Review

Electrochemical vs Optical Biosensors for Point of Care Applications: A Critical Review

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Electrochemical vs Optical Biosensors for Point of Care Applications: A Critical Review

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Abstract: Analytical chemistry applied to medical and diagnostic analysis, recently focused on the development of cost-effective biosensors able to monitor the health status or to assess the level of specific biomarkers that can be indicative for several diseases. The improvement of technologies relating to the possibility of non-invasive sampling of biological fluids, as well as sensors for the detection of analytical signals and the computational capabilities of the systems routinely employed in everyday life (e.g. smartphones, computers, etc.), make the complete integration of self-standing analytical devices more accessible. This review aims to discuss the biosensors that have been proposed in the last 5 years focusing on two principal detecting approaches, optical and electrochemical, which have been employed for quantifying different kind of target analytes reaching detection limits below the clinical sample levels required. These detection principles applied to Point-of-care (POC) devices, have been extensively reported in literature and even the limited examples found on the market are based on these strategies. This work will show the latest innovations considering the integration of optical and electrochemical detection with the most commonly reported analytical platforms for POC applications such as paper-based or wearable and implantable devices.

Keywords: biosensor; electrochemical detection; optical detection; Point-of-care; lab-on-chip; microfluidic device; paper-based device

1. Introduction

New technologies for the development of portable and self-standing devices for rapid diagnosis of health disorder are emerging every day and they are linked to the advances in different areas including chemistry, bioengineering, physics and medicine. Indeed, the latest generation biosensors benefited from an interdisciplinary approach that has allowed the development of sophisticated and

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innovative analytical systems that could potentially improve human health, particularly in the developing world. In this context, the Point-of-care testing (POCT) approach represents a set of diagnostics that can allow the rapid detection of diseases leading to a prompt execution of the necessary treatments. The International Organization for Standardization (ISO) defines POCT as 'testing that is performed near or at the site of a patient with the result leading to possible change in the care of the patient' [1]. Recently, POCT is used across a variety of settings, including general practice, nursing homes, pharmacies, outpatient and off-site clinics and in-home patient care [2]. The immediate goal of POCT is to use the information it can provide to directly influence the timely and proper care of patients [3] and to enlarge the accessibility to clinical-diagnostics regardless of existing medical and laboratory infrastructure [3-6]. Several POCT devices were proposed for different applications, such as detection of bacteria [7-9], viruses [10-13], biomarkers related to specific disease or health conditions [14-17]. Their increased availability is partly due to technological advances that have made them more robust, easy-to-use and cost-effective, including advances in smartphone based technologies, lab-on-a-chip platforms, novel assay formats, e.g. automated assays and fully integrated assays (which include all the required reagents and equipment), and advances in the long term storage of reagents needed for POCT [4]. Indeed, these devices are based on biosensors able to combine in a unique platform bioanalytical assays and transduction systems for the acquisition and elaboration of analytical signals (Figure 1). The integrated platform should provide all the analytical steps, from the pretreatment of the sample, to analysis and data processing in order to obtain a qualitative and/or quantitative information. In light of these considerations, it is important that the optimization of the analytical assay, which should be based on the use of small volumes of sample and reagents and on simple procedures, is accompanied by the development of portable and easy-to-use detectors.

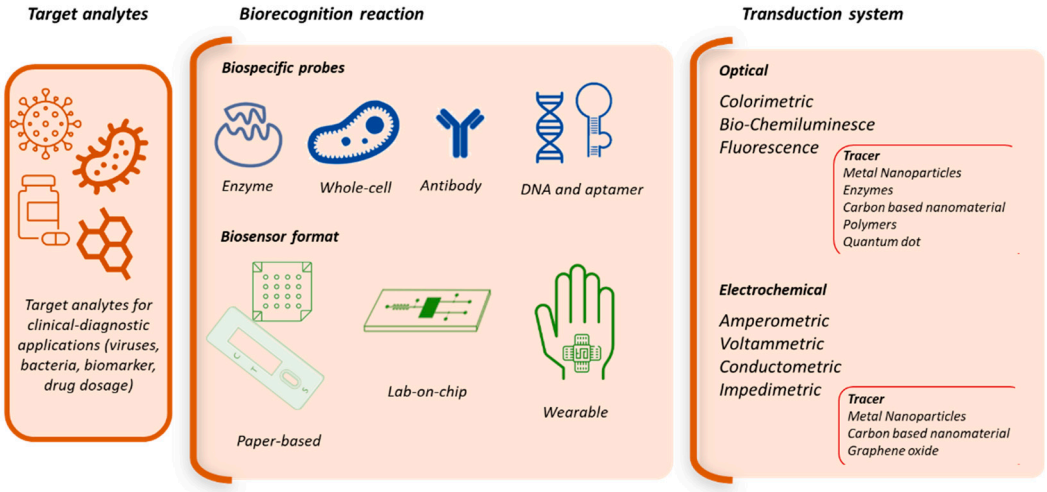

Figure 1. Scheme of the POCT devices considering the target analytes of interest, the biorecognition reactions and the transduction systems that are most widely employed and can be selected depending on the needed performances and instrumental requirements of the analytical method.

As it concerns bioanalytical assays, the most widespread are those based on immunoassays. Indeed, the high specificity and selectivity make them ideal candidates for detecting low traces of target analytes even in complex matrices and small volumes. In the same way, enzymatic assays already well established in laboratory practice, have been extensively proposed for POCT platforms. Several devices actually spread on the market for clinic-diagnostic purposes are based on these biorecognition reaction principles [18.]. Recently also biosensors based on DNA or aptamers probes as well as whole cell biosensors are gaining interest among the scientific community. These innovative systems are very promising taking advantage of their implementation with new nanomaterials, but until now their use is limited to academic literature and are not well established in the market [19, 20]. Detection techniques combined with these bioassays and exploited for

