Precision medicine, bioanalytics and nanomaterials: Toward a new generation of personalized portable diagnostics

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Abstract

The customization of disease treatment focused on genetic, environmental and lifestyle factors of the individual patients, including tailored medical decisions and treatments, is identified as precision medicine. This approach involves the combination of various aspects such as the collection and processing of a large number of data, the selection of optimized and personalized drug dosage for each patient and the development of selective and reliable analytical tools for the monitoring of clinicals, genetics and environmental parameters..

In this context, miniaturized, compact and ultrasensitive bioanalytical devices play a crucial role for achieving the personalized medicine expected goals. In this review, the latest analytical technologies suitable for providing portable and easy-to-use diagnostic tools in clinical setting will be discussed, highlighting new opportunities arising from nanotechnologies offering peculiar perspectives and opportunities for precision medicine.

Keywords: Precision medicine, Point-of-care, Biosensors, Nanomaterial, Wearable, Smartphone, Paper-based biosensor

1. Introduction

Precision medicine aims to make available to everyone the possibility to access to tailored medical care, minimizing individual variability caused by various factors such as genetic makeup, environment and lifestyle.[1] Indeed, it has already been demonstrated that each single person has the predisposition to develop particular diseases, with a consequent different response to pharmacological treatments and different side effects.[2,3] For this purpose, interdisciplinary approaches are necessary in order to achieve significant progress

in the field of precision medicine. The disciplines involved include, among others, advanced diagnostic techniques, new instrumentation (e.g., new high performance techniques, instrument components and materials), (nano)sensors, biosensors, smart materials, new drugs and delivery systems, as well as big data management procedures.

Initially most of the efforts were mainly focused on the collection of a large data set regarding the clinical story of a given patients including a full record of any kind of biochemical and instrumental examination in their life also connected with its parents. This will allow to gather useful information and correlations for a given disease. Today the challenge consists in the development of new technologies which may allow to take advantage of the new concept of precision medicine in everyday life.[4] The success of this approach is related to the possibility of obtaining data f from the single patient in real time with a power full diagnostic significance for an early detection of a give disease. .

In this context, the availability of commercially diagnostic devices hold a crucial role in order to obtain the patient physiopathology and pharmacological/metabolic data, which could be also useful for monitoring specific clinical biomarkers. Point-of-care approach (POC) perfectly fits the urgency for novel diagnostic and analytical technologies, that can be performed by untrained personnel anywhere.

The demand of onsite new generation prompt diagnostics has become high since current diagnostic procedures are typically expensive, time consuming and performed in different health facilities, obliging the patients to wait a relative long time from the test to the results and limiting early pathology detection and prompt pharmacological treatment and follow up.[5,6]

To date, the most successful devices for commercial POC applications are those developed for the detection of glucose, pregnancy, hormones for fertility monitoring, viruses (i.e. HIV,

OraQuick In-Home HIV Test), malaria, typhoid analyzers[7,8], but recently many healthcare and pharmaceutical companies have targeted to produce diagnostic devices following the market need. Indeed, the demand for these products is strongly increasing and many prototypes have been developed for the detection of biomarkers using different analytical platforms as a part of the so called “theragnostic approach”.,For these reasons, a lot of interest is being raised in using the smartphone as a integrated platform with different kind of biosensors. Indeed, the widespread distribution of the smartphones is changing the concept of mobile health and promising to reshape the biosensor market. Smartphone-based biosensors have a great potential in the POC field and they have been explored using the smartphone as a detector or as an instrumental interface.[9] Moreover, the connectivity and data processing offered by smartphones can be exploited to perform analysis directly at home with simple procedures. The system could eventually be used to monitor patient health and directly notify the physician of the analysis results.

Another great opportunity in the field of biosensors for personalized medicine is the development of wearable (bio)analytical devices. This approach is the most suitable for non-invasive and continuous monitoring of physiological parameters and biomarkers collected non invasively in biofluids including saliva and sweats.[10]

Furthermore, the possibility of carrying out multiple tests of a give biomarkers panel, thus simultaneously obtaining the measurement of several analytes of interest, allows to realize highly predictive diagnostic tools for specific pathologies. Indeed, clinical diagnosis is based on set of information that, linked together, offers a precise clinical framework. For this reason, several works have proposed a multiplexing approach in which a single analytical device is implemented for the detection of panel of biomarkers.[11-12]

This review aims to critically make the state of the art of the most innovative and promising technologies and approaches reported in literature for application in the precision medicine field.

