ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

A Review On Thread Based Biosensor And Its Applications Focusing On Diagnosis.

Aiswarya K Anilkumar, Manasa K, Shashikala Malgan, and Shilpa Sivashankar*.

Department of Biotechnology, Acharya Institute of Technology, Affiliated to Visvesvaraya Technological University (VTU), Soldevanahalli, Bengaluru, Karnataka-560107, India.

ABSTRACT

A device or substance which can detect disease or medical condition is known as diagnostics and the use of threads for this purpose helps in the fabrication of thread-based diagnostic devices. Threads, with their excellent flexibility and wicking property, are widely used in biosensor fabrication. These economical microfluidic devices are cost-effective, long-lasting, with early and simple diagnostics. This review emphasizes the recent advances in thread-based diagnostics. These devices are economical and durable with diagnostics made early and easy. The threads used for this should be hydrophilic in property in order to transport both liquescent and non-liquescent fluids via capillary action. Since in the threads transfer of liquids occur by capillary action the need for an external pumping system or any other resources for sample propagation is minimized. These multifilament threads can be aligned based on our choice and the limitation of creating hydrophobic barriers as in paper-based diagnostics can be overcome by shifting to threads. Threads also showcase various advantages over paper and because of which they are becoming powerful tools. Thread-based point of care (POC) devices are uncomplicated, their wicking property makes them accountable. They can be used for qualitative as well as quantitative analysis and can also be incorporated with various other materials to make biological sensors. Thread-based diagnostic materials have inherent applications in early-stage diagnostics, food quality testing, environmental analysis, and in industries remarkably because of their minimal fabrication cost. These low-cost micro Thread based analytical devices (μTADs) have huge potential in developing countries because of their cost, easy fabrication, and a huge point of care diagnostics.

Keywords: Diagnostics, Thread-based diagnostics, point of care devices, biological sensors, paper analytical devices.

https://doi.org/10.33887/rjpbcs/2022.13.2.16

Corresponding author

ISSN: 0975-8585

INTRODUCTION

The use of miniaturized, easy portable devices has always fascinated scientists because of their better analysis, simplified operations, lower energy consumption, and cost-effectiveness. A notable example of this is the paper-based diagnostic approach initiated by the whiteside group. Currently, research is focused on textiles especially by using threads that are cost-effective and widely available. Various kinds of threads such as cotton and polyester can be used for diagnostic purposes but our primary focus is on cotton threads. Natural cotton consists of a wax layer on their surface and because of this, they do not get wet by aqueous liquids. This indicates that inherent cotton thread is hydrophobic and to make them hydrophilic, it has to undergo plasma treatment. Plasma treatment makes the thread ready for wicking by increasing the concentration of c and c-o on the thread surface[1]. Natural cotton thread flaunts a variety of characteristics that make it selectable for modeling the biomedical system. These are low-cost, widely available with a variety of characteristics in different sizes and colors. Notably, they are lightweight, highly flexible, and can be used properly according to the assay. The principle of capillary action helps in the proper wicking of threads for various detections and diagnostics.[2]

Various types of viruses and bacteria prevailing in our system can be detected by several molecular diagnostic measures such as the RT-PCR and serological tests such as the ELISA which helps in the identification of antibodies & immunoglobulin which occur in the body in response to the pathogenic infection. There are also various calorimetric techniques, fluorescence polarization, and electrochemical analysis methods which are used to identify different types of viruses and bacteria which cause infection in the human body. But all these techniques require highly skilled labor and it includes all time-consuming procedures and storage processes. Thread-based biosensors can replace these traditional detection methods by overcoming all the drawbacks. The incorporation of nanotechnology with the thread-based diagnostic devices has enormously helped with biosensor fabrication to increase the specificity, sensitivity, and response time which in turn helps in gaining better results with a higher degree of accuracy.[3]

Thread-based Point-of-Care (POC) is efficient compared to the use of paper and all the conventional methods. Paper-based biosensors require a hydrophobic wax layer coating to create detection zones and this, in turn, leads to undesired wetting that can lead to breakage of the working area on a paper. This issue of major concern can be overcome by shifting to thread-based biosensors which are very much productive, reproducible, and effective in action. In a thread-based system, the movement of the sample solution is via capillary force, and therefore no specific equipment is used as a pumping system for the streaming of the sample solution. These are also highly usable in two and three-dimensional configurations which in addition increases their efficiency. Threads also have the ability to quickly modify their properties by immobilization or in other terms with the incorporation of various substances for concatenation of events as well as the ability to lose less unused volume of solution due to greater fluid confinement. POC devices have been observed to be beneficial in portable diagnostic equipment, including smart binding & tissue engineering.[4]

A BRIEF HISTORY

Point of care (POC) diagnostics has always attracted scientists because of the fast results that they give with the limited use of resources precisely and rapidly. These quick detection techniques will help to fasten the treatment process because of the detection in an early stage and thus reduces the risk of death. More importantly, this diagnostic technique helps in the reduction of time required for the collection of the sample, to do the test, and to obtain the result thus reducing the need for a highly qualified professional to do the test. The POC method of diagnostics can help a developing country as well as a highly developed nation. In 1962 blood glucose analysis was developed which was a POC device. After 1977 self testing pregnancy kits were successful because of the POC detection technique.[5] All of these are termed POC's as the testing is done on-site without the need for an expert. On-site diagnostics is a trend in the current era and is used in telemedicine where the diagnostics are performed at home and shared with medical practitioners for advice on treatment and prevention of diseases. Hence emphasis should be laid to develop more advanced POC's.

Around 1700 litmus paper was being used as POC devices to test for acidic and basic characteristics which was a sensational invention at that time. In the 1930s and 1940s, West produced

metal spot studies based on this principle, the replacement of pH-sensitive chromophores with ligands that changed color in the presence of particular metals. This lacked sensitivity along with selectivity which led to the discovery of paper-based sensors such as LAF(lateral flow assay) in 1970. This was able to detect biomolecules from labelled antibodies by using a calorimetric and or a fluorometric device, also these were efficient in environmental monitoring. Paper-based sensors are very much effective, efficient, and they work due to capillary action on the paper strip which is preloaded with reagents. The quantitative and qualitative results can be obtained at the end of the reaction based on the intensity of color obtained by the addition of reagent on paper.[6]

The development of glucose-based biosensors was so much in trend from 1946 to 2012. Scientists developed various glucose monitoring biosensors to quantify the blood glucose level of diabetic patients[7]. The glucose detection paper biosensor will be coated with enzymes(glucose oxidase or dehydrogenase) whereupon the addition of a drop of blood the resulting enzymatic reaction helps in the analysis of glucose levels in patients. The stability of these strips was notably good for many years at a temperature of about 45℃. This was one of the best paper-based biosensors and it was fabricated in the 1970s. Another material that has similar or better properties than paper would be thread and hence we are providing an overview of thread-based diagnostics in various fields.

