Diagnosis Using Urine Samples

Subjects: Others

Contributor: Lung-Ming Fu

Urine is a by-product of kidney metabolism and is rich in many nitrogen-containing substances, including urea, uric acid and creatinine, which are excreted from the body as water-soluble chemicals during urination. The urine volume of normal healthy adults ranges from 0.6 to 2.6 L per day, where approximately 91–96% of this urine is composed of water. However, urine also contains various inorganic salts and organic compounds, such as proteins, hormones, and metabolites. The chemical composition of fresh urine mainly consists of nitrogen, ammonium, ammonia nitrogen, nitrate, nitrite, phosphorus, potassium, sulfate, sodium, magnesium, chloride, and calcium. Moreover, the urine of healthy individuals is clear or light yellow in color. However, in the presence of certain diseases or disorders, such as hematuria, diabetes, or kidney stones, distinct changes in the color, composition or smell of urine may occur. Therefore, urine serves as an important bio-rich resource for health monitoring.

Keywords: microfluidic ; paper-based devices ; lab-on-paper ; urine ; non-invasive samples

1. Overview

In recent years, microfluidic lab-on-paper devices have emerged as a rapid and low-cost alternative to traditional laboratory tests. Additionally, they were widely considered as a promising solution for point-of-care testing (POCT) at home or regions that lack medical infrastructure and resources. This review describes important advances in microfluidic lab-on-paper diagnostics for human health monitoring and disease diagnosis over the past five years. The review commenced by explaining the choice of paper, fabrication methods, and detection techniques to realize microfluidic lab-on-paper devices. Then, the sample pretreatment procedure used to improve the detection performance of lab-on-paper devices was introduced. Furthermore, an in-depth review of lab-on-paper devices for disease measurement based on an analysis of urine samples was presented. The review concludes with the potential challenges that the future development of commercial microfluidic lab-on-paper platforms for human disease detection would face.

2. Urine

Urine is a by-product of kidney metabolism and is rich in many nitrogen-containing substances, including urea, uric acid and creatinine, which are excreted from the body as water-soluble chemicals during urination. The urine volume of normal healthy adults ranges from 0.6 to 2.6 L per day, where approximately 91–96% of this urine is composed of water. However, urine also contains various inorganic salts and organic compounds, such as proteins, hormones, and metabolites. The chemical composition of fresh urine mainly consists of nitrogen, ammonium, ammonia nitrogen, nitrate, nitrite, phosphorus, potassium, sulfate, sodium, magnesium, chloride, and calcium. Moreover, the urine of healthy individuals is clear or light yellow in color. However, in the presence of certain diseases or disorders, such as hematuria, diabetes, or kidney stones, distinct changes in the color, composition or smell of urine may occur. Therefore, urine serves as an important bio-rich resource for health monitoring [1][2].

Unlike blood, urine is a non-invasive sample that can be easily collected without pain, or the need for special equipment. As a result, it has significant potential for point-of-care testing (POCT) or home health monitoring and diagnosis. However, current urinalysis diagnosis techniques still need the use of sophisticated laboratory apparatus and skilled personnel, which precludes their use in the home or in undeveloped areas of the world with poor medical infrastructure and lack of resources. Consequently, lab-on-paper diagnostic platforms have aroused great interest in recent years. Compared to traditional macroscale systems, microfluidic lab-on-paper devices have many advantages, including ease of manufacture, good portability, low cost, a simple diagnostic procedure, and good disposability [3][4][5][6][7][8][9][10]. As a result, they are expected to find increasing use for POCT applications in coming years based on a variety of samples, including urine.

Lab-on-paper devices perform the microanalysis process through patterned microchannels on paper substrates. The flow of the sample and buffer solutions through the device is driven mainly by capillary forces, and hence no external driving source is required. Furthermore, paper is a cheap and easily available material with many options and properties. For

example, chromatography paper [11][12][13][14][15] has the advantages of hydrophilicity, cleanliness, homogeneity, reproducibility and biocompatibility, while nitrocellulose (NC) membrane [16][17][18] has a high binding capacity for biomolecules, good stability, and stable reproducibility. Finally, ion-exchange paper and paper towel [19][20] have the advantages of selective separation and permeation, respectively. In fact, the choice of substrate material largely depends on the particular testing requirements.