2. Nanomaterials for biosensors development

The development on new materials and nanomaterials is fundamental for the development of innovative devices for biomedical and diagnostic applications.[13-25] Indeed, nanomaterials are used to improve the performance of analytical-diagnostic techniques and to achieve more efficient therapy (e.g., nanoparticles for targeted drug delivery, biocompatible and implantable materials). In the last years, it has been observed a continuous evolution from passive nanostructures (e.g., coatings, nanoparticles, nanostructured metals, polymers, ceramics), to active nanostructures (e.g., 3D transistors, amplifiers, targeted drugs, actuators, adaptive structures), and molecular nanosystems (e.g., molecular devices 'by design, atomic design) with medical applications including tissue engineering and smart prosthetic devices.

Concerning analytical methods, nanomaterials allow a closer interaction with the target molecules and increase the surface/volume ratio, making it possible to improve significantly their performances.

Nanomaterials are exploited in portable biosensors both as a support material to load signal markers, and as tracers that allow an ultra-sensitive detection of the analyte of interest.[26] The success of nanomaterials is due to their exceptional optical and electrical properties owing to electron and phonon confinement, high surface-to volume ratio, modified surface work function, high surface reaction activity, high catalytic efficiency and strong adsorption ability. In particular, metal nanoparticles have been largely exploited since their small size

combined with high surface area offer unique chemical, physical and electronic properties that are ideal for the development of POC assays.[27,28,19]

2.1 Nanoparticles

Gold nanoparticles (AuNPs) have been widely used as label and recently different shapes have been proposed as an alternative to traditional nanospheres. Indeed, there is a plethora of AuNPs that can be distinguished for the dimension: one-dimensional (nanorods, nanowires, nanotubes, nanobelts), two-dimensional (gold nanoplates such as stars, pentagons, squares/rectangles, dimpled nanoplates, hexagons, truncated triangles) and three dimensional (gold nanotadpoles, gold nanodumbbells (AuNDs), branched AuNPs such as nanopods, nanostars and gold nanodendrites).[29] According to the sensing strategy, the AuNPs sensors can be colorimetric, fluorescence-based, electrical and electrochemical, surface plasmon resonance, surface enhanced Raman scattering (SERS)-based, quartz crystal microbalance-based and Bio-barcode assay sensors.

Song et al.,[30] recently demonstrated a natural cotton thread immunoassay device for a rapid, sensitive and quantitative detection of human ferritin using gold nanorod as reporter probe for labelling the detection antibody. The immunoassay format of the device was more like a lateral flow strip biosensor with the sample solution and running buffer added drop wise directly on the sample pad to rehydrate the conjugates. The detection can be performed both by visualizing the presence of a coloured band on the test zone or by an electrochemical measure for obtaining a quantitative result. Another possibility is to exploit gold nanoparticles to quench a fluorophore, as proposed by Fakih et al.[31] (Fig. 1A). They reported a multiplex platform for the detection of viral DNA in which the probe is based on a

fluorescently labelled DNA hairpin (molecular beacon) complementary to a specific target and immobilized on gold nanoparticles decorating micro-sized polystyrene beads. In the absence of the target, the folded oligonucleotide will bring the fluorophore in close proximity to the gold nanoparticle surface and the dye is completely quenched. Upon hybridization to a complementary target, the hairpin will open up and the fluorescent signal is restored. To achieve multiplexing capabilities, the polystyrene beads, are decorated with multiple probes and single file through the laser path and trigger the detection of the target of interest. The sensing platform could be easily modified to detect any sequence of interest.

A recent approach for the detection of nucleic acid and protein targets without PCR is represented by the bio-barcode assay.[32-35] This method is based on the use of a magnetic nanoparticle with recognition elements for the target of interest and a AuNP with a second recognition agent (which can form a sandwich around the target in conjunction with the magnetic particle) and hundreds of thiolated single-strand oligonucleotide barcodes. After reaction with the analyte, a magnetic field is used to localize and collect the sandwich structures. In addition to the AuNP, also silver and magnetic nanoparticles have been exhaustively used as label for sensitive analysis.[36-41]

Furthermore metal NPs can be used as electrode modifiers providing a facilitate electron transfer processes at the sensing surface and high sensitivity.[42] These advantages are due to the fact that the immobilization of the capture probe occurs in a suitable orientation on the electrode allowing an efficient biorecognition. [43,44] As a consequence, several works reported the use of these sensing elements for the electrochemical detection of a wide range of target analytes, including nucleic acid,[45] proteins,[46] cells,[47] amino acids,[48] and pathogens.[49]

