

REVIEW

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Small-volume detection: platform developments for clinically-relevant applications

Wei-Hsuan Sung^{1†}, Yu-Ting Tsao^{1†}, Ching-Ju Shen², Chia-Ying Tsai^{3,4*} and Chao-Min Cheng^{5*}

Abstract

Biochemical analysis of human body fluids is a frequent and fruitful strategy for disease diagnosis. Point-of-care (POC) diagnostics offers the tantalizing possibility of providing rapid diagnostic results in non-laboratory settings. Successful diagnostic testing using body fluids has been reported on in the literature; however, small-volume detection devices, which offer remarkable advantages such as portability, inexpensiveness, capacity for mass production, and tiny sample volume requirements have not been thoroughly discussed. Here, we review progress in this research field, with a focus on developments since 2015. In this review article, we provide a summary of articles that have detailed the development of small-volume detection strategies using clinical samples over the course of the last 5 years. Topics covered include small-volume detection strategies in ophthalmology, dermatology or plastic surgery, otolaryngology, and cerebrospinal fluid analysis. In ophthalmology, advances in technology could be applied to examine tear or anterior chamber (AC) fluid for glucose, lactoferrin, interferon, or VEGF. These approaches could impact detection and care for diseases including diabetic mellitus, dry-eye disease, and age-related maculopathy. Early detection and easy monitoring are critical approaches for improving overall care and outcome. In dermatology or plastic surgery, small-volume detection strategies have been applied for passive or interactive wound dressing, wound healing monitoring, and blister fluid analysis for autoimmune disease diagnosis. In otolaryngology, the analysis of nasal secretions and mucosa could be used to differentiate between allergic responses and infectious diseases. Cerebrospinal fluid analysis could be applied in neurodegenerative diseases, central neural system infection and tumor diagnosis. Other small-volume fluids that have been analyzed for diagnostic and monitoring purposes include semen and cervico-vaginal fluids. We include more details regarding each of these fluids, associated collection and detection devices, and approaches in our review.

Introduction

Biochemical analysis of human body fluid is a frequently employed strategy for effective disease diagnosis. The most common approach, widely used in hospitals and

clinics, is an examination of blood chemistry. Blood sampling, whether from blood drawing or finger pricking, is relatively more invasive than tear or urine sampling [1]. Urine analysis is often an appropriate analytical strategy, especially for nephrological and urological disease diagnosis. While there are viable strategies that use large-volume samples such as blood or urine, some body fluid sources have limited volume, including those from the eye, blisters, and the cerebrospinal area, as well as body fluids such as semen and cervico-vaginal fluids. The challenges of obtaining and using samples from these areas are twofold. The first challenge is related to low volume

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availability, which facilitates only a limited number of tests and necessitates a suitable sampling tool as well as a sensitive and reliable testing tool. The second challenge is related to the potential health risks involved in sample collection, especially for fluids collected from the eye and the cerebrospinal area. Collecting AC fluid from the eye and aspirating cerebrospinal fluid from the interspinous space requires strict disinfection and professional personnel, and every collected sample must be carefully evaluated. Traditional, central lab analysis for disease detection requires extended time periods and large sample volumes. Finger prick blood sampling provides small volume of blood in a more convenient and less painful way. This technique has been applied to blood glucose self-monitoring in diabetic patients for many years, and is now popular worldwide. Recent studies have also described a variety of different devices that could detect infectious diseases or drug concentration from finger prick blood sampling, such as cryptococcal antigen screening [2], hepatitis C virus RNA detection [3], and infliximab concentration monitoring in inflammatory bowel disease patients [4]. These medical applications could determine infectious condition and medical concentration within a short period of time, and could provide precious information for doctors to adjust treatment immediately.

Advanced, point-of-care (POC) detection devices using small sample volumes have been developed to simplify disease detection and monitor disease state and treatment efficacy. The two primary benefits of such devices are: (1) decreased analysis time and clinical sample volume requirements; and (2) non-invasive, non-serological sample collection methods that reduce discomfort/pain and simplify collection. Non-serological approaches provided precedence that gave rise to the development of a variety of POC diagnostic devices [1]. There are, in fact, several collectable human body fluids that have demonstrated superior diagnostic ability for specific diseases that are hardly detected by traditional serum analysis [5, 6]. The current array of human body fluids suitable for fluid-based diagnostic analysis is provided in Fig. 1. Most of these fluids have shown great promise and impact for clinical diagnosis and follow-up analysis. Furthermore, many of them, including tear, nasal fluid, sweat, breast milk, semen, and vaginal fluid can be collected in non-invasive ways. Advances in analytical techniques and sensitive, portable platforms, have inspired greater research into non-invasive and minimally invasive methodology to meet the rising clinically relevant demand for POC diagnosis.

Portable biosensors capable of working with low-volume samples, such as those used in POC devices, not only present remarkable advantages such as portability,