The fabrication of microchannels or patterns on the substrates of lab-on-paper devices is mainly performed by filling the holes in the paper base with hydrophobic materials to form microchannels and impermeable barriers, or by cutting. The patterning process can be performed using many different methods, including laser printing, inkjet printing, plotting, wax printing, process cutting, flexographic printing, wet etching, laser cutting, screen printing, photolithography, chemical vapor deposition, knife drawing, spray coating, plasma treatment, sol-gel, handheld corona treatment, imprinting, 3D printing, embossing, and so on [21][22][23][24][25][26][27][28]. Each method has its own specific merits and drawbacks. For instance, the screen printing method is capable of producing large-scale devices with a simple process but has a poor hydrophilic-hydrophobic patterning resolution. In addition, the hydrophilic-hydrophobic patterns of paper-based devices made of wax are not suitable for the analysis of organic solvents. As a result, the choice of manufacturing method depends on the specific use and complexity of the device.

Generally speaking, the detection limit and resolution of the lab-on-paper platform depends on the choice of the detection method. Many different detection methods have been adopted, including the fluorescence method, colorimetric method, chemiluminescence (CL) method, electrochemical (EC) method, surface-enhanced Raman spectroscopy (SERS) method, electrochemiluminescence (ECL) method, spectrometry method, and distance-based method [29][30][31][32][33][34][35][36]. Among these methods, distance-based methods and colorimetric methods are the most convenient and do not need the use of any expensive detection apparatus. As a result, they are regarded as particularly promising diagnostic techniques for POCT applications.

3. Conclusions

Microfluidic lab-on-paper platforms are a rapidly developing and promising solution for the realization of next-generation preventive health care and detection analysis tools. Microfluidic lab-on-paper devices have many practical advantages over traditional laboratory systems, including a low cost, a simple manufacturing process, a straightforward operating procedure, good portability, high reliability, and a diagnostic performance close to that of benchtop methods. Furthermore, their use is compatible with many common biomedical samples, such as blood and urine. As a result, they have aroused great attention for POCT in the home or areas of the world with underdeveloped medical resources and infrastructures.

Urine has many advantages as a biomedical sample, including natural abundance, non-invasiveness and biological richness. Consequently, many microfluidic lab-on-paper platforms for urine analysis and disease detection have been proposed in recent years [100,108,164,165]. Many devices have also been successfully commercialized around the world, including the HiPee S2 smart urine analyzer (Tianjin Guo-Shih Tech. Co., Tianjin, China) and YH-1200 portable urine analyzer (Yao-Hua Co., Hebei, China). The MSLUA13 automatic urine analyzer (Medsinglong Medical Equipment Co., GuangZhou, China) has the ability to perform 14 different urine tests, including creatine, protein, microalbumin, specific gravity, calcium, nitrite, urobilinogen, pH, occult blood, glucose, bilirubin, ketone bodies, white blood cells, and vitamin C. Similarly, the Multistix 10 SG reagent strip device (Siemens Medical Solutions Inc., Malvern, PA, USA) can perform 10 urine tests, such as leukocytes, nitrite, urobilinogen, protein, pH, occult blood, specific gravity, ketone, bilirubin, and glucose. The test multi-drogues device produced by NarcoCheck Co. (Montluçon, France) provides the ability to test for 12 common drugs in urine, namely cannabis, cocaine, morphine, heroin, amphetamine, ecstasy, fentanyl, synthetic cannabinoids, ketamine, lysergic acid diacetamide, methylcystine and methamphetamine. Many simple urine test strips have also been commercialized for the detection of leukocytes and nitrites (Scanwell Health Co., San Diego, CA, USA), nicotine (Easy Healthcare Co., Willowbrook, IL, USA), pregnancy (Wondfo Biotech Co., GuangZhou, China) and ovulation (Femometer Co., Hong Kong, China). However, most of these devices provide only qualitative outcomes. Therefore, further research on the development of quantitative methods for the rapid analysis of urine samples using microfluidic lab-on-paper devices is still required.