Recent researches imply that thread-based sensors can be well substituted for paper-based point-of-care devices. The convenience of using thread instead of paper to build microfluidic sensors is that thread comes in a wider range of materials. While paper can be made from fibers other than cellulose, using current papermaking technology will be more costly and difficult. Thread is therefore an impactable alternative for paper in the production of accountable microfluidic sensors. Currently, various types of threads are being used and some of which include cotton, polyester, and much more. The capillary action of the threads along with a better wicking property helps in the proper usage of threads in point-of-care diagnostics. The usage of natural cotton threads is restricted if they are untreated with plasma treatment because as to remove the cellulose coating in order to get its wicking property.

APPLICATIONS

MEDICAL DIAGNOSTICS

Micro Paper-based analytical devices (μPADs) were introduced around 2007 which has much more potential as POC tools for the diagnostics of various diseases and pathogens, including Hepatitis C, understanding the genetic material of various organisms, glucose, and Blood Urea Nitrogen concentrations(BUN), and NO2 in saliva. Thread-based diagnostic processes can be used in the detection of glucose and urea nitrogen in the blood. Contemplation of the blood glucose level and BUN is very much required for diabetic patients and also for patients suffering from renal diseases. For the detection of BUN and blood glucose levels, thread-based diagnostic systems have been devised. The threads are coated with Polyvinylchloride (PVC). This helps in the on-site diagnosis of glucose and urea nitrogen electrochemically in whole blood. Paper-based microfluidic detection and cotton thread-based detection systems are currently in trend because of their speed of detection, easy fabrication, low cost in terms of production and they also possess high compatibility. But they are conjugated with practical problems which include breakage and the amount of the wetting capability of cotton threads. The thread which is being used for the BUN and glucose detection is coated with a variety of enzymes which includes glucose oxidase, urease, and catechol which is used as a moderator prior to coating with PVC in order to prevent the vaporization of these enzymes and this also helps to bring down the Joule's heating effect. The sample should be applied to the thread at -0.28 V. The principle of this system is the sample digestion by the immobilized enzymes on the thread and then the product obtained by this process can be detected by various downstream processes. Before adding the PVC to the thread, the thread is dopped with various enzymes and is put into a buffer solution. This is done to halt the penetration of PVC into the fibers of the thread. Notably, this water-based buffer solution has an immiscible nature with PVC and thus it helps in the accomplishment of a narrow layer on the thread surface. Once this is done an electric field can be applied to the thread dropped with the sample for capillary electrophoresis separation. Coating the enzyme soaked thread with PVC has helped in the better propagation of the sample and progressing results were obtained. In comparison to the normal glass microfluidic channel, PVC layer coating on the thread displays good EOF mobility, as per the results. The designed thread-based microfluidic device also has a wide linear range for detecting GLU

ISSN: 0975-8585

and BUN, according to the results. The haemocytes were concurrently lysed and filtered using the thread to detect GLU and BUN in full blood. The mode of detection is represented in [9] **Fig. 1.**

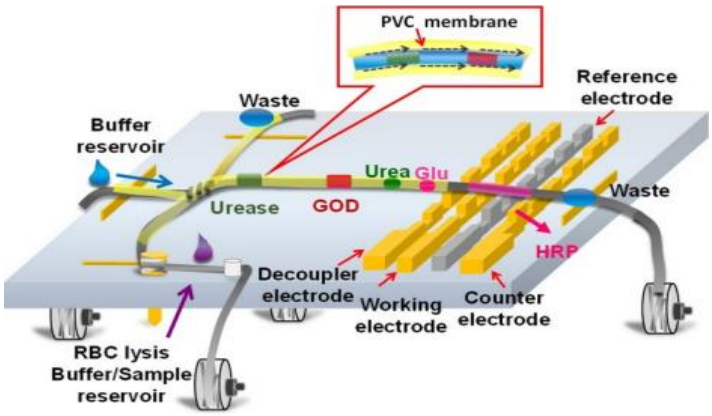

Figure.1. Schematic of the enzyme-doped thread coated with PVC membrane for high-performance CE-EC detection.[9]

Antibodies are special proteins that help our body to fight against the foreign invaders that are the antigens and enhance the working ability of our immune system to fight the approaching harmful microbes in the near future. Determination of various antibodies is very much important for various therapeutic applications, diagnosis of autoimmune diseases, and contagious diseases. But the detection of antibodies in the laboratory requires all the sophisticated technology along with a highly skilled workforce making it cumbersome. This scenario can be eliminated by switching to thread-based detection techniques which can be used as a Point-of-Care system in the lab. Microfluidic thread-based analytical devices (μTADs) use bioluminescence resonance energy transfer (BRET) switching detector proteins to detect antibodies. This device consists of a vertical arrangement of layers. In this a blood separation layer and plastic sheet are present. The plastic sheet has threads woven onto it onto which protein (BRET sensor protein) and substrate (furimazine) is pre-immobilized and this is depicted in **Fig. 2**. This is based on intensity-dependent analysis and results can be obtained on smartphones. These μTADs in amalgamation with BRET helps in the quantification of antibodies present in a single prick of blood (5 μl). [10]

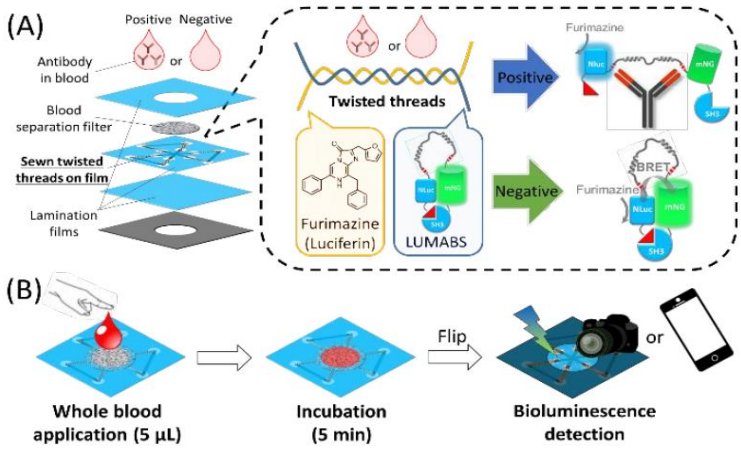

Figure 2. (A) Schematic illustration of the mechanism of bioluminescence reaction on μTADs where LUMABS and its bioluminescent substrate (furimazine) are pre-deposited separately in dry form on two intertwisted cotton threads; the emitted bioluminescence color shifts from green to blue in response to a specific antibody. (B) Schematic illustration of the analytical procedure for antibody detection with proposed μTADs: applying a single drop of whole blood sample to the device (5C), followed by capturing the bioluminescence signal by a digital camera or a mobile phone camera 5 minutes after sample application [10]

ISSN: 0975-8585

A thread-based diagnostic approach has also been used for blood grouping. Using 2 µl of blood from a single prick the ABO and Rh blood grouping can be achieved with the use of µTADs. The fibers of the threads help in the separation of agglutinated red blood cells from plasma. Also in this system with the use of chromatographic techniques has improved the quality of detection. The further development of this system can be helpful for diagnostics even in remote areas of the world, obliging better and faster results. For this process, polyester threads were used due to the outstanding color projection properties so that the propagation and reaction which occurred can be seen early in the capillary channel of the thread.[11]