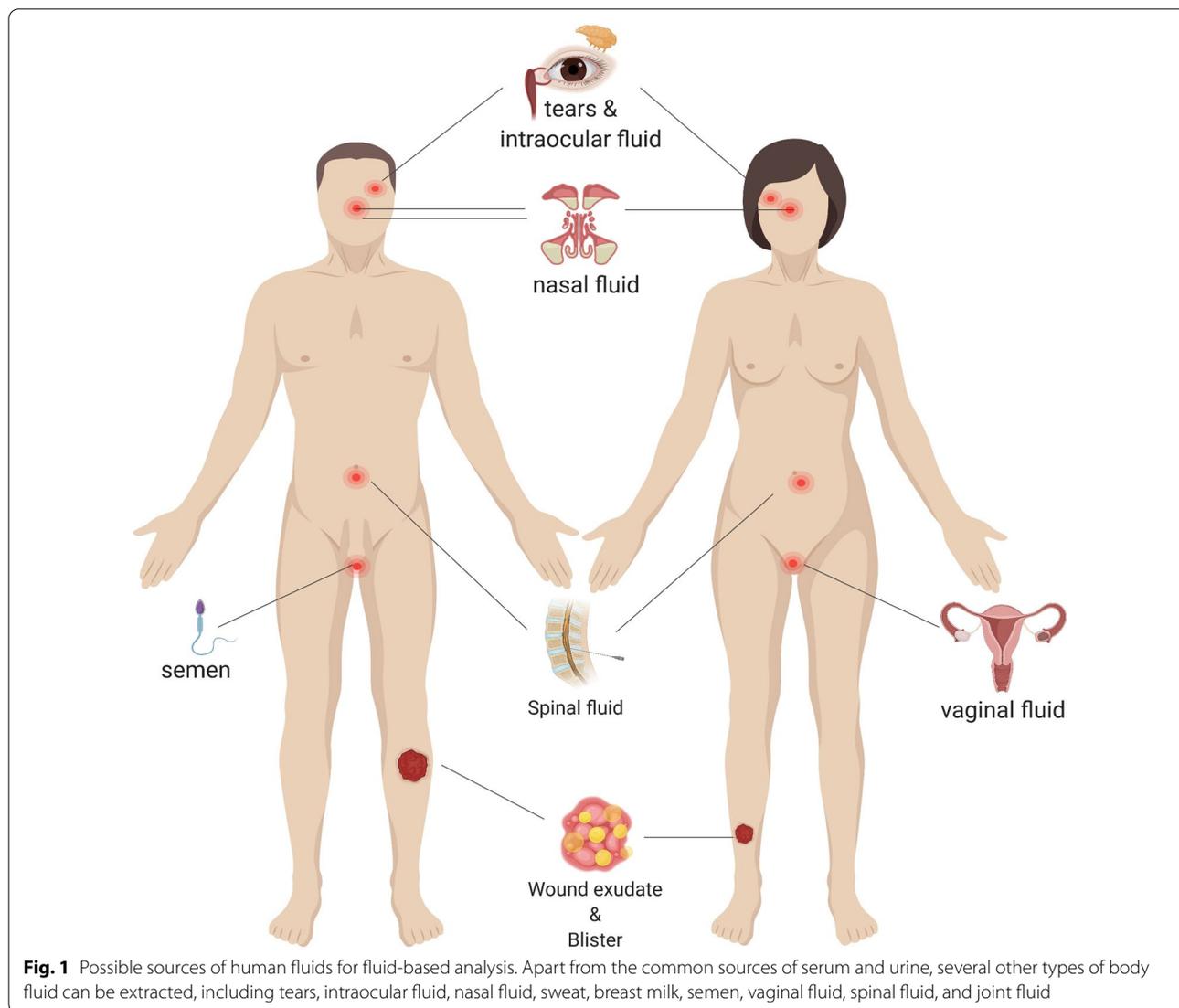
inexpensiveness, capacity for mass production, and small sample volume requirements, they also retain or advance diagnostic sensitivity and specificity [7] as evidenced by paper-based ELISA, a procedure that relies on a simple filter paper substrate [8]. Combining small-volume detection devices with other modern technology, e.g., smartphones, for optical and electrochemical analysis creates “all-in-one” approaches with great potential for use in laboratories, among the general population, and in remote, or resource-limited regions [9, 10]. We would, therefore, like to summarize the developments and current status of small-volume detection/diagnosis devices for clinically relevant applications.

Applications in ophthalmology: small-volume sample analysis of tear and AC fluid

The number of microfluidic and biosensor devices suitable for ophthalmological condition assessment has increased over the last 10 years. Fluids secreted by the eye, i.e., tears and AC fluid, contain a number of detectable molecules, proteins, and antibodies. While these fluids are available in only small amounts, highly sensitive devices can be used to detect and even quantify specific fluid components as a means of diagnosing ophthalmological diseases and disease states. To date, such microfluidic approaches have been successfully used to determine glucose level, diagnose dry eye disease (DED), detect infection, and measure levels of vascular endothelium growth factor (VEGF).

Current developments in small-volume tear sample analysis for examining diabetic mellitus (DM)

Diabetic mellitus (DM) is characterized by high blood glucose levels, and affects vessels throughout the body. In severe cases, patients experience blindness, amputation, and may require hemodialysis [11–14]. According to the World Health Organization (WHO) and other global data, an estimated 415 million people were diagnosed with DM in 2015 [15], a number that grew to 420 million in 2020 [16]. Finger-pricking is a traditional method to monitor blood glucose, but is invasive and painful. Several studies have found a correlation between glucose concentration in blood and tears [17–20], but others have found no or an indeterminate correlation [21, 22]. Despite the conflicting results, scientists still consider detection of tear glucose as a possible alternative to finger pricking. A wearable and disposable fluorophore-doped contact lens [23, 24] and a contact lens embedded with an electrochemical have been used to determine tear glucose level [25]. Badugu et al. developed a glucose-sensitive silicone hydrogel contact lens to continuously monitor tear glucose level. In this glucose level sensing contact lens, the range of new boronic acid containing



fluorophores (BAFs), based on the quaternized form of the quinolinium nucleus, responded to glucose within the contact lens [24]. Several review articles have noted the noninvasive merits of continuous monitoring with glucose-sensitive contact lenses [26]. A recent study showed a nanoparticle embedded contact lens (NECL) that leveraged oxidation–reduction to reflect different spectra detectable by spectrometry at different glucose levels [27]. In addition to contact lens devices, paper-based colorimetric biosensors have also been developed to detect tear glucose level. The idea for using noninvasive biosensors to determine tear glucose level was first examined by Romano et al. in 1987 [28]. Later Kim et al. demonstrated a paper-based analytical device (iPAD) to quantify tear glucose by colorimetric detection [29]. Gabriel et al. demonstrated an iPAD using Chitosan to

detect tear glucose level in the normal population and in DM patients as a means of providing analytical reliability and accuracy [30]. In 2018, Kownaka et al. developed a NovioSense glucose sensor to continuously detect glucose level in tears. This device used electronic components and utilized glucose oxidase as enzymatic element [31]. Tear glucose level is affected by different collection methods. Mechanical stimulation requires compression of the conjunctiva. Tear fluid may be contaminated with intercellular fluid leakage from damaged conjunctival cells, resulting in higher fluid glucose concentration. In the chemical stimulation approach, a lachrymator such as an onion is used, but this process may result in over-dilution of tear glucose. Non-stimulation approaches are the safest and most reliable methods. This process uses capillary tubes to collect 1–10 μ L tear without touching

the ocular surface, but is more challenging to execute than the other two approaches described above [20, 32].