The problem of improving the analysis accuracy of microfluidic lab-on-paper devices is also an ongoing concern. Studies have shown that the detection performance of colorimetric methods can be improved by inducing the aggregation of nanomaterials in order to enhance the detection resolution [119,149,156]. Similarly, in the EC and SERS methods, the detection performance can be improved by patterning nanomaterials with good electrical conductivity on the electrode surface [120,125,135,166,167]. In CL and fluorescence methods, the intensity of the detection signal can be amplified through the catalytic reaction of nanomaterials and the induced fluorescence quenching [132,168,169,170], respectively.

Many studies have confirmed that the detection performance of microfluidic lab-on-paper platforms can be improved through the use of sample pre-concentration techniques (such as ICP, ITP, EKS, and so on) [72,73,75,115,171], or amplification devices (e.g., LAMP, PCR, CHR, HCR, and so forth.) [78,81,83,172,173]. For example, the authors in [84] showed that the detection performance of a lab-on-paper platform for urine microalbuminuria diagnosis in diabetic patients could be significantly improved by incorporating EKS with a 100-fold amplification capability in the sample pre-treatment stage.

Although lab-on-chip devices that use human urine samples for human disease diagnosis have achieved rapid development in recent years, there are still several key challenges that need to be resolved. For instance, due to the irregularity of the fiber structure of paper-based materials, achieving precise fluid control and quantitative sample transportation is extremely difficult, and leads to potentially serious reproducibility and stability issues. Accordingly, the development of porous materials with a uniform pore size distribution is essential in improving the reproducibility and detection sensitivity of future lab-on-paper devices. Recent studies have reported that glass fiber paper and nitrocellulose membranes have a uniform pore size distribution [5,10,16,17,18], and therefore offer an improved detection sensitivity and reproducibility. However, the manufacturing process for such materials is complicated and is more expensive than that for regular paper. In addition, although paper-based devices have many advantages over traditional benchtop systems for clinical diagnosis purposes, many of the sample pretreatment processes required to enhance the detection performance of these devices still require the use of large-scale laboratory equipment. As a result, further research is required to develop on-chip sample pretreatment methods in order to fully realize the commercial potential of paper-based microfluidic devices for POCT applications. Finally, there are still several remaining target analytes for detection by using urine samples and lab-on-paper platforms that have not been considered, including ketone body, Leu. esterase, epithelial cells, occult blood, and casts. In addition, lap-on-paper has insufficient research on virus infection or COVID in urine sample, mainly due to the detection limit. If lap-on-paper can be combined with amplification system [77] or immunosensor [174], it is research that can be developed. Hence, future studies are also needed to develop effective lab-on-paper platforms to detect such analytes.