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developing POCT devices are generally based on optical and electrochemical principles [18, 21-24]. Optical biosensors can exploit different phenomena, depending on the tracer employed in the bioanalytical assay. One of the most widespread approach well known for routinary applications is based on the use of metal nanoparticle (MNPs) which are characterized by strong surface plasmon resonance (SPR) bands, which frequency depends on the size and shape of the nanoparticle and on the nature of the metal together with the composition of the surrounding medium [25]. This phenomenon is widely exploited by commercially available Lateral Flow Immunoassay (LFIA) which are well known for their application in pregnancy test and more recently for SARS-COV-2 diagnosis. LFIA is one of the most successful colorimetric assay because of the advantages of simplicity, rapidity, cost-effectiveness and no requirement of equipment or technical expertise for operation. It is based on immunoassays in which the sample and a suitable labeled probe flow by capillary forces along a porous membrane that contains immobilized reagents with molecular recognition properties. These are placed in specific areas of the membrane, that are usually defines as Test and Control lines, where the former gives information about the target analyte, while the latter ensures the correct functioning of the test. The occurring of immunoreactions leads to the development of detectable bands with naked eyes in correspondence of the Test and Control lines, due to the accumulation of the label in such zones which allow to obtain qualitative information about the presence or the absence of the target analyte [26]. Otherwise, chemiluminescence (CL) is another optical technique alternative to colorimetric approach and it is triggered by a chemical reaction in which an atom or molecule passes from the excited state to the steady state, releasing photons as a by-product of the reaction [27]. CL-based biosensors usually rely on the use of enzymatic tracers that are able to catalyze CL reaction in presence of a suitable substrate. The measurement of the produced photons can be related to the concentration of the target analyte enabling quantitative analysis, but it implies the use of an external detector able to detect luminescent signals. Nowadays, there is a wide choice of miniaturized and compact CL detector that can be implemented into portable and easy-to-use analytical devices, since the main requisite is the ability to collect as much light as possible for achieving the highest detectability [28]. Besides the traditional photomultiplier, silicon p-i-n photodiode have been extensively proposed since they guarantee small size, low noise together with fast response time [29-31]. In additional, if it is necessary to obtain spatial information, charge coupled device (CCD) [32,33] and complementary metal-oxide semiconductor (CMOS) can be exploited for developing compact imaging systems. In particular, the use of CMOS is attracting great attention since this technology is integrated into smartphone photocamera enabling the smartphone to be used as a portable CL detector [34-36]. In the context of CL detection, recently also thermochemiluminescent (TCL) approach was proposed in which light emission is triggered by heating [37]. Therefore it is required the same instrumentation for acquiring photon emission with the addition of an heating system to induce the luminescent phaenomenon. As optical tranducing system, also fluorescence (FL) has been extensively proposed for developing clinical-diagnostic biosensors thanks to its high sensitivity. In this case, however, the analytical device must be integrated with both the luminescent detector and a light source for exciting the fluorophore used as label for the bioassay [38].

On the other hand, electrochemical transducers usually are based on the measurement of the current developed during a redox reaction (amperometric and potentiometric methods) or of the change in surface conductivity and impedance (conductometric and impedimetric methods) [39]. Amperometric-based transducers are one of the most frequently used and in this case the measured current results from the oxidation and reduction reactions of electroactive species. Amperometric measurements are performed by exploiting the passage of the current of the working electrode at the constant amplitude voltage against the reference electrode through the sample keeping the potential unchanged. If the current is measured applying controlled variations of the potential, the method is called voltammetry. Among the most widespread amperometric sensor, the glucose meter is one of the major applied for routinary analysis for people who suffer from diabetes mellitus or hypoglycemia. The assay is started by depositing a drop of blood on test strips equipped with an electrode containing glucose oxidase or dehydrogenase. Glucose present in the sample is oxidized by the enzyme which is regenerated by the presence of a mediator reagent generating an electric current.

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The total charge passing through the electrode is proportional to the concentration of glucose in blood that has reacted with the enzyme. Also potentiometric-based biosensors focus on redox processes but in this case it is measured the potential difference between working and reference electrode by a voltammeter. Impedimetric methods rely on the measurements of electrical changes that occur after a biorecognition reaction on the surface of a modified electrode. In particular, electrochemical impedance spectroscopy (EIS) is widely applied in developing biosensors based on the interaction between the biological probe and the target analyte that are selectively adsorbed on a modified electrode surface [40]. These adsorption cause changes in the electron transfer kinetics between a redox species present in the solution and the electrode. By monitoring the charge transfer resistance (Rct) it is possible to quantitatively determine the amount of target analyte bound at the electrode surface. Finally, also conductimetry can be exploited for developing biosensors. An ohmmeter is used for measuring the change in conductance between two metal electrodes at a certain distance from each other. Since an alternating current is applied to the electrode, the change of ionic content causes a conductance that can be measured. Generally, the change of ionic strength occurs as a result of an enzymatic reaction in the system, so this technique is employed for developing enzyme-based biosensors.

To combine the advantages of electrochemical and optical detection, electrochemiluminescence (ECL) has been propose which is very wide employed for analytical routinary applications in laboratory settings. ECL is a luminescent phenomenon triggered by an electrochemical stimulus on a specific molecular system [41,42]. Compared with FL and CL, ECL provides high temporal and spatial control of light emission, low background, high sensitivity, wide dynamic range, and rapid measurement [43, 44], while with respect to other electrochemical techniques it is less sensitive to electrical interferences and can exploit simplified detector, since photon emission is measured instead of current [45]. Thanks to these advantages, ECL is particularly interesting for the development of portable biosensing devices [46, 47].

This review aims to do the state-of-the-art about the development of POC biosensors for clinical-diagnosis purposes based on optical and electrochemical detection. The review will focus on the major latest achievements in this field showing the advantages and the weakness of the two different approaches as well as the possibilities for future development with a critical comparison with the widespread and commercialized systems that are already available.

2. Lab-on-chip based biosensors

The expression Lab-on-chip (LOC) refers to a technology based on the integration of fluidic, biosensing probes and measuring components (electrodes or optical apparatus) onto the same chip [48-53].

As it concerns electrochemical biosensors, the methods require a set of electrodes (working, reference and counter electrodes), their respective connectors, a potentiostat for regulating the electric processes and a laptop (or similar) to analyze the data [54]. Computer and potentiostat have been already replaced by portable and handheld wireless all-in-one devices which are suitable for POCT approach [55-61]. Following this trend, also electrodes are moving to easy-to-use and low-cost platforms such screen-printed electrodes (SPEs) that are developed by depositing or pasting a combination of layers onto a wide variety of flat substrates [62]. Electrochemical techniques combined with SPEs have proved to be an ideal candidate for replacing conventional benchtop equipment with affordable, rapid and portable devices thus offering versatility in terms of electrode design, materials and compatibility with different applications [63]. For developing biosensors, the immobilization of the recognition element onto the electrode surface is generally required. The immobilization processes on SPEs include different approaches such as adsorption, covalent coupling, entrapment or cross-linking for obtaining the formation of a self-assembled monolayer of biospecific probes [64-67]. Hu et al. proposed an electrochemical immunochip based on MoS2/ poly (diallyldimethylammonium chloride) (PDDA) hybrid film produced via layer-by-layer self-assembly method [68]. An electrochemical impedance detection platform was developed for the detection of alpha-fetoprotein (AFP) by integrating a three-electrode system in the micro-channel, which