Current developments in small-volume tear sample analysis for examining dry eye disease (DED)

Tears may also be used to measure electrolytes, enzymes, and protein. The presence and type of dry eye disease (DED), a common ocular disease affecting a number of people worldwide [33], can be evaluated by examining the level of lactoferrin, a glycoprotein in tear fluid. Autoimmune-related diseases, graft-versus-host disease (GVHD), and some medications, may lead to DED. Further, lack of lactoferrin is associated with increased overall tear concentration and corneal epithelial disorders [34]. In one experiment, terbium (Tb^{3+}) was complexed to lactoferrin and subsequently used to determine lactoferrin level via fluorescence detection using a digital camera [35]. This antibody-free method had only 6% error compared to ELISA, and required only 15 min, and a 2.5 microliter sample collected by polyethylene pipette, thus providing for convenient, inexpensive, and time-saving quantification of tear lactoferrin [35]. To prevent potential error derived from different light sources and cameras, Yamada et al. developed a distance-based scheme for lactoferrin quantification using an iPad [36]. This distance-based approach has been used to study tear components, which were collected by polyethylene pipette and stored in Eppendorf tubes under $-30^{\circ}C$ [36]. Sonobe et al. investigated the correlation between tear lactoferrin and DED severity using both an iPad and ELISA. In their study of tear fluid collected by microcapillary tubes from 24 patients, they found that tear lactoferrin was positively correlated to DED severity, and the patients with GVHD had lower levels of tear lactoferrin than non-GVHD ones [37]. The limitations of this device includes the inability to detect the extremely low lactoferrin level in severe c-GVHD-related DED, and it lacks clarification regarding tear collection methods [37]. Other approaches useful for detecting sub-types of DED, include determining anion (Na^{+} , K^{+} , Ca^{2+}) concentration and tear osmolality [38].

Current developments in small-volume sample analysis for examining other ocular diseases

In recent years, some microfluidics techniques have been designed using contact lens technology to provide diagnostic or theranostic effects [39, 40]. Guan et al. designed a contact-lens-on-a-chip as a diagnostic tool for personalized medicine. This contact-lens-on-a-chip provides a way to quantify protein and microbial bioburden using only a tiny amount of tear fluid [39]. Mak et al. developed a noninvasive, theranostic contact lens for the modulation and detection of herpes simplex virus (HSV)

infection. This contact lens captures and concentrates interleukin-1a (IL-1a) upregulated in HSV-1-infected patients [40]. In other research, patients with Zika virus infection demonstrated virus shedding from the lacrimal glands that was detectable in tear fluid [41]. Fungal infection may lead to pathogen-induced host response, such as a change in tear protein, after *Apergillus flavus* infection [42]. The presence of virus causes changes in tear protein composition that can be evaluated, via microfluidic techniques, to detect infection in a novel and innovative manner.

Current developments in small-volume sample analysis based on AC fluid for examining diabetic macular edema (DME) and age-related macular degeneration (AMD)

In addition to tear fluid, AC fluid, or aqueous humor, can be used in microfluidic detection/diagnostic methodology. Unlike noninvasive tear collection, aqueous humor collection requires needle aspiration. This procedure comes with a higher risk of trauma and infection. No more than 200 μL of aqueous humor can be safely removed from the anterior chamber, but even small samples can be used to determine intraocular component concentration. Two major vision-threatening retinal diseases, diabetic macular edema (DME) and age-related macular degeneration (AMD), can be evaluated by examining intraocular VEGF level, which plays a critical role in the pathogenesis of macular edema and neovascularization in both DME and AMD [43]. The standard first-line treatment for DME and AMD is intravitreal injection of anti-VEGF, which ameliorates the increased VEGF concentration found in both conditions. Detection of aqueous humor VEGF level can be used to reflect disease condition and activity and thus monitor and appropriately treat disease early on, before further changes in macular structure and increased threat of vision loss [44, 45]. Paper-based ELISA (p-ELISA) has been used to detect small amounts of aqueous humor VEGF and could provide a rapid and accurate POC approach [44].

Applications in dermatology and plastic surgery: current developments in small-volume sample analysis of exudate from skin

Skin, as the largest organ of the human body, protects the body against exogenous chemicals and acts as the first-line physical barrier against pathogenic microorganisms [46]. Skin lesions may compromise the barrier functions of the skin, providing potential portals of entry for infection. Wound infections may lead to a significant increase in attributable costs, higher mortality, longer hospitalization, and delayed wound healing [47]. Therefore, prevention and ascertainment of infection as well as determination of severity are critical for appropriate

wound management and classification [48]. Continuous monitoring of the wound is the primary concern for patients dealing with non-healing wounds, which characteristically fail to complete the normal stages of healing, i.e., vascularization, granulation, and re-epithelialization [49]. Qualitative wound assessment is based upon gross clinical appearance via visual inspection, which could be subjective and inconsistent due to variations in lighting and distance [50]. Over the three healing stages, the relevant parameters for wound healing such as pH value, uric acid, Flightless I protein and C-reactive protein (CRP) concentration in wound exudates change accordingly. These parameters can be used to indicate healing stage and may identify healing difficulties [50–53]. Wound sensors have been more fully developed than detection sensors for other body fluids. This may be due to the fact that they can be applied directly to a wound for sample collecting and ample wound exudate exists. Notably, the range of wound fluid sample volume required for analyte detection, 0.5 to 50 μL , is relatively broad [54]. Approaches that employ wearable biosensors for monitoring wound exudate components continue to be investigated for potential use in medical diagnostics [55].