References

1. Ma, L.; Zhang, Z.; Li, X. Non-invasive disease diagnosis using surface-enhanced Raman spectroscopy of urine and saliva. Appl. Spectros. Rev. 2020, 55, 197–219.
2. Aitekenov, S.; Gaipov, A.; Bukasov, R. Detection and quantification of proteins in human urine. Talanta 2020, 233, 121718.
3. Jemmeli, D.; Marcoccio, E.; Moscone, D.; Dridi, C.; Arduini, F. Highly sensitive paper-based electrochemical sensor for reagent free detection of bisphenol A. Talanta 2020, 216, 120924.
4. Li, F.; You, M.; Li, S.; Hu, J.; Liu, C.; Gong, Y.; Yang, H.; Xu, F. Paper-based point-of-care immunoassays: Recent advances and emerging trends. Biotechnol. Adv. 2020, 39, 107442.
5. Mao, M.-X.; Zheng, R.; Peng, C.-F.; Wei, X.-L. DNA–Gold Nanozyme-Modified Paper Device for Enhanced Colorimetric Detection of Mercury Ions. Biosensors 2020, 10, 211.
6. Ng, H.Y.; Lee, W.C.; Kung, C.T.; Li, L.C.; Lee, C.T.; Fu, L.M. Recent Advances in Microfluidic Devices for Contamination Detection and Quality Inspection of Milk. Micromachines 2021, 12, 558.
7. Baek, S.H.; Park, C.; Jeon, J.; Park, S. Three-Dimensional Paper-Based Microfluidic Analysis Device for Simultaneous Detection of Multiple Biomarkers with a Smartphone. Biosensors 2020, 10, 187.
8. Tseng, C.C.; Kung, C.T.; Chen, R.F.; Tsai, M.H.; Chao, H.R.; Wang, Y.N.; Fu, L.M. Recent advances in microfluidic paper-based assay devices for diagnosis of human diseases using saliva, tears and sweat samples. Sens. Actuators B Chem. 2021, 342, 130078.
9. Ireta-Muñoz, L.A.; Morales-Narváez, E. Smartphone and Paper-Based Fluorescence Reader: A Do It Yourself Approach. Biosensors 2020, 10, 60.
10. Silva, R.F.; Paixão, T.R.L.C.; Torres, M.D.T.; de Araujo, W.R. Simple and inexpensive electrochemical paper-based analytical device for sensitive detection of Pseudomonas aeruginosa. Sens. Actuators B Chem. 2020, 308, 127669.
11. Primpray, V.; Chailapakul, O.; Tokeshi, M.; Rojanarata, T.; Laiwattanapaisal, W. A paper-based analytical device coupled with electrochemical detection for the determination of dexamethasone and prednisolone in adulterated traditional medicines. Anal. Chim. Acta 2019, 1078, 16–23.