The thread-based diagnostic approach has also paved the way for the estimation of lactate present in saliva. It is a discomfort for patients to retrieve blood for various tests to determine any particular disease which they suffer from. The collection of the saliva samples is very effortless and can be cumulated by even an inexperienced worker. Test sample assemblage in the form of saliva is found to be useful for people with haemophilia, neonates, patients undergoing chemotherapy specially-abled people, and also in the case of certain elderly patients. Knowing the amount of lactate present in the body is very much required for patients in the medical crisis units, patients having lactic acidosis which is a condition that can weaken the muscles which in turn can cause a heart attack.[12] Lactate concentration in the saliva can be determined by using a cotton fabric biosensor. Primarily the cotton fabric used for this experiment was treated with $Na_2Co_3$to make it more hydrophilic in nature. Using the template patterning process all necessary electrodes for a three-electrode configuration system were incorporated on the treated cotton fabric. Using AutoCAD 2010 software, a template for patterning electrodes was created, with the counter(CE) having a significantly higher surface area than the working electrode(WE) and Reference electrode(RE). To enable optimal charge transmission within the device, the three electrodes were built with a minimum spacing between them. A digital craft cutter was used to print the template on self-adhesive vinyl sheets. The printed template was bonded to the cotton fabric surface, and the template apertures for the WE and CE were filled with Carbon graphite paste modified with Prussian blue (C-PB), while the RE was filled with Ag/AgCl paste. This was followed by placing the cotton fabric in the oven at 60°C. Using the included connector clips, the three electrodes on the device were connected to the mSTAT400 portable potentiostat and the electrochemical impulses were subsequently analyzed and exhibited via DropView software. The template method, regardless of the uneven and non-planar surface of the substrates, produces a rapid and high-quality transfer of the electrode patterns, making it suitable for extending the molding process to a variety of materials that are inconsistent with basic screen-printing protocols.[13] . The process of electrode fabrication and detection is enumerated in **Fig. 3 and Fig. 4.**

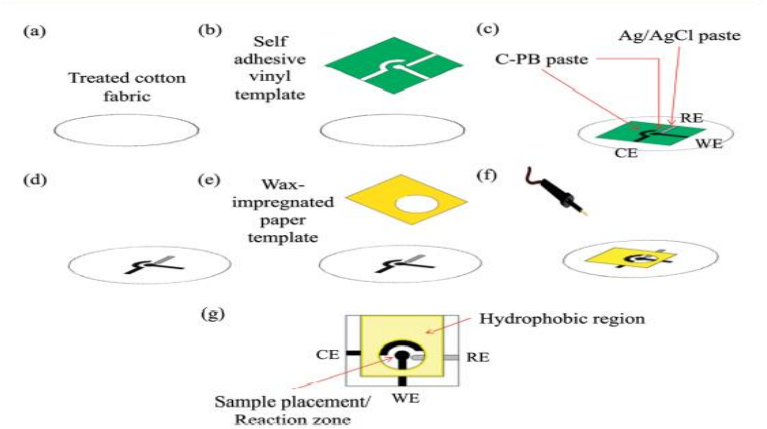

Figure 3. Schematic illustration of the fabrication process of the FED. (a) The platform for FED is treated cotton fabric. (b) For patterning the electrodes, the self-adhesive vinyl template was used. (c) C-PB paste was applied for both the WE and CE, while Ag/AgCl paste was applied for the RE. (d) After the template was removed, the substrate was cured at 60 C for 30 min in the oven. (e) The template for patterning the sample placement/reaction zone was printed on wax-impregnated paper. (f) The wax-impregnated paper template was placed accordingly and heat treatment was used to transfer the wax onto the substrate at 150 C using a soldering iron. (g) The ready-to-use device. RE, reference electrode; WE, working electrode; CE, counter electrode[13]

ISSN: 0975-8585

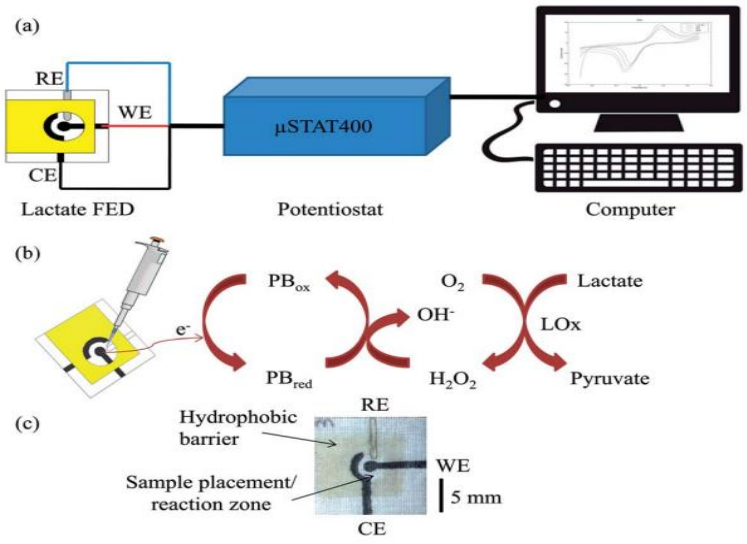

Figure 4. Overview of FED technology. (a) The instrumental setup for lactate determination. (b) The reaction that occurs at the C-PB/LOx electrodes of the FED. (c) Picture of the fabricated FED (15-15 mm). RE, reference electrode; WE, working electrode; CE, counter electrode.[13]

THREAD BASED BIOSENSOR FOR DISEASE DIAGNOSTICS

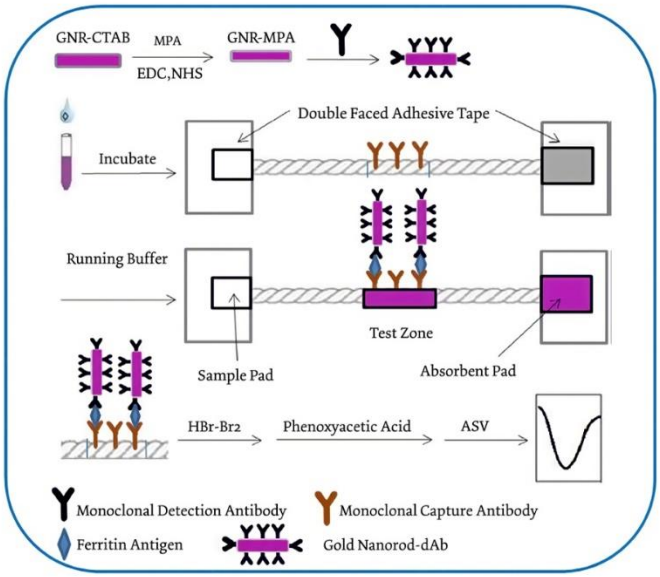

Figure 5. The principle of the nature cotton thread-based immunoassay device for electrochemical detection of ferritin[15]