Wound monitoring with passive dressings

Passive non-occlusive gauze and tulle dressings can be used to cover the wound and restore function [56]. However, such dressings are permeable toward bacteria and may adhere to the wound sometimes causing fresh trauma upon removal [56]. As for bandage-like biosensors, they are usually lightweight, flexible, breathable, easy to apply, and disposable, making them useful for both collecting and analyzing wound fluid [57]. Omphobic paper-based smart bandages (OPSBs), for example, are designed to monitor the status of chronic wounds and to detect the formation of pressure ulcers, even before the pressure-induced tissue damage becomes visible. Moreover, they wirelessly report wound status to the user or to medical personnel [57]. Smart bandages for not only monitoring but also treatment have been presented in several studies. Mostafalu et al. integrated temperature and pH sensors into flexible bandage with a stimuli-responsive, drug-releasing system comprising a hydrogel loaded with thermo-responsive drug carriers and an electronically controlled microheater [58]. Chen et al. demonstrated a smart bandage comprising luminescent porous silicon (LuPSi) particles loaded with ciprofloxacin [59] (Fig. 2). The oxidation of LuPSi in this bandage simultaneously triggers a drug release process synchronous with fluorescent intensity change in infected wounds with higher ROS and pH.

Gauze-like and pad-like biosensor dressing system have also gained much attention and have been reported

on in several recent studies. A robust electrochemical sensor-on-gauze system has been developed for quantitative measurement of uric acid that demonstrated high specificity and good linearity. This system was characterized by excellent reproducibility and consistent measurements for up to 7 h [60]. A pad-like biosensor dressing system has also been developed for simultaneous pH and glucose concentration detection. This system used a fluorescent pH indicator dye, carboxynaphtho-fluorescein, and a metabolite-sensing enzymatic system based on glucose oxidase and horseradish peroxidase for wound monitoring [61].

When it comes to designing wearable wound sensor, electrode design plays an important role. Although glass electrodes are widely used for pH detection, graphene shows promise due to its high reactivity to oxygen-bearing groups such as OH^- and its improved electrochemical response compared to glassy carbon electrodes, graphite, and CNTs [62]. Salvo et al. developed a pH sensor using a graphene oxide (GO) layer toward monitoring diabetic foot wound [63]. In addition to graphene, polyaniline has been used to fabricate flexible and thin nanopillar array-based pH sensors by employing soft lithography using a polymeric blend to create a flexible nanopillar backbone film [64]. Moreover, flexibility and comfort have been added to the design equation as means of bridging the gap between laboratory-tested systems and clinical applications. For example, one biosensor designed to detect vascular endothelial growth factor (VEGF) was printed with functional electrodes on flexible fibroin substrates using contact photolithography [65]. In addition to factors of comfort that improve compliance for wound biosensors, consideration must be given to material cytotoxicity as a means of evaluating true biocompatibility for continuous wound monitoring applications [66].

Wound monitoring with interactive dressings

Interactive dressings are semi-occlusive or occlusive, available in forms such as films, foam, hydrogel, and hydrocolloid [58]. Different interactive dressings have various properties. For example, foam dressings are suitable for deep wounds, diabetic ulcers, minor burns, and venous insufficiency ulcers due to heavy wound exudate absorbance [67]. The transparency of the film allows clinicians to visualize the wound healing process. Hydrogel dressings maintain the moisture level in the wound environment by using a swelled-water hydrophilic polymer [68]. The anaerobic environment provided by hydrocolloid dressings has been shown to improve the hypertrophic graduation of tissue [69]. From these findings, it is clear that selection of proper interactive dressings in combination with biosensors capable of monitoring