2.2 Carbon based nanomaterials

Carbon nanomaterials (CNMs), including fullerenes, carbon nanotubes and graphene, are considered to be ideal candidates to interact with biological systems because of their excellent physical and chemical properties including high carrier mobility, high surface-to-volume ratio, and robustness.[50,51] CNMs have shown superior potential for the development and improvement of biosensors, for example, a multitude of CNMs-based biosensors have been explored and applied in ultrasensitive biosensing.[13] Among the CNMs, carbon nanotubes (CNT) have been widely used as electrode materials in electrochemical biosensing[52,53] and they have been applied to the recognition of many cancer biomarkers, such as prostate-specific membrane antigen,[54] carcinoembryonic antigen (CEA)[55] and carbohydrate antigen 19-9 for pancreatic cancer.[56]

D. Cai et al., employed an array of CNT tips imprinted with a non-conducting polymer coating for protein recognition (human ferritin and human papillomavirus derived E7 protein) with subpicogram per liter sensitivity using electrochemical impedance spectroscopy and offering an alternative to biosensors based on biomolecule recognition.[57] The same research group investigated the mechanism underlying the ultrasensitive performance of this kind of systems using both a theorical and experimental approach. In particular, it was found the possible formation of interface, and its involvement in the high affinity of the imprinting. By artificially enriching the functional compounds with high binding energy to optimize interface composition in the electrosynthetic protein imprinting, the protein sensing performance is correspondingly increased in the electrochemical nanosensor (Fig. 1B).[58,59]

CNTs based sensors were tested also for in vivo applications, as reported by Yang et al.[60] It has been found that CNTs-coated niobium (CNTs-Nb) microelectrode exhibited higher sensitivity than the CNTs grown on other metal wires and it was employed to detect

stimulated dopamine release in anesthetized rats allowing rapid measurements in vivo. The CNTs-Nb sensor was stable for more than 4.0 h of non-stop measurement. Zhang et al. have designed an electrochemical ascorbic acid sensor for accurately measuring ascorbic acid levels in live brain using aligned carbon nanotube fiber (CNF) as a microsensor.[61] This sensor provided a simple methodology for the integration of high-performance biosensors with other neurotransmitters, which might inspire new sensing techniques in brain medical research.

Recently multi-walled carbon nanotubes (MWCNTs) have attracted a lot of interest since they allow an improvement in biosensors performances by increasing the conductivity and active surface area of the sensing platform. As example, Yu et al.[62] reported an electrochemical biosensor for the ratiometric determination of the human cellular prion protein (PrPC) using a glassy carbon electrode (GCE) modified with MWCNTs/ β-cyclodextrin nanocomposite obtaining a sensitive and selective detection of PrPC (LOD of 160 fM). In another research, a label-free miR-141 sensor based on a network of CNT and electro-active polymer for prostate cancer diagnosis was described. This one is a probe of miR-141 that bound to Poly (JUG-co-JUGA)/MWCNT-modified electrodes. Due to quinone group embedded in polymer backbone, polymer film presented very well electro-active property in neutral aqueous medium. Addition of target miR-141 increased current because of enhancement of the polymer electro-activity, but non-complementary miRNAs could not cause any significant current changes.[63] Exploiting the possibility to covalently attach the nucleic acid probes to CNTs, nucleic acids[64] and proteins[65] have been detected. Another biosensor based on MWCNT-lateral flow technology has been proposed for rapid sensing of specific DNA sequences for future on-site and point-of-care diagnostics of genetic and infectious diseases. In particular, Qiu et al.[66] exploited a capture DNA probes functionalized

to oxidized MWCNTs in which the target DNA sequence was hybridized with the capture probe forming a sandwich structure (Fig. 1C). A detection limit of 40 pM for the target DNA was obtained which benefited from the efficient lateral flow chromatographic separation and the large surface interaction area of the MWCNT.

Another platform for sensitive clinical detection and POC diagnostics is represented by graphene and its derivatives. Graphene possesses significant advantages such as rich anchoring sites, extraordinary surface area, brilliant biocompatibility, and low-priced production cost in comparison to other kinds of carbon nanomaterials, including CNTs, fullerene, carbon dots, and nano-diamond.[67] The functionalized graphene using various materials, including organic and biomolecules, metal and metal oxide nanoparticles, polymers and enzymes are often employed for achieving improved sensing and biosensing performance towards biomedical applications.[68,69]

Graphene found applications for a wide variety of analytes, such as cancer biomarkers as reported by Wu et al.[70] who reported the combination of graphene-based amplification strategies with microfluidic paper-based electrochemical immune-device for a multiplex analysis (Fig. 1D). Another example is represented by a functionalized graphene gated biochip suitable for measuring cardiac Troponin.[71] This label free biosensor allowed to measure up to 1 pg mL^{-1} and it could be employed for applications in routine monitoring of this biomarker in blood samples.