Due to a lack of suitable screening technologies, early detection and monitoring of cancer progression are limited. For the early identification and screening of cancer biomarkers at the front with precision as well as specificity of diagnosis, supersensitive and accurate point-of-care cancer diagnostic technologies are required. Followed by heart disease, cancer is the second largest cause of death around the globe.[14] . Thread-based assays have been an upcoming diagnostic tool in the field of cancer research. The urge for rapid, portable, affordable, and facile detection devices is always essential. Because of this in recent years the use of threads for the detection of malignancy as clinical and point of care application is in high consideration. For this, there were also approaches to determine the biomarker ferritin which is related to lung cancer electrochemically using a gold nanorod reporter probe that works on the principle of cotton thread-based immunoassay. This device was able to quantify 1.58ng/ml of ferritin within a duration of 30 min. The fabrication of this device was in the

form of a biosensor with a lateral flow strip to which the sample solution along with the running buffer could be added dropwise to absorb with the conjugates. The principle along with the procedure is given in **Fig. 5.** The cotton thread was immobilized with capture antibodies. The application of sample solution which contains GNR-Ab-ferritin complex to the sample pad subjects the solution to migrate due to capillary action and reaches the part of the thread which is immobilized with capture antibodies and this encounter results in the threads turning purple in color. The region of the purple band on the thread is cut out and treated in an electrochemical cell containing HBr-Br2 solution for quantitative detection. The result indicated that the sensitivity of the device was enhanced by using gold nanorods and this device can provide a huge impact on the detection of human ferritin even when it is present in a minimal amount.[15]

In the United States, chronic wound care has become a significant economic and health burden. Chronic wounds affect over 6.5 million people. Estimations of temperature, oxygenation, and wound severity can be used to determine the wounds healing status during the healing process. A wound becomes severely infected when the pH changes from 4-6 to 7-8; thus early diagnostics help in quicker treatments. However, wound tracking involves time-to-time dressing which reduces the observation of infections. This development of smart bandages has attracted scientists so that early detection of wound infection is so possible. Recent research fabrication is on thread-based sensors which can detect pH changes in wounds. This polyalanine-based biosensor with a High Surface Area (HSA) has been designed to accomplish this purpose. The Polyalanine (PANI) threads are swede onto the bandages and thus there is intimate contact between the threads and the wound because of which pH measurement is done. Readout electronics are condensed into a 25mm distortion-free button that can be easily integrated with our thread-based sensors. These buttons which can be used again are permanently attached to the bandages as a thread-changing interface. The local pH value is determined by measuring PANI's open circuit potential (E) in relation to a reference electrode, most commonly Ag/AgCl. The process of wound healing is depicted in **Fig. 6.**[12]

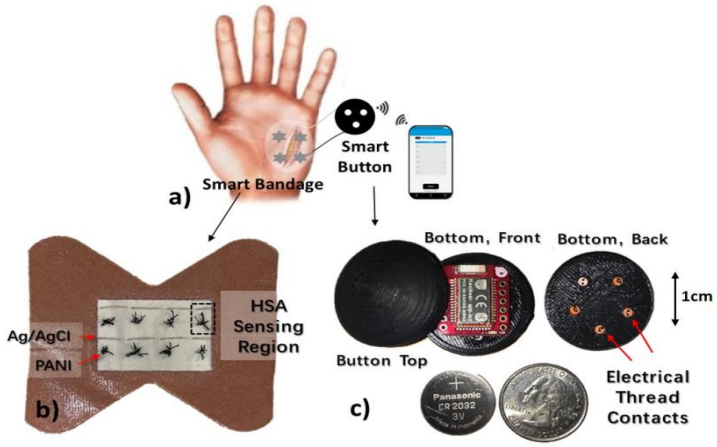

Figure 6. System Overview. Wireless data transmission from smart bandage to phone (a). Closer view of fabricated thread-based pH sensing bandage with HSA sensing regions as working electrodes and Ag/AgCl thread as reference electrode (b). Readout electronics encased in a 3D printed button and smartphone application for data acquisition © ref[12]

Sweat is a clear fluid produced in the human body which contains an ample amount of components including a number of biomarkers. It includes ascorbic acid, uric acid, and electrolytes such as Na+ and K+ with notable biomarkers such as glucose and urea. Both Glucose and urea can be used in the estimation of Diabetics and Kidney health respectively. To achieve the same a cotton thread-based biosensor was fabricated which can be used as a POC tool in monitoring urea and glucose concentration. This wearable has paved the way for an easy, affordable, highly comfortable, and non-invasive analytical diagnostic approach. Notably, this device could either be incorporated into the cloth or could even be attached to the skin for continuous monitoring. The experiment uses artificial sweat to monitor the disease condition. At first, the selected cotton thread was soaked in Cellulose Nanofiber (CNF) solution for 1hr by ultrasonication. After drying the treated threads further coated with Chitosan-Graphene oxide(GO) solution. After these two processes, the detection zones for both glucose and urea were made on the selected cotton threads. On the addition of the sample solution here

ISSN: 0975-8585

artificially made sweat solution color change was observed on the threads which were then captured using a mobile phone camera. The principle and procedure is given in **Fig. 7** The obtained picture was analyzed quantitatively in grayscale using the ImageJ software. The result indicated that with a detection limit of 0.1 mM, this sensor can detect glucose in the range of 0.1-3 mM. The sensitivity of urea was found to be 30 mM, with a linear range of 30 – 180 mM. Finally, this approach correctly detected the glucose and urea cut-off levels, allowing it to distinguish between normal and abnormal people.[16]

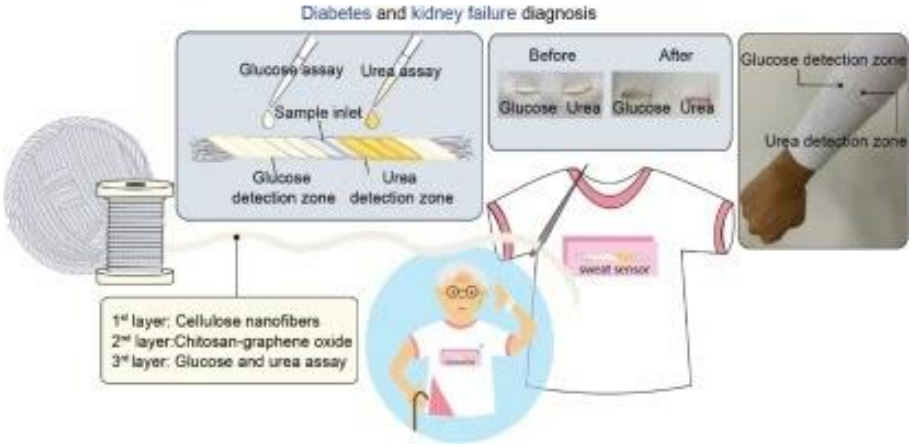

Figure 7. Abstract procedure of the fabricated cotton thread-based biosensor. [16]