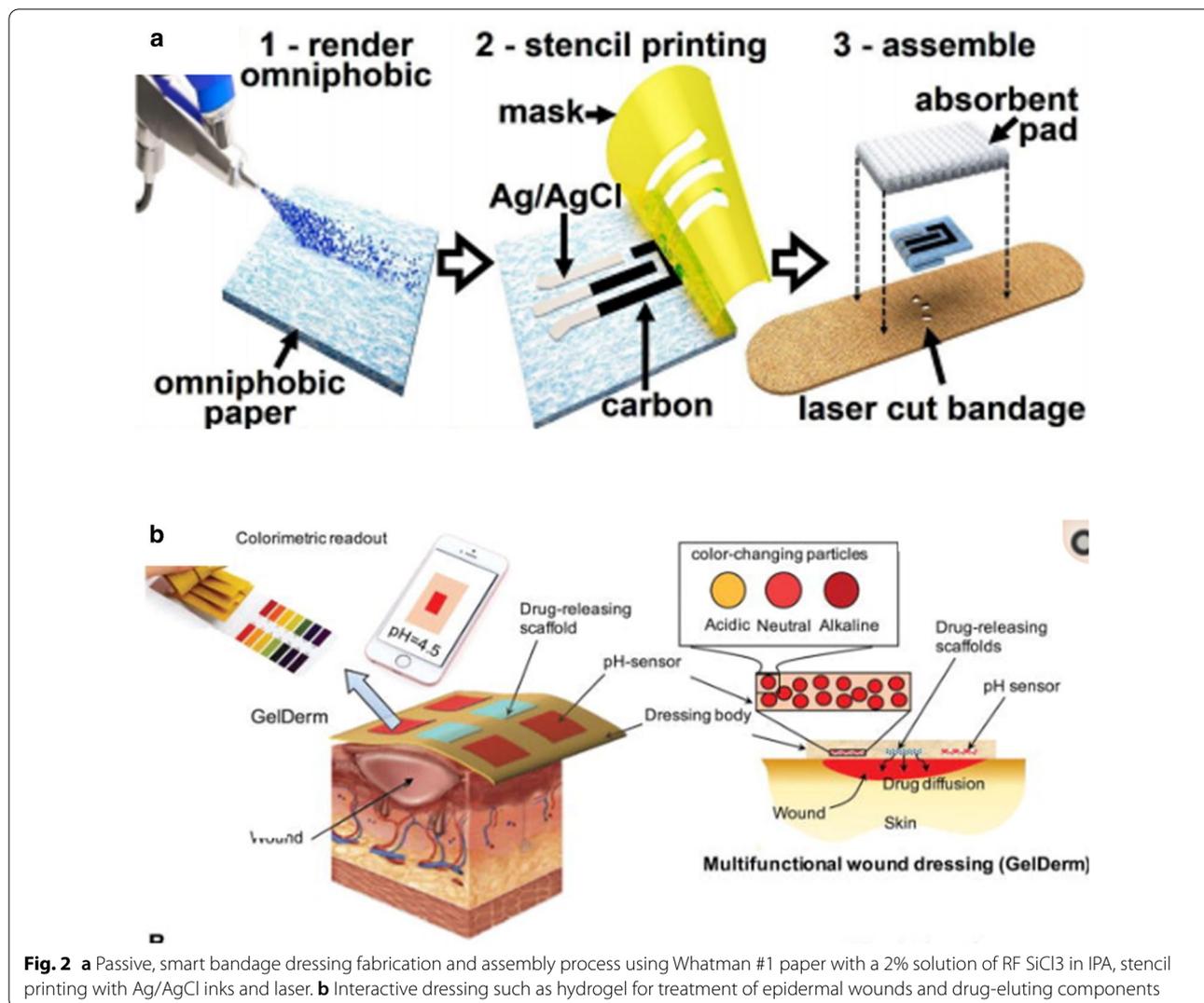


Fig. 2 **a** Passive, smart bandage dressing fabrication and assembly process using Whatman #1 paper with a 2% solution of RF SiCl₃ in IPA, stencil printing with Ag/AgCl inks and laser. **b** Interactive dressing such as hydrogel for treatment of epidermal wounds and drug-eluting components

physiological information of wounds are technologically possible and can improve wound healing.

Hydrogels offer an advantage over other wound dressing materials for wound monitoring because they easily entrap and minimally affect active molecules, they assist in wound moisture maintenance, their transparency facilitates wound observation, and they don't have to be removed during wound closure [58]. Hydrogels have consequently been intensively studied as carriers of sensing modalities for wound status screening [70]. Recently, a pH-responsive multifunctional hydrogel dressing (GelDerm) capable of releasing antibiotic agents at the wound site has been suggested for monitoring and managing trauma-, surgery- and diabetes-related chronic and acute injuries [71]. Another cleverly designed hydrogel-based wound dressing leveraged the pH-responsive fluorescence resonance energy transfer transition of Cyanine3 (Cy3) and Cyanine5 (Cy5) to monitor bacterial infection

and provide on-demand treatment in the presence of infection via near infrared (NIR) light-triggered antibiotic release [72]. Poor tissue oxygenation is linked to wound healing. As a result, Wisniewski et al. developed tissue-integrating oxygen sensors for tracking oxygen in real-time with injectable, tissue-integrating, biocompatible, and small hydrogel-based microsensors that have been shown to overcome the foreign body response for long-term sensing [73].

Current developments in small-volume sample analysis of blister fluid for examining autoimmune blistering skin disease

Bullous pemphigoid (BP) is one of the most common bullous autoimmune diseases. Laboratory diagnosis of BP relies on direct immunofluorescence (DIF) examination of linear antibody or complement deposition at the basement-membrane zone of a skin biopsy sample,

as well as serologic tests with indirect immunofluorescence (IIF) studies and ELISA for the detection of circulating antibodies in serum. Advances in biomaterials and biomedical devices are making great strides toward developing less complex and comparably suitable methods for BP testing. Paper-based ELISA, for instance, has been used to detect the NC16A autoimmune antibody, with only 2 μ L of blister fluid [74]. Further, as an alternative to serology performed on blood serum, serology may be performed on blister fluid. Biochip-based tests using blister fluid to detect antibodies, such as BP180, are appropriate initial approaches to the diagnostic workup of patients with suspected BP, especially for fragile elderly patients with poor venous access [75].

Applications in otolaryngology: small-volume sample analysis of nasal secretions and nasal mucosa