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introduces MoS2/PDDA film modified with antibodies specific for recognition of AFP as the working electrode, Ag/AgCl wire as the reference electrode and indium tin oxide (ITO) as the counter electrode. This approach showed a linear range from 0.1 ng/mL to 10 ng/mL and a detection limit of 0.033 ng/mL. Timilsin et al. described a multi-electrode sensor chips suitable for detecting several analytes in complex biological fluids based on sandwich enzyme-linked immunosorbent assay (ELISA) method [69] (Figure 2a). Functionalized electrodes coated with immobilized capture antibodies were exposed to samples containing the antigen and a secondary biotinylated detection antibody. Upon incubation, the specific antigen is sandwiched between the capture and biotinylated detection antibodies. Finally, streptavidin poly-horseradish peroxidase (spHRP) was added in order to bind the biotinylated detection antibody and the measurment step was performed exploiting the TMB substrate which results in the production of an insoluble electroconductive product that locally precipitates at the reaction site and that can be electrochemically read using cyclic voltammetry. A dual sensor for simultaneous detection of free and total prostate specific antigen was developed on SPE surfaces for detecting human chorionic gonadotropin hormone [70]. The authors described an electrochemical-based immunosensing method, gold-linked immune-assay based on the direct electrochemical activity of Au nanoparticles (NPs) on SPEs. A primary antibody was immobilized on an electrode surface, and a non competitive immunoassay was carried out. Then a secondary antibody conjugated with Au NPs was employed and upon application of approximately 1.2 V, the Au NPs are pre-oxidized from Au0 to Au3+. A differential pulse voltammetry (DPV) from 0 V to 1.0 V immediately after the pre-oxidation step reduces the oxidised NPs back to Au0. The reduction process is detected at approximately 0.48 V.

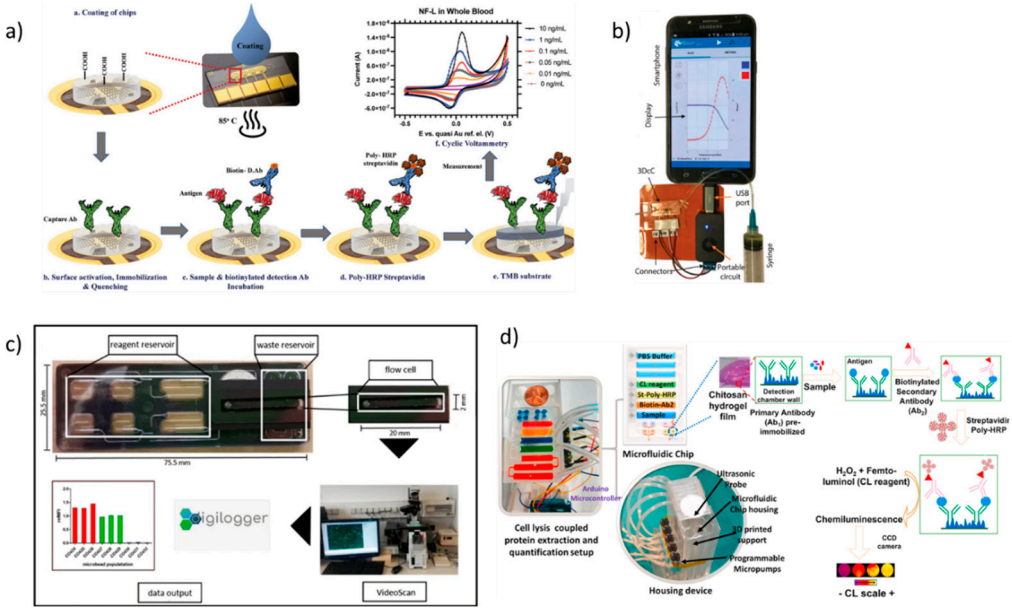

Figure 2. a) Scheme of working principle of the analytical device: a) 3D schematic of sandwich immunoassay on biosensor coated with antifouling nanocomposite. a. Electrochemical sensor with gold electrodes b. Immobilization of capture antibody and saturation with BSA c. Incubation of sample mixed with biotinylated detection antibodies. d. Addition of poly-HRP streptavidin. e) Addition of TMB followed by precipitation over the gold electrode f) Results of the cyclic voltammetry. Reprinted with permission from Ref [69]; b) The electrochemical analyticaldevice interfaced with a portable potentiostat connected to a smartphone via a USB-C connection to measure the signal. Reprinted with permission from Ref [79]; c) Microbeads immobilized in microfluidic chip and reagent reservoirs filled with assay reagents. Pumps inject reagents during the assay. Measurements were performed using VideoScan technology (Bioimage informatics). Reprinted with permission from Ref [83]; d) Device design comprising a microfluidic chip with 5 inlets connected to peristaltic micropumps, sample and rectangular prism reagent chambers with capacity of 80 ± 5 µL, and 8 cylindrical detection chambers with 8 ± 1 µL capacity each.. A microfluidic chip is designed to house sample and reagents and deliver them sequentially to detection compartment. The assay

protocol is based on poly-HRP and ultra-bright femto-luminol to produce CL that is measured using a CCD camera. Reprinted with permission from Ref [87].

Electrochemical LOC devices has been exhaustively proposed also for the detection of viruses [71-76] and recently they were applied for diagnosing SARS-CoV-2 [76]. Seo et al., functionalized graphene with SARS-CoV-2 spike antibody and the detection of the virus was performed both in the transport medium and clinical sample with a LOD of 1 fg/ml [77]. Zhao et al. [78], proposed the use of calixarene functionalized graphene oxide for detecting RNA of SARS-CoV-2 without nucleic acid amplification by exploiting a portable electrochemical smartphone. The LOD of the clinical specimen was 200 copies/mL and only two copies (10 μL) of SARS-CoV-2 were required for starting the assay. Azahar et al. [79] reported about the development of 3D nanoprinted electrodes, coated by nanoflakes of reduced-graphene-oxide (rGO) on which specific viral antigens were immobilized (Figure 2b). The electrodes were then integrated with a microfluidic device and used in a standard electrochemical cell. The antibodies present in the sample selectively binded the antigens, changing the impedance of the electrical circuit which was detected by impedance spectroscopy using a smartphone-based user interface. Furthermore the sensor can be regenerated within a minute by introducing a low-pH solution suitable for eluting the antibodies from the antigens, allowing successive sensing of test samples using the same sensor.