Another study showed the use of thread-based diagnostics to detect single base mismatched DNA, related to human genetic disease tyrosinemia Type I. As a reporter probe, an adenosine-based molecular beacon (ABMB) probe modified on gold nanoparticles was used. The ABMB would form a hairpin structure in the presence of coralyne, a small molecule that can react with adenosines. The hairpin structure of ABMB modified on gold nanoparticles will open in the presence of target DNA sequences, and the biotin group added at one tip of the DNA probes will now be liberated and react with the streptavidin mounted on the cotton thread's test zone. This produces a color change, red color is obtained based on the reaction which was visible with the naked eyes. Quantitative detection was done with the help of a scanner by which the image is scanned and results are analyzed with ImageJ software. The principle is projected in **Fig. 8**. Over the range of 2.5–100 nM complementary DNA, the thread-based DNA test equipment responds linearly.[17]

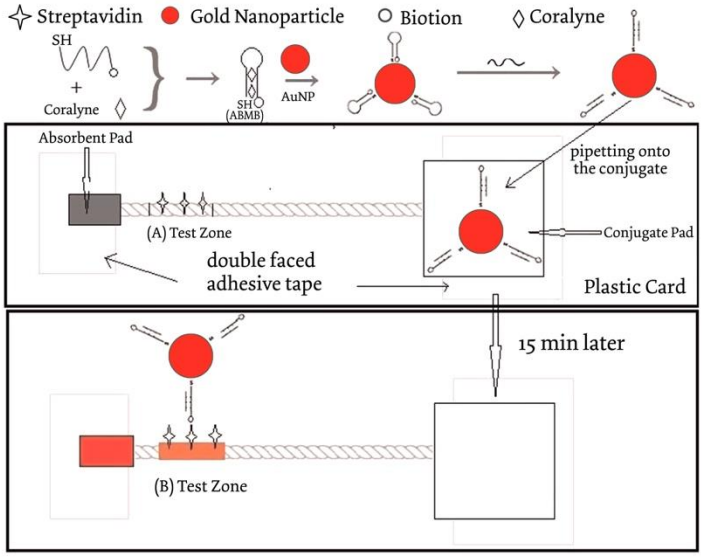

Figure 8. The principle of the cotton thread-based device for DNA detection[17]

ISSN: 0975-8585

ENVIRONMENTAL TESTING

Environmental monitoring is crucial and is important in recent years due to increase in pollution (air, water and soil). This pollution indeed leads to imbalance in the atmosphere, micro and macro nutrients in soil and water and indirectly leads to death of aquatic and human life. Hence, It's critical in environmental analysis to regularly track and analyze sample solution concentrations in the surrounding environment including air, water and soil.[18] As technology has progressed, heavy metals, microorganisms, minerals, organic compounds, chemicals, and other impurities have become highly contaminated. Most of these contaminants seem to be particularly hazardous to human health, necessitating the development of engineered tools able to detect analyte concentrations in a variety of sample matrices on a parts per million scale (ppm) or even parts per trillion (ppt), that had piqued researchers' attention in past few decades.[6]

Because of their portability, μPADs are suited for in-field analysis in the environmental field. A μPAD was introduced in 2015 to detect ammonia in industrial effluents via gas-diffusion separation on paper. An ammonia sample is mixed with excessive solid NaOH deposited in a hydrophilic reagent zone, which then dissipates across a hydrophobic microporous polytetrafluoroethylene (PTFE) membrane into a detection zone containing an acid-base indicator solution in the proposed gas diffusion reagent zone to produce molecular ammonia. The ammonia concentration as in original sample can be attributed to changes in the detection zone's colour intensity. [19] Some of the analytes that are tested in air, water and soil samples including nitrite, ammonia and metals. Paper-based elemental analysis has exhibited a great potential, particularly in the environmental field.

The initial description of the Thread-based analytical technique for diagnostics was in 2010.Thread has emerged as an effective option for building microfluidic analytical devices for analysis purposes. Thread has the advantages of being biodegradable, low-cost, versatile, and light-weight. Additionally, because the thread is hydrophilic, fluids also could run down thread due to capillary force, obviating any requirement for external fluid driving mechanisms. A single device was fabricated that can detect several analytes as well as provide rapid results in the field.[20]Nitrite is an ubiquitous component found in food, especially in fruits and vegetables, and it's also used as a dietary supplement to prevent Clostridium botulinum from growing in refined meat. Furthermore, It can be identified in solid wastes from dye production and in agronomic runoffs because it is an alternative result of nitrification and denitrification processes in soil including ammonium and nitrate from dye manufacture and agronomic runoffs. The Griess assay can be used with a UV–vis spectrophotometer to quantify nitrite levels more easily. Griess-based assays have become common for detecting nitrite. Thread-based microfluidics has recently gained a lot of interest for semi-quantitative research of a variety of target chemicals resulting in low sample volume consumption, high efficiency and commonly accessible platform, and field-based abilities. Microfluidic thread analytical detectors (μ-TAD) were developed for the identification of innumerable substances utilizing electrolytic and fluorescent detectors. To make a thread platform, pour 90μL of chromotropic acid in sulphate solution onto a 6-cm altered yarn piece. The yarn was unknotted into the 9 individual threads after it had been dried at room temperature for 30 minutes. A knot has been created in the center of the threads to provide a region for standards and sample insertion. After that, a 6-cm thread is then sandwiched between two acrylic polymer sheets. For detection of nitrite, 3μL of sample solution was added on to the middle of a thread's knot. Beginning at knot, the inserted solvent dispersed across the length of the thread. A three-minute gap was used between each sample introduction to repeat the procedure. After placing a nitrite solution on the thread platform, an azo dye has been created throughout the length of a thread, resulting in a pink coloured band. The length of the colour band was measured and photographs of the threads were acquired in a studio box 20 minutes following the final sample preparation. The entire thread colour remained steady for a couple of hours. The concentration of acid, the chromotropic acid concentration, the standard sample preparation , and the interactions all were studied.[21]

ISSN: 0975-8585

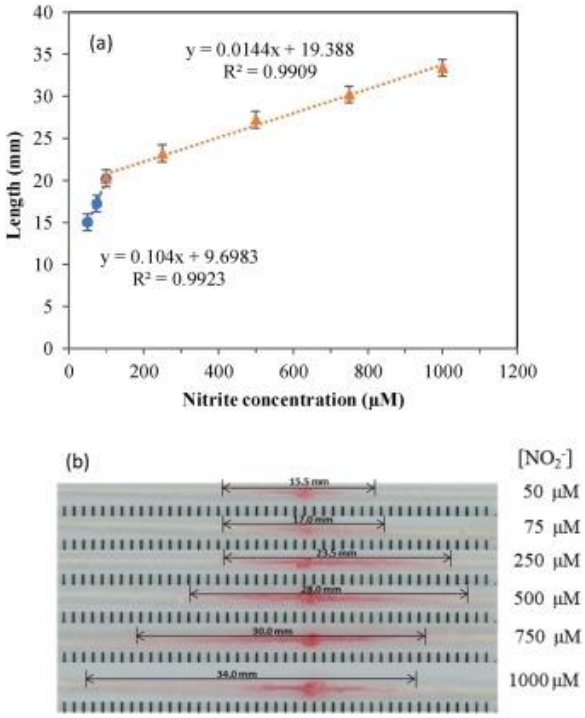

Figure9. (a). Thread-based platform calibration curve for nitrite measurement and (b). Image of threads is used to detect nitrite at concentrations. [21]