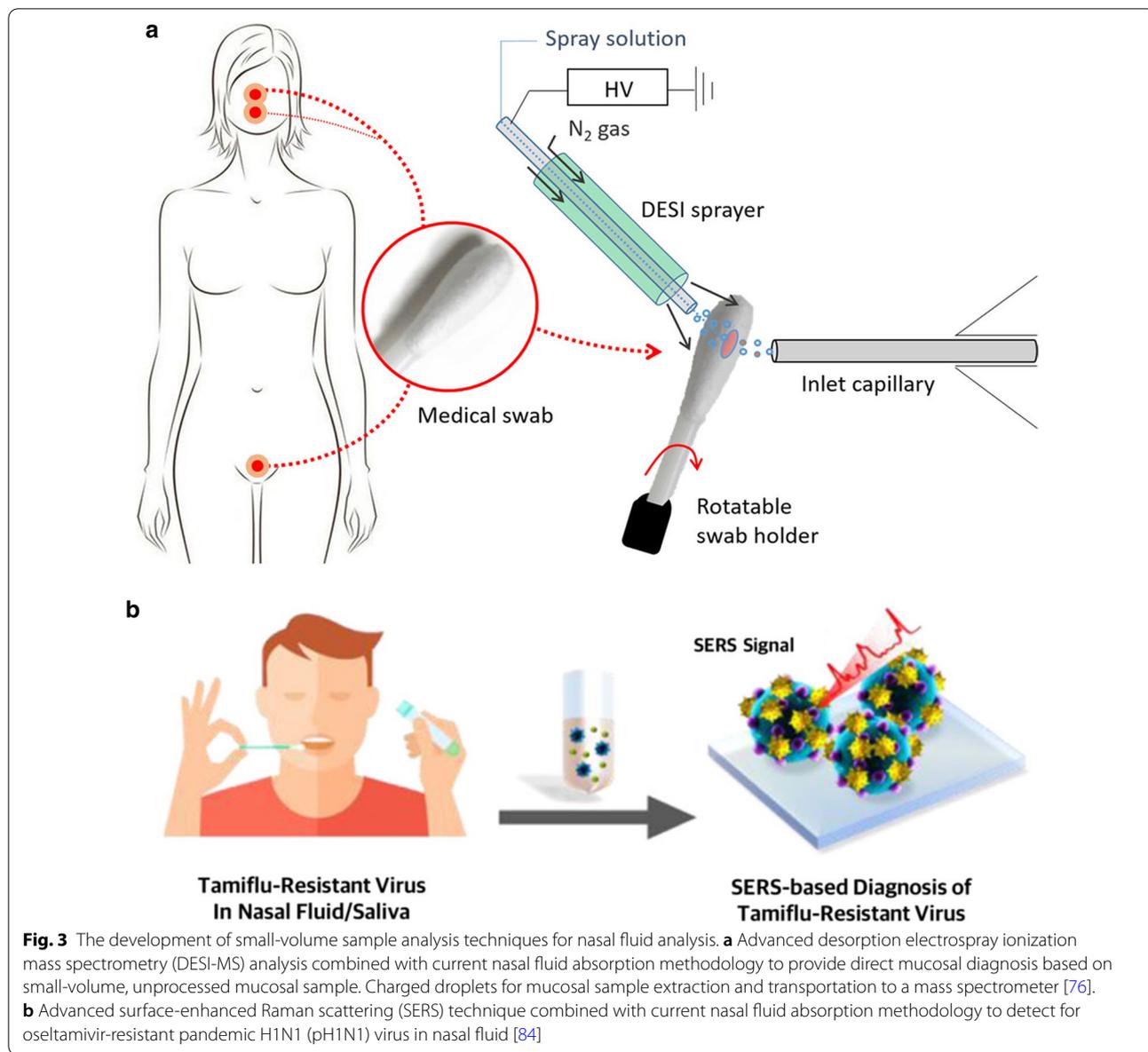
Nasal mucosa and nasal secretions are the first line of defense for the respiratory tract and are responsible for airborne pollutant clearance and microbial prevention. Therefore, analysis of the abundant immunological markers, cytokines, and proteins in the nasal mucosa and nasal secretions may provide clinical value for diagnosis and monitoring of respiratory tract diseases [76]. Furthermore, nasal secretions, which can provide direct information about local inflammatory activity, have been widely used in studies of nasal and sinus diseases, especially allergic rhinitis [77–79] and upper airway infections [80, 81]. However, advances in the development of nasal secretion biochemical analysis has stagnated as a result of the high variance in nasal secretions between individuals, as well as individually unstable, daily changes [82] and unpredictable dilution factors associated with traditional collection methods. A novel nasal secretion collection method without dilution accompanied with precise small-volume sample analysis has yet to be developed.

Current developments in small-volume sample analysis of nasal secretions for examining allergic and infectious disease

Among the diverse range of nasal secretion collection methods, absorption seems to be the best approach for providing sufficient undiluted sample for the detection of low-concentration immunoglobulins and inflammatory mediators [83]. Current nasal fluid analysis studies have employed existing absorption methods to develop small-volume sample analysis or developed their own undiluted sample collecting methods (Fig. 3). Jochems developed an adsorptive matrix strip and another nasal curettage method to noninvasively collect nasal lining fluid and cells without dilution [84]. These methods showed superior ability to analyze immune cells and detect cytokines

compared to traditional sample collection methods. Additionally, Pruski introduced a desorption electrospray ionization mass spectrometry (DESI-MS) method as a potential POC diagnostic device approach for rapid mucosal analysis [85]. Other researchers tended to combine simple sampling methods with advanced multiplex analysis of cytokines in nasal discharge to assist the diagnosis and differential diagnosis of respiratory-related allergic or infectious disease [76–89]. Because the amount nasal mucosa acquired from general nasal swab use is very limited (a few microliters) and sticky, diluting the sample with buffer is required. Unfortunately this drastically reduces target substance concentration. For this reason, polymerase chain reaction (PCR) is typically used because it increases the amount of target analyte to provide ultra-high sensitivity. This approach has been a clinical mainstay for low-volume sample testing. However, it requires the use of sophisticated laboratory equipment and a long analytical time that limit its practicality for rapid testing and for testing in large numbers. The emergence of the COVID-19 pandemic has underscored the urgent need for a more rapid and convenient POC platform for nasal secretion testing. The solution may be found in several recent, state-of-the-art studies showing that POC platforms could be developed using a variety of alternative methodologies including nucleic acid amplification tests (NAAT), rapid antigen detection tests (RADT), and fluorescent immunoassay analyzers (FIA) [90]. Sun developed a smartphone-based microfluidic chip that used an NAAT to return live virus from nasal sampling in 30 min [91]. Abdulrahman et al. and Young et al. have found that combining a paper-based lateral flow platform with RADT or IFA could yield rapid and cost-effective results that showed good consistency with the traditional PCR results (> 90%) [92, 93].

In addition, electrochemical biosensor would be another powerful POC platform for rapid detection of SARS-CoV-2 that would be worthy of large-scale investigative studies [94]. As a novel approach for respiratory tract infectious disease diagnosis, Yam combined the easy-to-manipulate nasal swab sample collection method and a commercially available LightCycler MRSA (methicillin-resistant staphylococcus) test to provide rapid detection of nasal MRSA colonization with high sensitivity and specificity [95]. This study showed the potential of nasal secretion microanalysis for screening and controlling specific infections for public health strategies. Chotiprasitsakul further verified that the MRSA nasal swab test was helpful in infection control practices [96]. Eom used the surface-enhanced Raman scattering technique to detect the Tamiflu-resistant influenza virus in patients who got the flu [97]. This study was the first to demonstrate the diagnosis of the antiviral drug-resistant virus based on only a nasal secretion. Nasal



fluid-based noninvasive screening techniques have the potential to prevent the transmission of drug-resistant bacteria and viruses.

Meng recently showed that detection of sIgE levels in nasal secretion could provide a quick diagnostic result capable of replacing blood testing as an effective and non-invasive way to assist the diagnosis of some allergic diseases while most other immunoglobulins or cytokines are not highly specific for targeted disease detection [98].