As for the electrochemical detection, also optical detection requires additional components that has to be integrated into the LOC device. Indeed, while electrochemistry needs the presence of electrodes and potentiostat, optical equipment comprises detector suitable for quantifying the colorimetric, CL or FL signals (in the last case also an excitation source is required for exciting the fluorescent specie). In an optics of integration with LOC devices, recently several detectors characterized by an adequate sensitivity combined with portability have been proposed leading to the development of ultrasensitive POCT assays [80,28]. For example, optical biosensors were designed exploiting new generation of (thermally cooled) back illuminated (BI) CCD [32] and smartphone BI-CMOS camera [36] or thin film photosensors, such as single photon avalanche photodiodes [81] or amorphous silicon (a-Si:H) photosensors [82]. Fluorescent detection has been widely proposed for the development of LOC devices. Dinter et al. [83] developed an adaptable platform to detect biomarkers using a microfluidic technology (Figure 2c). They were able to distinguish fluorescently labeled biomarkers exploiting an immunoassay based on physically and spectroscopically different functionalized microbeads. This multiplexing approach allowed to quantify four cardiovascular disease biomarkers using a commercial video imaging detector. Also Chang et al. [84] proposed a FL immunoassay developing a 3D microfluidic chip composed by a nichel mesh that acted as bead trap comprised between two layers sealed together by an adhesive of the PET sheet. Photonic crystal beads (PCBs) immobilized with capture antibodies are introduced into the microfluidic chip by injection and trapped into the grids of the nickel mesh. FL immunoassays were performed into the microfluidic chip and the signals were acquired exploiting a FL microscope equipped with a CCD. The PCBs were encoded by their colors coming from the photonic structure and the analysis of the target analytes (human immunoglobulin G, carcinoembryonic antigen and alpha fetoprotein) were performed by the identifying PCB colors and quantifying the FL intensity. Another work based on FL detection was proposed by Yuan et al. [85] The authors presented a bead-based assay for the detection of one of the four serotypes of dengue virus non-structural protein (DENV-NS1) as well as its cognate human IgG. In this system, the FL microspheres containing the classification fluorophore and detection fluorophore are imaged through a microfluidic chip using an infinity-corrected microscope system. To capture dengue virus non-structural protein (DENV-NS1), the magnetic beads with higher dye concentration were coupled with the anti-DENV-NS1 specific antibody, while the beads with lower dye concentration were loaded with DENV-NS1 to capture anti-DENV-NS1 antibodies. The coupled beads were then introduced into microfluidic channels for analysis using a laser and portable imaging setup These examples show a difficult integration between the LOC device and the detector for the measurement of the analytical signals. Indeed, the detection system is generally bench-top instrumentation applied for acquiring FL signals that occur into a microfluidic chip. The implementation of LOC device is

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better achieved in the field of CL. Dei et al. [86] developed a small-sized instrument for CL detection and signal analysis. They proposed a pump-free microfluidic chip, in which flow is self-initialized by capillary pumping and continued by imbibition of a filter paper. Microfluidic units in teardrop shape ensure that flow passes through the reaction areas at a reduced flux to facilitate the association between antigen and antibody and speeds up after the reaction areas. By spotting different antibodies into the reaction area, four types of biomarkers can be measured simultaneously in one microfluidic chip. The system was validated by testing four biomarkers of colorectal cancer (carcinoembryonic antigen, alpha-fetoprotein, carbohydrate antigen 125 and carbohydrate antigen 19-9) using plasma samples from patients. Sharafeldin et al. [87] used a system of micropumps and an Arduino microcontroller to create an automated 3D-printed device that lysed cells and performed a sandwich immunoassay for four metastasis biomarkers. The chip was composed by a detection chamber in which the inner walls coated with chitosan hydrogel film were functionalized with capture antibodies (Figure 2d). Non-competitive immunoassays where performed and the CL signals were acquired a CCD camera. In addition to FL and CL, also bioluminescence (BL) can be exploited for the development of LOC devices. Mirasoli et al. [88] described an integrated lab-on-chip, in for the amplification of viral DNA composed of a disposable 10-µL polydimethylsiloxane reaction chamber coupled to a glass substrate that hosts a thin-film metallic resistive heater and (a-Si:H) photosensors which act as light detector. A loop-mediated isothermal amplification (LAMP) technique was developed for specifically amplifying parvovirus B19 DNA and it was coupled with Bioluminescent Assay in Real Time (BART) technology to provide real-time detection of target DNA.

As an alternative, the ECL approach was employed by Calabria et al [89] for developing a 3D-printed miniaturized biosensor for glucose detection. The system employed a two-electrode configuration and it relied on the glucose oxidation catalysed by glucose oxidase. The developed hydrogen peroxide was detected by the addition of luminol and the ECL signal was acquired employing a smartphone.

As emerged from the reported works, the development of LOC devices has required in recent years a great effort mainly addressed to the resolution of problems such as the implementation of bioassay on microfluidic platform and the increase in the sensitivity of analytical methods (e.g. use of nanomaterials, innovative tracers, signal amplification). From this point of view, LOC biosensors have significantly improved their potential, reaching performances that are comparable with those obtained using laboratory settings. To date the most critical aspect remains the complete integration of the microfluidic device with the signal detector. Indeed, many works report the development of miniaturized and easy-to-use microfluidic platforms, but the detection step is very often performed with bench top instrumentation. From this point of view, the use of the smartphone (both for electrochemical and optical detection) represents a very important possibility to make these devices more usable even by non-specialized personnel. Instead, limited to the optical detection, the use of low-cost photosensors is also a significant step forward in achieving full integration of the analytical device.

3. Paper-based diagnostic devices

Paper-based devices have represented a real revolution in do-it-yourself diagnostics and most of the biosensors currently on the market for POCT applications are based on this platform. In addition to accessibility, affordability, and ease of disposal compared to traditional materials used in microfluidics, paper is also suitable for being processed in many different ways including printing, coating, cutting and lamination. This peculiarity offers a great versatility and the opportunity to design different devices that potentially can be mass produced with low cost [90, 91]. By changing properties such as hydrophobicity, conductivity, porosity and reactivity (modifying the chemical structure) paper could be adapted to the specific need [92]. Furthermore, the use of paper as sensing element allows to simplify the required instrumentation since capillary force can drive fluid without an external pump.

Indeed, the most successfully commercialized devices with both electrochemical and optical detection are based on the use of paper as the analytical platform. Most glucometers, for example, are

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based on the use of disposable strips on which the reagents necessary for performing the test are immobilized. The detection is carried out by adding a drop of blood on the strip and then inserting it into the portable detector. Since the introduction of the first prototype of glucometer, many electrochemical biosensors were proposed in literature and recently some new nanomaterials were proposed for increasing the analytical performances [93-101].

Similarly, also the most common optical detection tests commercially available for POCT (pregnancy tests and COVID-19 tests) are based on LFIA technique. Traditionally, LFIA are employed for qualitative analyses, which involved the user's conclusion about the presence or absence of a specific target analyte in the sample based on visual evaluation of the colored bands formed upon the strip. Recently this technique was implemented in order to obtain a quantitative output thanks to the combination with different detection principles [102]. Indeed, over the past decade, several affordable and compact detector were designed for integrating the LFIA strip with portable and easy-to-use system [103-105]. Even if the inclusion of instrumental measurement can complicate the analytical procedure, the possibility of obtaining quantitative informations for several biomarkers without the need of an equipped laboratory is attracting great attention [106].