FOOD QUALITY

Food safety has become another major public health problem in recent years. According to the United Nations (WHO), every year nearly 2 billion people die because of diarrheal disorders caused by pathogens, bacterial infections, and viruses transmitted via contaminated food and water. Effective food standards monitoring strategies that prevent food contamination become more important as food production grows.[22]These outbreaks were mainly caused by food pathogens such as microbes and pollutants. In 2011,an outbreak of Escherichia coli O104:H4 caused by tainted fenugreek seeds in Germany resulted in 386 illnesses and 54 deaths. The serious occurrence was attributed to the unauthorized use of artificial chemical di(2-Ethylhexyl) phthalate (DEHP) as a cloudifier in Chinese food products and drinks, implying poor food safety measures. The public's awareness of food safety has increased as a consequence of these incidents. To resolve these issues, a variety of emerging point-of-care technologies, including paper-based and chip- based sensors, were designed to detect food contamination instantly, sensitively, and precisely for food safety monitoring.

Several research studies were conducted on the production of highly integrative paper-based sample-to-answer technologies for identification of food pollutants. Adkins et al. employed two different forms of µPADS to find Enterococcus and Escherichia coli. To achieve this, E. coli produced proteins (-galactosidase, -glucuronidase, and -glucosidase) were combined with substrates to synthesize p-aminophenol (PAP), p-nitrophenol (PNP), and o-nitrophenol (ONP).A colorimetric spot analysis and an electrolytic ePAD were used to identify the PAP, PNP, and ONP. These two µPADs, satisfactory in-laboratory calibration curves were established while keeping a small, affordable, and simple to use device.[6]

Currently, threads are being considered as a moderate, field-based detecting alternative substrate. A thread-based colorimetric detector was created for the identification of Salmonella enterica in food products like lettuce, milk and orange juice.[22] Silver enhancing was implemented in a previous research to improve the overall detection sensitivity of a well plate-based ELISA. To improve the colorimetric pattern of AgNP-Ab for the identification of a specific analyte, a silver enhancement solution is added to the sample. As a result, this sensor has a high ability to detect antimicrobial residues in milk, including tetracyclines and quinolones. Polysiloxanes were also used

in another study to improve the detection sensitivity of a thread-based colorimetric device**(Fig10)**.Under ideal conditions, the polysiloxanes-modified thread-based device was 10-fold more sensitive than the original device in colorimetric detection of S.enterica in food items such as lactose, lettuce and orange juice. a thread-based device requires less sample volume (20µL) for testing than a standard test strip (50µL), indicating that it might be used in POC applications rather than a standard test strip.

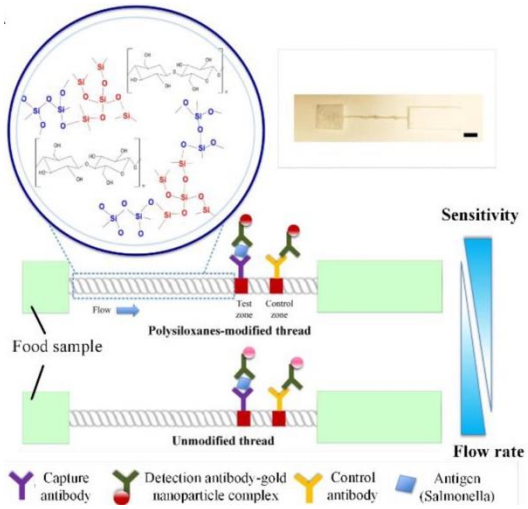

Figure 10. Analysis of food safety via new point-of-care (POC) technologies. A thread-based colorimetric detector was developed to detect Salmonella enterica in food items includes lactose, lettuce and orange juice.[20]

Capacitively connected continuous conductivity detection (C^4D) has become increasingly popular for detecting analytes that lack a chromogenic or fluorogenic functional group.[23] Capacitively connected continuous conductivity detection (C^4D) has been widely used for both standard and microchip capillary electrophoresis systems. C^4D is focused on conductivity measurements with a couple of electrodes substantially detached from the sample solution(contactless).The high frequency alternating signal (hundreds to kHz) is applied between the conductors and the solution to reduce the resistance between them. The conductivity of the test solution determines the alternate current that travels between the conductors. In comparison to traditional (contact) conductivity testing, C4D has a significant advantages. C^4D electrodes can be made out of low-cost materials because there are no chemical interactions between the electrodes and the solution to be tested.

µTADs (microfluidic thread-based analytical devices) were used in a number of applications, including bioassays and food quality testing. To illustrate the CE-applicability C^4D's on µTAD, the researchers measured K^+ and Na^+molecules in dietary soft drink samples. The concentrations of these metal ions in dietary soft drinks used to be higher than that in non-dietary versions due to the additional sweeteners such as potassium (acesulfame) or sodium (cyclamate and saccharine) salts. The results of analysis shows that the concentrations of Na+ in both samples were higher than that of K+. The use of sodium salts as flavourings and additives in non-alcoholic beverages explains this discrepancy. The sweetener acesulfame (as potassium salt) is only added by the manufacturer to the diet cola soft drink. As a result, K^+ concentration in this sample was higher than in the lemon diet soft drink. [24]

INDUSTRIAL APPLICATIONS

While several conventional paper-based tools have been successfully industrialized, such as pH strips, dipstics and LFAs, there are currently few instances of µPADs on the market. Biomedical diagnostic assays, which are expected to be worth more than $8 billion by 2022, are being developed by a number of companies. Scientific Frontiers interacts across a wide range of sectors, but the firm has now assisting in the development of tuberculosis and malaria diagnostics using paper based techniques. Accessible Sensor devices Technologies has developed a series of portable analytical devices (µPADs) that can detect heavy

metal pollution in water. A smartphone application analyzes the radial region of colour change and reports concentration when a little water sample is deposited on a test card.

Threads can be used to manufacture analytical equipment for use in chemical education. In a recent work, a transverse channel thread based microfluidic analytical device was used to demonstrate electrophoretic separation.[6] To measure analytes in samples, chromogenic reactions were commonly used. A camera or a digital scanner is used to capture images of detected zones. The contents of analytes were then measured using image processing software based on the color intensity. Environmental factors like temperature, humidity and lighting condition may also have an impact on accuracy.