Expanded use of small-volume sample analysis for examination of nasal secretions

In recent years, microanalysis of nasal secretions has expanded in use to not only examine local allergic disease and upper respiratory tract infection, but also to provide impact in preventative medicine, otorhinolaryngology, environmental quality evaluation, and forensic investigations.

Hirvonen provided a great example of using nasal secretion analysis to evaluate environmental exposure to molds [99]. As a primary contact surface area for airborne elements, nasal mucus easily traps and retains foreign particles. It has been used as a tool to detect a variety of things including the gunshot residues of suspected shooters in forensic investigations [100, 101]. Nasal mucus can also trap inhaled medicine. Recent studies have shown the advantages of nasal mucus analysis for cocaine abuse confirmation. It provides for noninvasive analysis and precise detection of the prototype compound instead of its metabolites [102, 103]. Even though there is no strong correlation between nasal secretions and blood, nasal fluid analysis can provide a noninvasive, biochemically analytical tool for a variety of breathing-related disease and investigations.

Small-volume sample analysis of cerebrospinal fluid (CSF)

Cerebrospinal fluid (CSF) is the most useful biological specimen for diagnosing central nervous system (CNS) diseases, including neurodegenerative and infectious diseases. However, CSF samples are collected by an optimized, but invasive procedure, lumbar puncture [104, 105]. Further, given that most of the neurodegenerative diseases are preventable in the preclinical stage but irreversible once the symptoms appear [6], several recent studies concentrated on early stage diagnosis of CNS disease based on the biochemical analysis of CSF.

Neurodegenerative diseases

Mohammad et al. successfully improved the diagnostic sensitivity of a test for Parkinson's disease (PD) by using protein misfolding cyclic amplification (PMCA) technology to amplify the signal of α -synuclein (α Syn), reaching detection limits of 0.1 pg/mL for α Syn oligomers. This work demonstrated a useful biochemical tool to preclinically identify patients who may develop PD [106]. Another inspiring work is that Chao et al., who developed a label-free optical nanosensor to simultaneously detect beta-amyloid (A β 42) and total tau (T-tau). Their chip comprised four sensors, and the optical signals were produced by the functional surface coated with 10 nm Au and either A β 42 or T-tau antibody. As a novel POC testing device with detection limits of 7.8 pg/mL of A β 42 and 15.6 pg/mL of T-tau, it can provide early diagnosis toward Alzheimer's disease [107].

CNS infectious disease

CNS infectious diseases have fulminant symptoms and high prevalence in developing countries. Siyuan et al. introduced next-generation sequencing techniques to examine CSF to assist in the diagnosis of neurobrucellosis

[108] and Anna et al. demonstrated real-time multiplex PCR of CSF to assist in the differential diagnosis of meningitis or encephalitis [109]. Regarding the development of CSF POC testing devices, Emily LH et al. developed a treponemal immunochromatographic strip test to assist the diagnosis of neurosyphilis [110], Geoffroy et al. introduced the use of a glucometer, Bertrand et al. introduced the Multistix 10 SG strip and iSTAT to provide rapid diagnosis of bacterial meningitis, [111, 112] and Robin exploited lens-free microscopy to assist the diagnosis of meningitis [113]. All of these POC testing devices demonstrated the advantages of being rapid, easy to handle, and diagnostic accuracy equal to that of existing gold standard methods. The development of advanced or innovative diagnostic tools may play a critical role in guiding treatment of infectious diseases and reduce the misuse of antibiotics in CNS infections.

CNS tumor

In addition, noninvasive POC analytical approaches have been developed in recent years for examining CSF to diagnose CNS tumors—these approaches would replace invasive CNS biopsy methods [114]. The development of POC diagnostic devices for CSF analysis remains very promising.

Small-volume sample analysis of other body fluids

Semen analysis

Semen analysis is primarily used in the field of male infertility and the median semen volume per ejaculation is about 3–4 mL. The clinical standard methods are manual microscope-based counting and computer-assisted sperm analysis (CASA) [115]. In recent years, studies have focused on developing POC diagnostic devices to overcome the psychological resistance of men who would otherwise have to go to a hospital for infertility testing. Su developed a lightweight automated semen analysis platform combining lensfree, on-chip microscopy to conduct semen analysis, which was the precedent for developing POC devices in the field [116]. Based on these technologies, automated smartphone-based semen analytical assays have been developed. Kanakasabapathy has designed a portable smartphone-based POC diagnostic device that could distribute the unprocessed semen sample on the microchip by a capillary-based tip and a rubber bulb to create negative pressure. This allowed them to image and analyse small semen sample volumes (<35 μ L) within 5 min [117]. Furthermore, a biochemical-based POC semen analytical device that combined a microfluidic paper plate with a colorimetric reaction has been developed: Matsuura developed paper-based assays for sperm counting and sperm motility assessment [118, 119]. Tsao developed a paper-based MTT assay to

differentiate normal and low total motile sperm concentration for semen screening by self-assessment [120]. Using a different approach than traditional image-based analytical techniques, these biochemical-based POC diagnostic devices all loaded the reaction reagent onto the paper platform first and then directly inserted the test strips into the semen sample, which largely reduced the duration and cost (0.1 USD per test) for semen analysis (Fig. 4). This study also introduced the combination of universal image readout devices (smartphone or camera) and imaging processing software to streamline the process. The integration of microfluidic platforms, simple image readout devices, and the internet may pave the path for future advances in semen analysis.