One of the applications on which more publications based on colorimetric LFIA have been reported is about the detection of the Sars-CoV-2. Indeed, from 2020 several analytical devices have been released in commerce for the diagnosis of this disease. Literature focuses on the possibility to improve the detectability of these systems. Lee et al. [107] developed a colorimetric LFIA exploiting a specific linker for immobilizing antibodies on the cellulose membrane in an oriented manner. The proposed system allowed to increase the sensitivity with a detection limit of 5 × 104 copies/mL. Another approach to improve the performances of the method relied the combination of different detection principle. For example a colorimetric and FL dual-functional LFIA biosensor was developed for the sensitive detection of spike 1 (S1) protein of SARS-CoV-2 by Han et al. [108]. The dual approach allowed to combine a rapid visual detection for a screening of suspected SARS-CoV-2 infection on sites while the FL signal allowed the quantitative detection of virus infection at the early stage. The authors coated a single-layer shell formed by mixing 20 nm Au NP and quantum dots (QDs) on SiO2 core to produce a dual immune label that simultaneously produced strong colorimetric and fluorescence signals. The detection limits of detecting S1 protein via colorimetric and fluorescence functions of the biosensor were 1 and 0.033 ng/mL, respectively. Also Roda et al. [109] proposed a dual optical/CL format of LFIA immunosensor for IgA detection in serum and saliva exploiting a recombinant nucleocapsid antigen which specifically captured SARS-CoV-2 antibodies in patient specimens. As detection system, a smartphone-camera-based device measures the colour signal provided by nanogold-labelled anti-human IgA while for the CL measurements a CCD was employed for acquiring the light signal resulting from the reaction of the HRP-labelled anti-human IgA with a H2O2/luminol/enhancers substrate. The use of the smartphone has been widely proposed in the field of optical detection associated with the LFIA technique [110-112]. Li et al [113] designed a QD LFIA for the FL detection of specific IgG for SARS-CoV-2 in human serum or whole blood samples. In this case the smartphone was employed as interface for elaborating the FL signals, indeed the detector was a portable fluorescence strip reader connected via WIFI to a smartphone platform. Mahmoud et al [114] exploited the smartphone directly as a FL detector, by developing a 3D printed smartphone imager with a built in UV-LED light source. Interleukin-6 and thrombin were measured by using FL green and red QDs as labels for the two target analytes, respectively. Through separation of RGB-channels, the acquired images can be processed to simultaneously quantify two analytes on the same test line enabling the optical multiplexingapproach. Also Rong et al. [115] developed a smartphone-based FL-LFIA platform for the detection of Zika virus nonstructural protein 1 (ZIKV NS1) exploiting QD microspheres as FL probes. A device to be integrated with the smartphone comprising optical and electrical components was designed and 3D-printed (Figure 3a). The implementation of CL detection with smartphone camera has been also investigated. Chabi et al [116] reported a LFIA platform employing phage-based CL reporters, combined with smartphone detection and applied to the diagnosis of SARS-CoV-2. Exploiting the same approach Ren et al. [117] trying to increase the sensitivity of the system, proposed a CL-LFIA based on the synthesis of the Au

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NP–antibody–HRP–polyethylene glycol conjugate for detecting cardiac troponin I (Figure 3b). Due to the presence of polyethylene glycol that allowed a controlled orientation of the immunocoplex, the accuracy of the LFIA was improved significantly allowing a detection limit of 10 pg mL^{-1}. In the context of CL detection, Calabria et al. [29] employed an origami paper-based analytical device (μPAD) format for allowing the preloading of all the reagents in the dried form on the paper substrate. The analytical protocols were started by injecting a buffer solution in the μPAD, and the device was designed to be hosted in the AstroBio CubeSat (ABCS) nanosatellite. This biosensor represented the first step to develop an innovative technology to conduct research in space, implementing different kind of bioassays on the same platform to verify their compatibility with the effect of deep space conditions. An example of paper-based device was also proposed combined with TCL detection. Roda et al. [118] reported a TCL vertical flow immunoassay (VFIA) in which a one-step test was performed for detecting valproic acid. The VFIA sensor was composed by several layers layers functionalized with reagents stored in a stable form and by by 3D printing, accessories were produced to turn a smartphone into a biosensing device that provides a power source for the heat shock required to trigger the TCL reaction and a sensitive camera for measuring emitted photons.