COMMON THEMES: STRENGTHS, CHALLENGES AND FUTURE DIRECTIONS

Low-cost optical detection systems, chemical substrates, threaded channels and reduced sample preparation techniques, when used at residence or by public health specialists, have the potential to enhance healthcare in resource-poor areas. While microfluidic thread-based devices come in a variety of designs, response types, and detection methodologies, as seen in the examples above, certain common characteristics emerge. This review article describes state-of-the-art research in Thread-Based POC technologies for various applications, including medical diagnostics, food quality testing, environmental testing, and industrial applications. POC devices are widely used in variety of applications because of their efficiency, simplicity, cost-effectiveness, and portability. The benefits of using these strategies in low-income neighbourhoods include providing patients with reasonable clinical testing, raising poor people's health awareness and empowering regional healthcare providers. The emergence of novel thread-based procedures is likely to change point-of-care diagnostics, allowing anyone to do preventative health-care diagnostics and enhance telemedicine consultations. Diagnostic tool affordability and accessibility are significant challenges in underprivileged and rural communities. Transportable, efficient, and low-cost elevated(TADs) devices can help to enhance much-needed healthcare in these locations. Future research should focus on automating fluidic distribution to produce a simplified sample-to-answer process.[25]

SENSITIVITY AND REPRODUCIBILITY

The most common problems associated with the μPADs for semi-quantitative or quantitative analysis are the poor sensitivity and lack of reproducibility. These two issues are very significant in visual colorimetric detection, hence developing more accurate diagnosis systems is likely to continue as a significant area of research. The electrochemical and luminescence detection methods have proved to be potential in terms of sensitivity but they are more sophisticated and costlier. In the electrochemical and luminescence detection method, as a result of calibration a linear curve was obtained for the model analyte. In comparison to other natural methods utilized in paper-based sensing methods, the detection limit is excellent. The increased complexity can be ideal and incorporated into the device's design and production without increasing its cost or reducing its "user-friendliness". The μPAD should be simple and not involve multiple steps or manipulations that provoke user error and variability. The innate property and varying dissolution of dry reagents stored on the paper matrix is a major source of faults in many μPADs. There are cases where the immobilized enzyme or the reagent gets accumulated on the paper and sometimes the process of dispensing is quick and steady. Factors like rate of reagent dissolution, entirety of the dissolution, homogeneity of the final solution all affect a variety of variables which are difficult to be handled in a non- laboratory setup. The reagent's physical and chemical properties can all have an effect on rate of dissolution. Some of these properties can be controlled during the manufacturing process, while others will change during use of the μPAD and its long-term storage, especially in unsupportive laboratory and storage conditions. In some devices, the homogenous mixing of two or more moving fluids within a paper matrix should also be a problem other than dissolution. It is not possible to assume that two miscible fluids combined in a paper matrix will form a homogenous mixture. Other non-ideal properties of paper, such as analyte adsorptive retention, contamination, or reactivity under extreme conditions, will continue to be important considerations, even if they are not typically primary concerns.[6]

SPECIFICITY AND MULTIPLEXING

All the point of care diagnostics test's precision and dependableness are very much required for its successful functioning. The factors which affect the performance studies of the device include

susceptibility and selectivity studies, positive and negative test results, and predictive values. False positives are less damaging than false negatives in diagnostic efforts because μPADs are typically used as an initial screening before more robust confirmatory tests, which will detect false positives. Since μPADs are used for initial diagnosis faulty negative results are more damaging than the faulty positive results.[6]

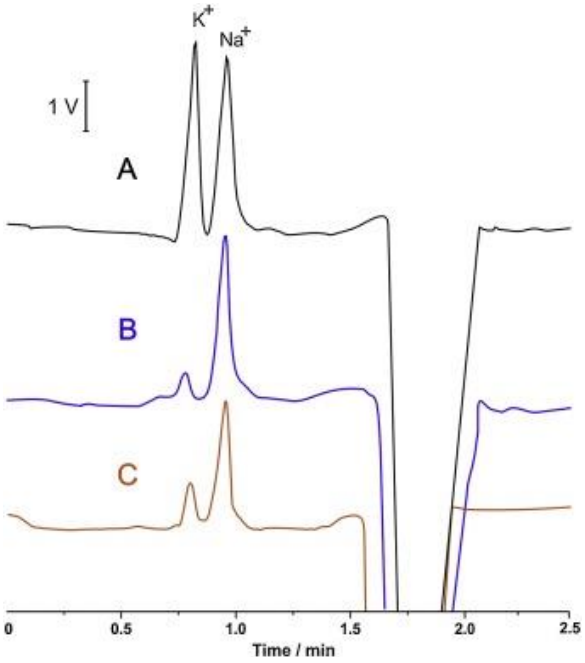

Figure 11. Electropherograms of (A) a standardized solution of K+ and Na+ (75 mol L1); (B) a 100-fold diluted lemon and (C) a 100-fold diluted cola diet soft drink..[24]

Other feature that needs to be improved is that a single device should be able to test many analytes(multiplexing). Many factors like specific pathogen, pollutant, or other substances needs to be checked for proper diagnosis of the disease. Such a test can be ideally performed with a single device that tests for multiple key markers at the same time. Multiplexing would not only increase efficiency but also reduces costs. However, all this can be achieved only when the instrumentation, operation of the device remains simple by making required manipulation.[6]

STABILITY

Unsupported laboratory facilities storage ability should be considered a serious problem before the PADs are considered to be commercially viable. Storage locations which cost less may not have suitable temperature and humidity conditions.[26] Selectivity and susceptibility, which are the typical values required for a test can be compliant to comparisons made with respect to various populations and disease existence, and are the most convincing statistical data for diagnostics precision in binomial test findings. Hence designing devices that can withstand any physical conditions is important.

When compared to a laboratory, the accuracy of any diagnostic test conducted in a clinical setting can be compromised. Any POC test analyses that depend on laboratory test procedures may portray erroneous pictures of a test device's performance in a real-world situation. Contrary predictive values are directly proportional to the prevalence of the disease or disorder in the research population, and so are not regarded inherent to diagnostic test features.[6]

CONCLUSION

Threads can be used as a very promising tool for the making of POC due to their flexibility and easy portability. Based on various material comparisons threads have proved to be more cost-effective and also can be tuned out easily. The propagation of sample solution on the thread by capillary action eliminates the need for external devices to help with fluid proliferation. Various varieties of threads

are available who thus possess a choice of material based on the need. A large number of samples can be analyzed simultaneously with the usage of threads as biosensors. Mass production is possible which again makes it more affordable and making it available at a cheaper rate for the public.

REFERENCES

[1] X. Li, J. Tian, and W. Shen, "Thread as a versatile material for low-cost microfluidic diagnostics," *ACS Appl. Mater. Interfaces*, vol. 2, no. 1, pp. 1–6, 2010, doi: 10.1021/am9006148.

[2] X. Mao, T. E. Du, Y. Wang, and L. Meng, "Disposable dry-reagent cotton thread-based point-of-care diagnosis devices for protein and nucleic acid test," *Biosens. Bioelectron.*, vol. 65, pp. 390–396, 2015, doi: 10.1016/j.bios.2014.10.053.

[3] R. S. P. Malon, S. Sadir, M. Balakrishnan, and E. P. Córcoles, "Saliva-Based Biosensors: Noninvasive Monitoring Tool for Clinical Diagnostics," *Biomed Res. Int.*, vol. 2014, no. i, 2014, doi: 10.1155/2014/962903.

