads, which makes the standardization of their physical and chemical characteristics fundamental for the mass construction of µTADS. To achieve this uniformity, developing new treatments for the threads or adapting existing methodologies for large-scale applications is necessary. Additionally, many of the manufacturing methods described are manual, which must be improved and standardized to enable large-scale production, particularly of 3D devices with several channels to perform multiple and simultaneous analyses. Finally, enhancements in the construction of portable systems, mainly in the development and integration of microelectronic components and flexible energy sources, are welcome, because many of the proposed µTADS still have some components with large dimensions or high weight, or require some analysis step that can only be conducted in a laboratory.

6. Concluding remarks
Textile threads have great availability, ease of purchase, affordable prices, a wide offer in big and small cities, and diversity of composition (cotton, polyester, nylon, silk, wool, etc.), size, color, and affinity for liquids.40 Textile threads have advantages over other low-cost substrates, such as paper, a material widely studied as a cheap alternative to microfluidic devices. The main superior characteristics of textile threads include high mechanical strength and directional fluid transport, not requiring hydrophobic barriers.80 Although only recently explored for analytical purposes, textile threads have been presented as a highly versatile
material, with the development of microfluidic devices integrated with several analysis methods, such as electrochemical, colorimetric, electrophoretic, chromatographic, and fluorescence. Due to their high flexibility and good mechanical resistance, textile threads allow their integration with important substrates (e.g., 3D printed polymeric components or different fabrics), technologies (e.g., smartphones), and microelectronics, which allows the development of fully portable devices and consequently, the expansion of application options, as in the construction of point-of-care or wearable systems. These systems have an enormous appeal because they improve people’s quality of life and are useful in sectors such as sports and safety. These features are due to the continuous monitoring of the organism’s conditions (through biomarkers and chemical or physical parameters), allowing early diagnosis and effective treatment of diseases. Moreover, it can increase sports performance and the detection of toxic compounds in the environment.

Thus, due to their properties, versatility, diversity, and the possibility of integration with different types of system, textile threads present enormous potential in the improvement and widespread use of microfluidic devices, especially in remote regions and/or with few resources, where there is great demand for simple, fast and low-cost analysis and testing. The COVID-19 pandemic is the most recent example of this need, with locations or countries experiencing great difficulty or not performing rapid tests on a massive scale on their population.

However, as textile threads have only recently been studied and applied in microfluidic systems for analytical, medical, and clinical purposes, some issues and characteristics related to textile threads still need to be improved and some challenges overcome for the large-scale production and application of microfluidic devices based on textile threads. Among these necessary improvements, we highlight the variations in the solution flow and the analytical response of the μTAD, the standardization of threads from different sources, and the advancement of the manufacturing processes.

Conflicts of interest

There are no conflicts to declare.

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