12. Yang, T.; Pan, S.C.; Cheng, C.M. Paper-based human neutrophil elastase detection device for clinical wound monitoring. Lab Chip 2020, 20, 2709–2716.
13. Amin, R.; Ghaderinezhad, F.; Bridge, C.; Temirel, M.; Jones, S.; Toloueinia, P.; Tasoglu, S. Pushing the limits of spatial assay resolution for paper-based microfluidics using low-cost and high-throughput pen plotter approach. Micromachines 2020, 11, 611.
14. Kamel, S.; Khattab, T.A. Recent Advances in Cellulose-Based Biosensors for Medical Diagnosis. Biosensors 2020, 10, 67.
15. Murray, L.P.; Mace, C.R. Usability as a guiding principle for the design of paper-based, point-of-care devices–A review. Anal. Chim. Acta 2020, 1140, 236–249.
16. Chen, P.; Gates-Hollingsworth, M.; Pandit, S.; Park, A.; Montgomery, D.; AuCoin, D.; Gu, J.; Zenhausern, F. Paper-based Vertical Flow Immunoassay (VFI) for detection of bio-threat pathogens. Talanta 2019, 191, 81–88.
17. Hecht, L.; Rager, K.; Davidonis, M.; Weber, P.; Gauglitz, G.; Dietzel, A. Blister-actuated LIFT printing for multiparametric functionalization of paper-like biosensors. Micromachines 2019, 10, 221.
18. Aydindogan, E.; Ceylan, A.E.; Timur, S. Paper-based colorimetric spot test utilizing smartphone sensing for detection of biomarkers. Talanta 2020, 208, 120446.
19. Jarujamrus, P.; Meelapsom, R.; Naksen, P.; Ditcharoen, N.; Anutrasakda, W.; Siripinyanond, A.; Amatatongchai, M.; Supasorn, S. Screen-printed microfluidic paper-based analytical device (μPAD) as a barcode sensor for magnesium detection using rubber latex waste as a novel hydrophobic reagent. Anal. Chim. Acta 2019, 1082, 66–77.
20. Rahbar, M.; Wheeler, A.R.; Paull, B.; Macka, M. Ion-Exchange Based Immobilization of Chromogenic Reagents on Microfluidic Paper Analytical Devices. Anal. Chem. 2019, 91, 8756–8761.
21. Wang, C.H.; Wu, J.J.; Lee, G.B. Screening of highly-specific aptamers and their applications in paper-based microfluidic chips for rapid diagnosis of multiple bacteria. Sens. Actuators B Chem. 2019, 284, 395–402.
22. Batool, R.; Rhouati, A.; Nawaz, M.H.; Hayat, A.; Marty, J.L. A Review of the Construction of Nano-Hybrids for Electrochemical Biosensing of Glucose. Biosensors 2019, 9, 46.
23. Soum, V.; Park, S.; Brilian, A.I.; Kwon, O.S.; Shin, K. Programmable paper-based microfluidic devices for biomarker detections. Micromachines 2019, 10, 516.
24. Jaeger, J.; Groher, F.; Stamm, J.; Spiehl, D.; Braun, J.; Dörsam, E.; Suess, B. Characterization and Inkjet Printing of an RNA Aptamer for Paper-Based Biosensing of Ciprofloxacin. Biosensors 2019, 9, 7.
25. Sousa, L.R.; Duarte, L.C.; Coltro, W.K. Instrument-free fabrication of microfluidic paper-based analytical devices through 3D pen drawing. Sens. Actuators B Chem. 2020, 312, 128018.
26. Noviana, E.; McCord, C.P.; Clark, K.M.; Jang, I.; Henry, C.S. Electrochemical paper-based devices: Sensing approaches and progress toward practical applications. Lab Chip 2020, 20, 9–34.
27. Antiochia, R. Paper-Based Biosensors: Frontiers in Point-of-Care Detection of COVID-19 Disease. Biosensors 2021, 11, 110.
28. Katelakha, K.; Nopponpunth, V.; Boonlue, W.; Laiwattanapaisal, W. A Simple Distance Paper-Based Analytical Device for the Screening of Lead in Food Matrices. Biosensors 2021, 11, 90.
29. Fu, L.M.; Wang, Y.N. Detection methods and applications of microfluidic paper-based analytical devices. TrAC Trends Anal. Chem. 2018, 107, 196–211.
30. Rossini, E.L.; Milani, M.I.; Pezza, H.R. Green synthesis of fluorescent carbon dots for determination of glucose in biofluids using a paper platform. Talanta 2019, 201, 503–510.
31. Yang, J.J.; Oh, H.B.; Hwang, S.H. Paper-based speedy separation of amplified DNA (PASS-DNA): Potential for molecular point-of-care testing. Sens. Actuators B Chem. 2019, 286, 101–103.
32. Kung, C.T.; Hou, C.Y.; Wang, Y.N.; Fu, L.M. Microfluidic paper-based analytical devices for environmental analysis of soil, air, ecology and river water. Sens. Actuators B Chem. 2019, 301, 126855.
33. Fu, L.M.; Liu, C.C.; Yang, C.E.; Wang, Y.N.; Ko, C.H. A PET/paper chip platform for high resolution sulphur dioxide detection in foods. Food Chem. 2019, 286, 316–321.
34. Hou, C.Y.; Fu, L.M.; Ju, W.J.; Wu, P.Y. Microfluidic colorimetric system for nitrite detection in foods. Chem. Eng. J. 2020, 398, 125573.
35. Ng, J.S.; Hashimoto, M. 3D-PAD: Paper-Based Analytical Devices with Integrated Three-Dimensional Features. Biosensors 2021, 11, 84.

36. Tseng, C.C.; Ko, C.H.; Lu, S.Y.; Yang, C.E.; Fu, L.M.; Li, C.Y. Rapid electrochemical-biosensor microchip platform for determination of microalbuminuria in CKD patients. Anal. Chim. Acta 2021, 1146, 70–76.