Cervico-vaginal fluid

Cervico-vaginal fluid (CVF) is considered a potential source of noninvasive biomarkers for various female-specific diseases. It is characterized by its small volume, averaging only about 0.5 mL [121], and its capacity for significant influence by the microbial community [122]. Recent studies have found many cytokines and proteins that are correlated with the premature rupture of membranes including prolactin, IL-6, IL-10, TNF- α , GM-CSF, MCP1, neutrophil elastase, acetate, VDBP, TIMP-1, DKK3, RANTES, etc. [123–128]. Among these measured cytokines, IL-6 was most studied and showed an equally predictive value for intra-amniotic infection to amniotic fluid WBC count in some studies [129–133]. Musilova has used a paper-based lateral flow platform to detect vaginal fluid IL-6. The non-centrifuged vaginal fluid was

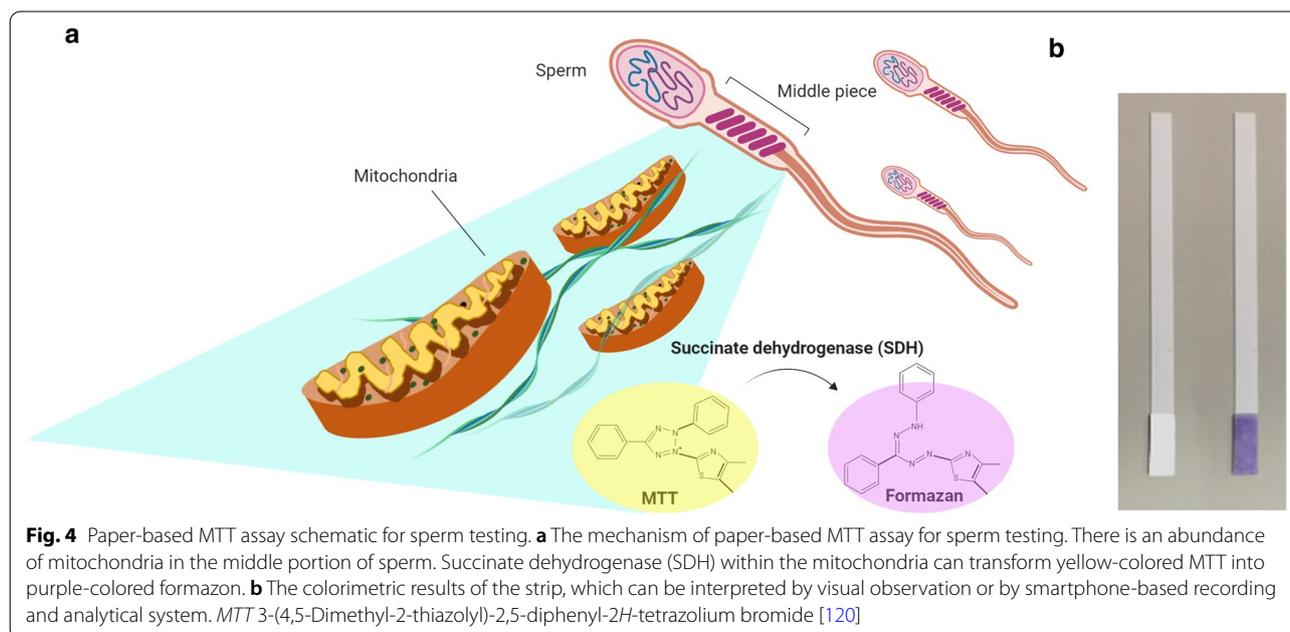
directed loaded onto the platform and approximately 100 μ L was adequate to conduct the test with good specificity and negative predictive value [134].

In addition premature membrane rupture, CVF cytokines can also be used to screen for lower genital tract infections, [135, 136] and cancer [137]. Appidi et al. developed a POC colorimetric assay to detect the cervical cancer. Cervico-vaginal swabs was used for sample collection and the samples were diluted in buffer to expend sample volume. After mixing the sample with H₂AuCl₄ and ascorbic acid in the plastic tube, colorimetric result developed within 1 min. This is a successful study that introduced label-free gold nanoparticle based POCT in CVF [138].

However, further development of POC CVF analytical devices is hampered by the relatively low sensitivity and specificity of independent cytokines and proteins. Integrating the abundant research results and picking out a cluster of parameters for accurate diagnosis of specific lower genital tract diseases is necessary for the future development of POC CVF analysis.

Interstitial fluid

Interstitial fluid is also informative regarding metabolites, pharmacokinetics and immunologic markers. Microneedle (MN) patch and tattoo biosensors have been used to extract and detect metabolite levels, such as glucose, cholesterol, and albumin, from the dermal interstitial fluid, which provide a minimal-invasive method to monitor diabetic mellitus and other metabolic syndrome [139, 140]. An animal study also showed that an MN patch



could be used to monitor Vancomycin level and polio antibody level from dermal interstitial fluid, an approach that could be used to replace blood draw methodology [141].

Conclusions

In laboratory diagnostics, research and developments in POC testing of small-volume samples is relatively young but offers abundant space for further progress. Studies using small-volume clinical sample detection and liquid biopsy approaches have become increasingly common and more refined. To accomplish POC detection of small volume samples, we need advanced tools. In this article, we summarized important recent advancements in POC detection technologies using human body fluids, including detection tools for AC fluid, contact lenses for examining glucose level in tears, and tools for analyzing wound and blister contents, nasal secretion, CSF, CVE, and sperm. Collection of tear fluid, nasal secretions, blister secretions, and even wound secretions from hydrogel dressings, can be executed non-invasively during routine medical care. Some procedures are invasive but would not leave permanent wounds or complications if carried out carefully. From this review, we are most encouraged and inspired to employ advanced technologies for collecting and examining AC fluids. We have published relevant related studies and our current work focuses on using AC fluid for detecting various ophthalmic diseases, such as age-related macular degeneration and dry eye. Additionally, tear fluid, which can be easily and non-invasively collected using only a paper strip, is a promising diagnostic fluid source that may become more reliable with advances in POC technology. This review provides a complete picture of the current development in this domain and further promotes the development of small-volume POC diagnostic products for both academic and commercial communities as a means of providing advances in early disease diagnosis and management.

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Authors' contributions

WHS, YTT and CJS reviewed the articles from the journal databases and drafted the manuscript. CYT reviewed the articles from the journal databases, drafted, and revised the manuscript. CMC highlighted the concept of this review article, and revised the manuscript. All authors read and approved the final manuscript.

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