Three dimensional (3-D) porous graphene (3D GN) is considered as new support for immobilization to improve the enzymelike activities towards the sensing of various biomolecules. The structural effects of metal oxide nanomaterials such as NiO, Co_3O_4, Fe_3O_4, etc. and peroxidase-like activity were examined. Wang et al. recently fabricated 3D

GN decorated with Fe_3O_4 nanoparticles for the detection of glucose with a low sensing limit of 0.8 μM.[72]

The functionalization of graphene sheets can be easily attained using numerous approaches such as mechanical mixing, hybridization, co-deposition, covalent or non-covalent interaction, etc. The incorporation of graphene sheets into screen printed electrodes (SPE) presents another approach for developing POC tests, as reported by Texeira et al.[73] They developed an electrochemical immunoassay based on a graphene-SPE for the detection of hCG, obtaining a detection limit of 0.286 pg mL^{-1} even in urine matrix.

The graphene oxide based nanomaterials have been employed extensively in electrochemical sensors and biosensors for biomedical, health care and clinical applications (e.g. cancer detection[74-76] biomarkers for monitoring the damage of DNA by oxidative stress[77]) and novel strategies for the fabrication of graphene nanocomposite based electrode materials are currently under investigation.

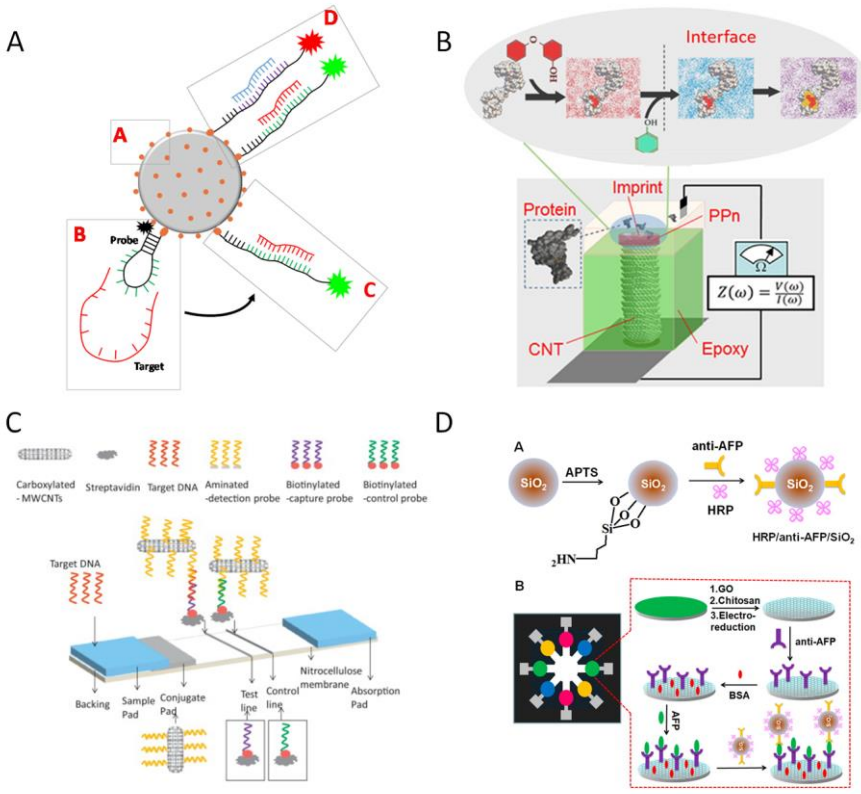

Fig. 1. Nanomaterials-based device. (A) Schematic representation of the operational scheme of the sensing platform divided into four major phases: Synthesis of monodispersed polystyrene beads coated with gold nanoparticles (a); Assembly of fluorescently labeled DNA hairpin onto the gold nanoparticles (b); detection of specific oligonucleotide targets (c); Multiplex detection of two or more targets is possible by modifying the DNA hairpin with spectrally resolved fluorescent dyes (d). Reproduced from ref. 31 with permission from Elsevier, copyright 2017. (B) Involvement of interface formation in the high affinity of the imprinting. Reproduced from ref. 59 with permission from American Chemical Society, copyright 2019. (C) Schematic illustration of the principle of DNA measurement on MWCNT-based lateral flow biosensor. Reproduced from ref. 66 with permission from Elsevier, copyright 2015. (D) Preparation of Nanobioprobes through the Coimmobilization of HRP and Antibody onto Monodispersed SiO2 Nanoparticles and schematic

Representation of the Fabrication and Assay Procedure Used to Prepare the Microfluidic Paper-Based Electrochemical Immunodevice. Reproduced from ref. 70 with permission from American Chemical Society, copyright 2013.