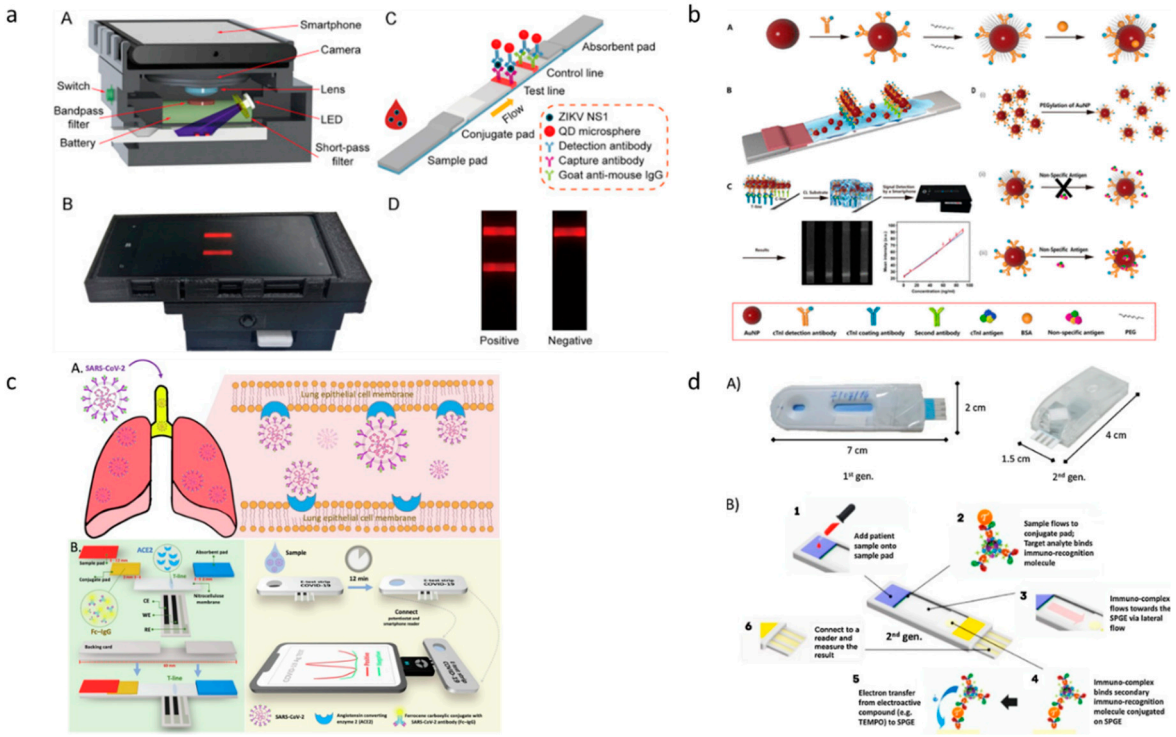

Figure 3. a) Scheme of the design and application of the smartphone-based fluorescent LFIA platform. a. Internal structure of 3D smartphone-based imaging device. b: Photo of the developed fluorescent LFIA reader. c. Schematic of the fluorescent LFIA for the detection of ZIKV NS1. d. Images of the test strips in the presence (left) and absence (right) of ZIKV NS1. Reprinted with permission from Ref [115] b) LFIA based on the PEGylation of AuNPs. a. Preparation of AuNP-Ab-HRP-PEG conjugates based on the PEG-modified AuNPs. b. Scheme of CL-LFIA in which the AuNP-Ab-HRP-PEG conjugates are applied for the detection. c. Light signal detection with a camera of a smartphone d. PEGylation of AuNPs for improving the performance of CL-LFIA biosensor. Reprinted with permission from [117] Copyright {2023} American Chemical Society." c) Schematic illustration of the E-test strip testing: a. SARS-CoV-2 binding with the ACE2 cellular receptor. b. Schematic of the E-test strip in which the SARS-CoV-2 spike antigen is detected using the ACE2 WE test zone on the nitrocellulose membrane. Reprinted with permission from Ref [124] d) a. Electrolateral flow immunosensor (ELLI) b. ELLI's assay based on modified PEG-stabilized and TEMPO-tagged AuNPs for an amperometric point-of-care detection of dengue NS1 protein. Reprinted with permission from Ref [125].

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As it concerns the electrochemical detection applied on paper-based devices, the electrode fabrication is a fundamental step for their development [119,120]. The first example was reported by Henry et al [121] who proposed carbon SPE onto filter paper. From this pioneering work, several devices have been presented, based on different electrode fabrication techniques [122]. Also LFIA has been extensively combined to electrochemical detection by implementing the nitrocellulose membrane with SPE. The main difference with the optical based LFIA is that in the electrochemical approach the membrane presents only the test line equipped with the SPE. The labels selected are generally molecules that undergoes an oxidation–reduction reaction on the electrode surface, or enzyme which can promote the oxidation–reduction reaction of the pre-existing reactant, resulting in an electrical current, voltage and other electrical changes. A portable potentiometer is then employed in order to measure the signal through the electrode and perform quantitative analysis [123]. Deenin et al [124] reported about the development of a biosensor for diagnosing COVID-19 based on an test strip integrated with a SPE (Figure 3c). The ferrocene carboxylic acid was used as tracer for SARS-CoV-2 antibody that binds the SARS-CoV-2 antigen in the sample before flowing on the strip. On the test line in correspondence of the electrode, angiotensin-converting enzyme 2 (ACE2) receptor was immobilized for capturing the immunocomplex formed in the presence of the target analyte. The electrochemical signal was measured with a smartphone interface achieving a detection limit of 2.98 pg/mL. Sinawang et al. [125] presented a work for the quantification of Dengue NS1 protein in human serum based on an electroactive immunonanoparticles that bind to the target biomarker and subsequently move along toward the biofunctionalized gold SPE to generate an amperometric signal measured by a potentiostat (Figure 3d). The gold SPE worked simultaneously as a signal transducer and a solid-state support for a sandwich ELISA-like immunoassay. Srisomwat et al [126] proposed an automated automated electrochemical LFIA device for the quantitative detection of the hepatitis B virus (HBV). The novelty is related to the use of a time-delayed microfluidic strategy fabricated on paper, which allowed a sequenced solution transfer. The device consisted of a straight nondelayed channel, a zigzag delayed channel, and a detection zone. In this configuration, the high-performance pyrrolidinyl peptide nucleic acid (acpcPNA) probe was immobilized on the test line of the test strip equipped with an electrode, while the Au3+ for gold metallization was deposited at the delayed channel zone. When the sample containing the HBV DNA was injected, the solution flowed across the nondelayed channel and hybridize with the acpcPNA probe at the test line. Meanwhile, the solution flowing through the delayed channel was retarded by the wax-printed barriers and gradually merged with the preceding flow from the nondelayed channel. Taking advantage of this differential flow behavior, DNA hybridization occured first, followed by Au3+ binding to the captured DNA through electrostatic interactions. The two steps of the assay proceed automatically and sequentially following the sample application and the buffer flow without the requirement of additional steps.

Also LFIA was combined with ECL detection. Tris(2,2′-bipyridyl)ruthenium (II) [Ru(bpy)32+], a photoactive species with optical properties, is frequently used in ECL-based sensing platforms due to its good solubility in water, high electrochemical stability, and capability for repeated regeneration in ECL reactions with tripropylamine (TPA). Hong et al. [127] proposed an ECL-LFIA for detecting troponin using Ru(bpy)32+-loaded mesoporous silica nanoparticles (RMSNs). Antibody-immobilized RMSNs were used for developing a non competitive LFIA and the detection was performed using a CCD camera.

The widespread diffusion of paper-based devices in commerce and in our everyday life demonstrates how this approach is functional and effective when combined with both electrochemical and optical detection. Certainly, in the future, qualitative detection technologies based on colorimetric detection could be replaced by more advanced quantitative systems which could give additional information and could therefore be applied to different types of target analytes including specific biomarkers for diagnosis or monitoring of several diseases. This will lead in an increasing interest in the research filed in improving the implementation of detectors on entirely portable platforms and increasing the sensitivity of the analytical methods involved.

4. Wearable technologies

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Wearable and implantable technologies have gained great interest since the introduction of smartphones and mobile devices, thanks to the possibility of providing a monitoring of performance and state of health [128-132] and enabling measurement of clinically-relevant parameters in real time [133, 134]. The first efforts in this field were mainly addressed on physical sensors for measuring parameters such as mobility, steps, calories burned or heart rate. With the implementation of new technologies, the demand for wearable devices changed requiring instrumentation suitable for performing non-invasive analysis on several biomarkers (such as metabolites, hormones, bacteria) in biofluids, like tears, saliva, urine, sweat and interstitial fluids. [135] Following this trend, researchers have proposed several examples of wearable biosensors mainly based on electrochemical and optical detection combined with the most innovative nanomaterials technologies and the use of flexible and advanced materials.