[4] X. Weng, Y. Kang, Q. Guo, B. Peng, and H. Jiang, "Recent advances in thread-based microfluidics for diagnostic applications," *Biosens. Bioelectron.*, vol. 132, no. January, pp. 171–185, 2019, doi: 10.1016/j.bios.2019.03.009.

[5] A. N. Konwar and V. Borse, "Current status of point-of-care diagnostic devices in the Indian healthcare system with an update on COVID-19 pandemic," *Sensors Int.*, vol. 1, no. 101, p. 100015, 2020, doi: 10.1016/j.sintl.2020.100015.

[6] C. Carrell *et al.*, "Beyond the lateral flow assay: A review of paper-based microfluidics," *Microelectron. Eng.*, vol. 206, no. January, pp. 45–54, 2019, doi: 10.1016/j.mee.2018.12.002.

[7] P. Martinkova and M. Pohanka, "Biosensors for Blood Glucose and Diabetes Diagnosis: Evolution, Construction, and Current Status," *Anal. Lett.*, vol. 48, no. 16, pp. 2509–2532, 2015, doi: 10.1080/00032719.2015.1043661.

[8] Y. Rasmi, X. Li, J. Khan, T. Ozer, and J. R. Choi, "Emerging point-of-care biosensors for rapid diagnosis of COVID-19: current progress, challenges, and future prospects," *Anal. Bioanal. Chem.*, pp. 4137–4159, 2021, doi: 10.1007/s00216-021-03377-6.

[9] Y. A. Yang and C. H. Lin, "Multiple enzyme-doped thread-based microfluidic system for blood urea nitrogen and glucose detection in human whole blood," *Biomicrofluidics*, vol. 9, no. 2,pp. 1–13, 2015, doi: 10.1063/1.4915616.

[10] K. Tomimuro, K. Tenda, Y. Ni, Y. Hiruta, M. Merkx, and D. Citterio, "Thread-Based Bioluminescent Sensor for Detecting Multiple Antibodies in a Single Drop of Whole Blood," *ACS Sensors*, vol. 5, no. 6, pp. 1786–1794, 2020, doi: 10.1021/acssensors.0c00564.

[11] D. R. Ballerini, X. Li, and W. Shen, "An inexpensive thread-based system for simple and rapid blood grouping," *Anal. Bioanal. Chem.*, vol. 399, no. 5, pp. 1869–1875, 2011, doi: 10.1007/s00216-010-4588-5.

[12] B. Lyu, M. Punjiya, Z. Matharu, and S. Sonkusale, "An improved pH mapping bandage with thread-based sensors for chronic wound monitoring," *Proc. - IEEE Int. Symp. Circuits Syst.*, vol. 2018-May, pp. 1–4, 2018, doi: 10.1109/ISCAS.2018.8351878.

[13] R. S. P. Malon, K. Y. Chua, D. H. B. Wicaksono, and E. P. Córcoles, "Cotton fabric-based electrochemical device for lactate measurement in saliva," *Analyst*, vol. 139, no. 12, pp. 3009–3016, 2014, doi: 10.1039/c4an00201f.

[14] P. Sandbhor Gaikwad and R. Banerjee, "Advances in point-of-care diagnostic devices in cancers," *Analyst*, vol. 143, no. 6, pp. 1326–1348, 2018, doi: 10.1039/c7an01771e.

[15] T. T. Song, W. Wang, L. L. Meng, Y. Liu, X. B. Jia, and X. Mao, "Electrochemical detection of human ferritin based on gold nanorod reporter probe and cotton thread immunoassay device," *Chinese Chem. Lett.*, vol. 28, no. 2, pp. 226–230, 2017, doi: 10.1016/j.cclet.2016.07.021.

[16] N. Promphet *et al.*, "Cotton thread-based wearable sensor for non-invasive simultaneous diagnosis of diabetes and kidney failure," *Sensors Actuators, B Chem.*, vol. 321, no. December 2019, p. 128549, 2020, doi: 10.1016/j.snb.2020.128549.

[17] T. E. Du, Y. Wang, Y. Zhang, T. Zhang, and X. Mao, "A novel adenosine-based molecular beacon probe for room temperature nucleic acid rapid detection in cotton thread device," *Anal. Chim. Acta*, vol. 861, pp. 69–73, 2015, doi: 10.1016/j.aca.2014.12.044.

[18] C. Kung, C. Hou, Y. Wang, and L. Fu, "Sensors and Actuators B : Chemical Microfluidic paper-based analytical devices for environmental analysis of soil, air , ecology and river water," *Sensors Actuators B. Chem.*, vol. 301, no. April, p. 126855, 2019, doi: 10.1016/j.snb.2019.126855.

[19] B. M. Jayawardane, I. D. McKelvie, and S. D. Kolev, "Development of a gas-diffusion microfluidic paper-based analytical device (μPAD) for the determination of ammonia in wastewater samples," *Anal. Chem.*, vol. 87, no. 9, pp. 4621–4626, 2015, doi: 10.1021/acs.analchem.5b00125.

[20] L. Cai *et al.*, "Visual Quantification of Fe on Cotton Thread Using a Ruler," *J. Chem. Educ.*, vol. 96, pp. 1532–1535, 2019, doi: 10.1021/acs.jchemed.8b00800.

[21] P. Singhaphan and F. Unob, "Thread-based platform for nitrite detection based on a modified Griess assay," *Sensors Actuators, B Chem.*, vol. 327, no. May 2020, p. 128938, 2021, doi: 10.1016/j.snb.2020.128938.

[22] J. R. Choi, K. W. Yong, J. Y. Choi, and A. C. Cowie, "Emerging point-of-care technologies for food safety analysis," *Sensors (Switzerland)*, vol. 19, no. 4, pp. 1–31, 2019, doi: 10.3390/s19040817.

[23] A. A. Elbashir, R. E. E. Elgorashe, A. O. Alnajjar, and H. Y. Aboul-Enein, "Application of Capillary Electrophoresis with Capacitively Coupled Contactless Conductivity Detection (CE-C4D): 2017–2020," *Crit. Rev. Anal. Chem.*, vol. 0, no. 0, pp. 1–9, 2020, doi:10.1080/10408347.2020.1809340.

[24] R. F. Quero, L. P. Bressan, J. A. F. da Silva, and D. P. de Jesus, "A novel thread-based microfluidic device for capillary electrophoresis with capacitively coupled contactless conductivity detection," *Sensors Actuators, B Chem.*, vol. 286, no. January, pp. 301–305, 2019, doi: 10.1016/j.snb.2019.01.168.

[25] S. P. Raju and X. Chu, "Rapid Low-Cost Microfluidic Detection in Point of Care Diagnostics," *J. Med. Syst.*, vol. 42, no. 10, 2018, doi: 10.1007/s10916-018-1043-1.

[26] J. L. Delaney, C. F. Hogan, J. Tian, and W. Shen, "Electrogenerated chemiluminescence detection in paper-based microfluidic sensors," *Anal. Chem.*, vol. 83, no. 4, pp. 1300–1306, 2011, doi:10.1021/ac102392t.