Electrochemical biosensors were the earliest platform that was exploited in the wearable field as they can benefit from easy readout and data processing [136, 137]. Lei et al proposed a stretchable, wearable, and modular multifunctional biosensor, implementing enzymatic assays on a MXene/Prussian blue (Ti3C2Tx/PB) electrodes for the detection of glucose, lactate and pH levels in sweat [138]. The patch-type wearable device consisted of three modules (a sweat sampling layer, a sensor layer and a cover layer) and it could be connected to an electrochemical analyzer in order to measure a current signal. Also Wang et al [139] measured glucose level in sweat using a gold electrode deposited on the surface of PET through a procedure that avoid expensive and complex fabrication processes. Zhao et al [140] reported a fully integrated self-powered smartwatch for noninvasive and continuous monitoring of glucose levels. The system integrated several components: amorphous silicon (a-Si) photovoltaic cells that can be stored in rechargeable flexible Zn-MnO2 batteries providing sufficient power for the operation of the whole system, an electrochemical sensor for measuring glucose in sweat, a controlling module consisted in a printed circuit board (PCB) and a display to allow a direct and real-time monitoring. Lin et al [141] developed a flexible electrode system based on graphene oxide nanosheets for the impedance measurement of lactate in sweat exploiting an enzymatic assay involving lactate oxidase. Exploiting the same enzymatic approach for the detection of lactate, also Wang et al [142] proposed a stretchable gold-fiber-based electrodes which were included in textiles in a planar configuration. A different approach was used by Zhang et al [143] who exploited molecular imprinted polymers (MIP) as recognition probe. They generated a film of MIP on Ag nanowires which coated carbon working electrode. MIP allowed to obtain a selective and sensitive detection of lactate by measuring differential pulse voltammetry response. Fiore et al [144] reported about an immunosensor paper-based microfludic device for monitoring cortisol in sweat (Figure 4a). Using wax printing technology and laser-cutter technique they designed a microfluidic pattern that allowed to perform a competitve immunoassay in which antibodies for the recognition of cortisol were immobilized on magnetic beads and captured on a reaction zone. The competition between cortisol in the sample and cortisol conjugated to tracer (acetylcholinesterase enzyme) allowed the formation of the analytical signal by folding the pad pre-loaded with the proper enzymatic substrate. Combining this system with Near-Field Communication wireless module it was possible to monitor the signal using a smartphone. The same research group also developed a flexible device for monitoring the pH in sweat by depositing an iridium oxide film onto graphite working electrode and combinig it with an integrated circuit board allows for data acquisition [145].

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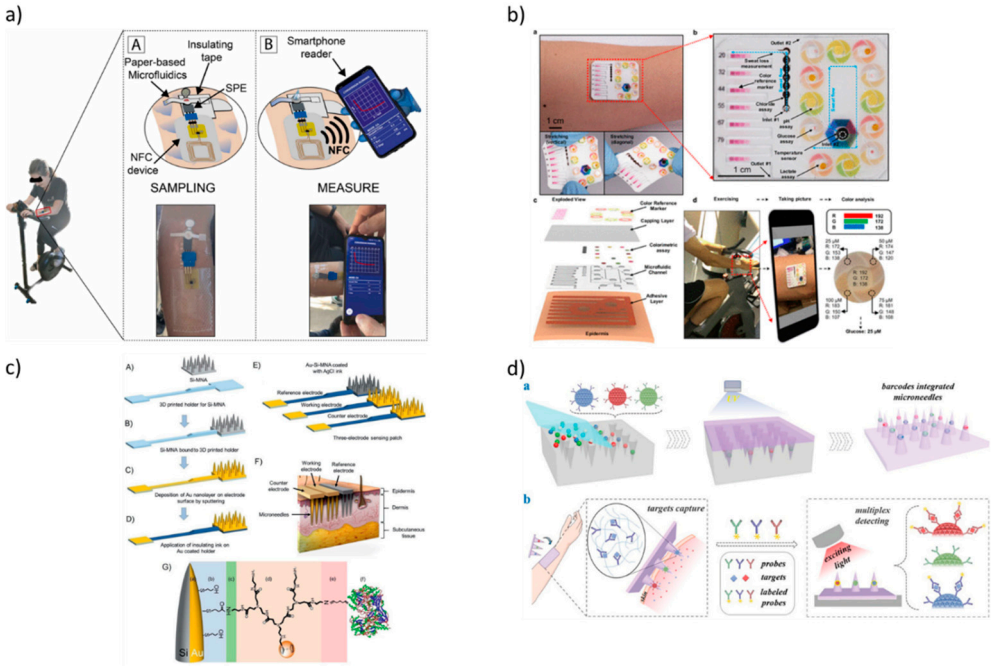

Figure 4. a) Illustration of cortisol during physical activity. a. sampling process and b. measuring signal and transmitting data to a smartphone via NFC. Reprinted with Permission from Ref [144]; b) Scheme of the microfluidic device for colorimetric analysis of sweat a. Optical images microfluidic devices for colorimetric analysis of sweat on the skin (top) and under mechanical deformation with bending (bottom left) and twisting (bottom right). b. Top view of microfluidic channels filled with blue-dyed water. c. Exploded scheme of a device and its interface with the skin. d. Procedure for collecting sweat samples and color analysis of digital images of the device. Reprinted with permission from Ref [150] Copyright 2019 American Chemical Society; c) Schematic illustration of the high-density silicon MNA electrode preparation. a. Si-MNA and 3D printed holder, b. Si-MNA substrate attached to the 3D printed holder, c. sputter deposition of a thin film of Au, d. insulating ink application, and e. three-electrode sensing patch with Au-Si-MNA as working and counter electrodes, and reference electrode with Au-Si-MNA coated with AgCl ink. f. Schematic of the three-electrode MNA patch penetrating the skin and interfacing the epidermis and superficial dermis. g. Schematic illustration of the modification of the working electrode. Reproduced with permission from Ref [187]; d) The generation and application of the encoded MNs. a) Schematic illustration of the fabrication of the encoded MNs by micromolding. b) Schematic illustration of the application of the MNs in ISF detection. Reproduced with permission from Ref [204].

As well as electrochemical detection, also optical detection has been proposed for its application on wearable biosensors [146-151] (Figure 4b). In this case some biomarkers have intrinsic optical properties like absorption and emission spectra that allows to selectively detect these species [152]. Among the applications of colorimetric approach, several works reported the use of acid-base indicators to measure the pH allowing a rapid and reversible detection [153-157]. Generally, these methods are based on naked-eye evaluation of the color formation which allows to work with a very simple equipment. If a quantitative analysis is required, the use of smartphone camera as detector is the most employed solution. A multiplex colorimetric assay for detecting creatinine, pH and urea was developed by Rogers et al [158]. The sensor was composed by different areas on which specific reagents for the detection of different target analytes have been immobilized. The colorimetric readout was performed using a smartphone that allowed the quantification exploiting the RGB analysis system. Optical detection based on FL principle has been proposed since the sensitivity offered by FL is higher respect to colorimetric methods. However, FL implies a more complex equipment and therefore analytical procedure. Xu et al. [159] proposed a wearable Cl− monitoring platform which combined a flexible cotton piece and two fluorescent materials (lanthanide metal–

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organic frameworks (MOFs) acting as test and control signals respectively) by simple ultrasonic loading. The two lanthanide-based fluorescent materials provide high color purity and accurate measurements. By designing Cl− and fluorescence signals for a logic circuit, a codec device was produced, exploiting a smartphone as detection platform.

The reported examples focus on the non-invasive analysis of sweat or saliva but these kind of samples can be not sufficient for determining some biomarkers. For this reason the minimally invasive sampling of intestitial fluid has been proposed since in comparison with the other peripheral biofluids, it contains several physiological information and exhibits a close correlation with blood samples due to the transcapillary exchange between blood and cells [160,161]. Recently, microneedles have gained great success due to the micron size and minimal invasion. [162, 163] and they have been widely exploited for sampling interstitial fluid for the detection of several target analytes [164-169] exploiting different principle of extraction (negative pressure, capillary action, swelling force, ionophoretic). One of the most important applications for mincroneedle devices is the integration in biosensors for the monitoring of glucose. Generally, the electrochemical glucose sensors are based on a reaction that occurs at the working electrode involving glucose [170, 171] and it can be classified as enzymatic [172] or non-enzymatic [173, 174]. In literature several devices have been proposed based on enzymatic reaction between glucose and glucose oxidase and exploiting oxygen as redox mediator (first generation of glucose monitoring devices) [175]. In these applications, selectively permeable membranes were used to confined glucose oxidase on the surface of electrodes and to prevent electroactive interference [176-182]. The second generation of glucose sensors employed artificial mediators to avoid the oxygen dependence, and is also widely integrated on microneedles based device for glucose monitoring [183-191] (Figure 4c). The possibility to exploit non enzymatic reaction allows to overcome limitations due to the use of enzyme and to obtain advantages such as good stability, reproducibility, and free from oxygen limitation. In this case, generally the glucose sensors are based on the direct electrochemical oxidation of nanomaterials, such as metals (Pt, Au, Ni, Cu, etc.), metal-oxides (NiO, CuO, etc.), metal sulfides, metal-organic framework, metal azolate frame work and carbon materials [192, 193]. Several works has been published about the development of microneedle-based monitoring of glucose exploiting these mechanisms [194-196].

The technology of minimally invasive sampling using microneedles for glucose monitoring in interstitial fluid has been extensively proposed also in combination with optical detection. The colorimetric approach is the one that gained much more attention since colorimetric signals can be directly evaluated by naked eyes, or measured by a smartphone camera, and finally elaborated through an RGB processing for obtaining a quantitative analysis. In this context, the most exploited mechanism is that involving the use of glucose oxidase to generate hydrogen peroxide which can be used for a subsequent reaction with a chromogenic agent. He et al [197] proposed a Polyvinyl alcohol/ Chitosan hydrogel integrated with microneedles suitable for the detection of electrolyte ions, glucose, lactate, and proteins even if the analysis procedure needs a substantial simplification in order to be compatible with the POCT approach. Paper-based platform have been applied also in combination with these technologies and several biosensors were reported in literature [198-201]. Besides the production of color on paper located upon microneedles, different colors can be directly produced on the microneedles patch or even on the skin surface. As an example, Gu et al. [202] developed a transdermal colorimetric microneedles patch consisted of two layers: glucose oxide embedded bottom needle layer, and calcium phosphate encapsulated HRP and TMB in the upper layer. The back of the patch turned to blue as a consequence of the reaction in presence of glucose and the quantification can be performed by scanning the color intensity with a miniaturized scanner. Yang et al. [203] exploited a microneedles patch made of glucose oxide-conjugated MnO_2/graphene oxide nanozymes and Gelatin methacryloyl. When the interstitial fluid diffused into the patch the presence of glucose induced the production of H_2O_2, which bind to MnO_2 and promoted the oxidation of TMB on the patch, causing a color change. However, many of the colorimetric system relies on the use of enzyme and chromogenic substrates, which can cause problems including enzyme denaturation, color quenching, byproduct toxicity, and non-reusability. As an alternative always exploiting optical detection, FL has been explored for these applications. Zhao et al [204] proposed an integrated

microneedles device with photonic crystal barcodes to enable the multiplex detection of tumor necrosis factor-alpha (TNF-), interleukin-1β (IL-1), and IL-6 . A FL conjugated antibody immobilized onto microneedles was used to detect these biomarkers and the quantification was performed by reading the FL signal of the barcodes (Figure 4d). Also Wang et al. [205], coated the surface of microneedles with antibodies linked to FL plasmonic fluorophore for the detection of biomarker. Zheng et al [206] reported about a reagentless biosensor based on FL labeled aptamer probe for the on-needle detection of target analytes (glucose, adenosine triphosphate, l-tyrosinamide, and thrombin). By employing a strand displacement, the aptamer-conjugated to fluorophore were bound to DNA competitor strand marked with a quencher specie. Sang et al [207] developed a biosensors based on microneedles array that enabled the emission of a FL signal using a glucose responsive FL monomer. The monomer has two moieties, one for the recognition of glucose and the other one conjugated to anthracene for the FL emission.

Even if the efforts in this field are increasing, the FL signal is affected by light intensity, probe stability and variation due to human tissue. Furthermore, complex procedures and the need of optical instruments limit their diffusion for routinary applications.

Up to now, the wearable biosensors based on the use of microneedles available on the market are those for the monitoring of glucose and they are all based on electrochemical detection. Several companies such as Medtronic (Dublin, Ireland), Dexcom (San Diego California, US) and Abbott (Chicago, Illinois, US) have proposed their devices [208] and they are gaining a great success thanks to the easy-of-use, the relatively low-cost and advantages in terms of real-time monitoring of the health status. With the improvements in technologies for the optical detection as it concerns the integration with the biosensing element, portability and reproducibility of the results, in the future also this detection principle will be more explored and exploited for the development of biosensors suitable for the market.

5. Conclusions

In this review are described the advances in the development of POC devices for clinical-medical applications based on optical and electrochemical detection. The most significant efforts have been made in the study of new nanomaterials to employed like platforms for bioanalytical assays, innovative strategies for the amplification of analytical signals, the integration within self-standing devices capable of performing the analysis outside centralized laboratories. In many cases, results have been reported that are comparable with those obtained with bench top instruments that make use of specialized personnel. Despite these enormous steps forward, the most common biosensors in everyday life continue to be the glucometer with predominantly electrochemical detection and the pregnancy or COVID-19 tests mostly based on optical detection. Given the high interest raised from this research field, we will certainly observe an increase in their diffusion and use in the coming years. One of the most significant advances in this sense is represented by wearable devices which have already attracted attention from researchers and which will probably find wide use also for routine applications.

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