3. Paper-based diagnostics

The development of a low-cost and robust diagnostic test for monitoring and controlling disease evolution in response to drug treatments represents one of the major challenges in the medical field.

POC devices or biosensors based on paper represent an interesting approach for rapid and routine diagnosis, improving patient care and providing low-cost healthcare solutions. Paper is composed by an easy-accessible and soft matrix able to self-pumping fluids when is in direct contact with them. However, thanks to intrinsic properties, such as porosity and hydrophilicity, combined to the presence of numerous capillaries, paper represent a potential substrate for low cost microfluidics offering an effective alternative for the generation of personalized biosensing. A new era for the development of easy and robust paper-based platform for various analytical and bio-analytical techniques was opened by Martin and Synge[78] in 1952 when the paper was used for the first time as diagnostic platform. Depending on the complexity of the paper-based microfluidic system, several analytical format have been developed and categorized; such us Disptick, Lateral Flow Assay (LFA) and µ-PADs genres.

Dipsticks devices represent one of the simple and well-established formats for POC in which the device contain the pre-deposited reagents. Unfortunately the colour change of the strip after the introduction of the sample provides only qualitative results with limited detectability.

Since The first dipstick paper for urine analysis developed by Jules Maunmenè[79] in 1850 many other have been developed for pH, glutathione, uric acid (UA) measurements and pathogen detection.[80,81]

More recently Kumar et al.[82] develop a semi-quantitative test exploiting the reaction of H_2O_2 with 3,5,3',5'-tetramethyl benzidine (TMB) in the presence of positively charged AuNPs. In the assay the bluish-green colour due to the reaction of H_2O_2 with TMB disappeared in the presence of UA (Fig.2B).

An evolution of the dipstick assay is represented by paper based Lateral Flow Assay (LFA) introduced in the 1970s. Routinely used by clinicians for point of care testing and by the general public at home, they are considered and remain a cornerstone of modern medicine able to detect pathogens and biomarkers. Colorimetric or luminescent are the most used readout allowing qualitative and/or semi-quantitative analysis with high detectability.[83,84]

LFAs provide an analysis platform for various types of clinical sample including serum, whole blood, saliva, urine and nucleic acids.[85-89] This device offers a sample and controlled fluid flow allowing its use for versatile and complex multistep assays. Generally, LFA consist in four components: a cellulose sample pad used for inlet and filtering the sample, a conjugate pad composed by glass fibres used for the deposition and the dry storage of reactive agents allows the interaction between the sample and the reagents, a nitrocellulose membrane used for analytes detection where the captures reagent leads to a development of signals, and finally an adsorption pad composed by cellulose fibres used as liquid actuation.

Pregnancy test strip is the most well-know LFA commercialized and is composed by AuNPs and micro-latex beads functionalized with specific antibodies to hormone β-hCG released by pregnant woman. A pink aggregate appears at the test line when these particles are in contact with urine samples containing β-hCG.[90] Typically, in the LFAS the biorecognition

element is represented by an antibody or nucleic acid. Damborsky et al. [91], proposed an innovative concept of LFA where special kind of glycan-binding protein called lectins are used as biorecognition element for the determination of the glycosylation of free serum proteins. In particular this system was used for the detection of the prostate specific antigen (PSA) a biomarker of prostate cancer (PCa). The lectin-based LFA test was performed within 10 min and evaluated by eye as variation of the signal faint pink colour to a clear and intense dark ruby red (Fig.2A).

The Lateral Flow Immunoassay (LFIA) approach coupled with chemiluminescence (CL) detection has been exploited to detect salivary levels of cortisol in astronauts, as marker of chronic stress. The CL-LFIA biosensor was used directly by astronaut Paolo Nespoli and is able to detect salivary cortisol down to 0.4 ng/mL directly on board the International Space Station, confirming the feasibility of performing sensitive immunological clinical chemistry analyses in microgravity conditions.[92]

A useful analytical tool for precision medicine was developed by Galaziou et al.[93] regarding a paper-based device that provides visual detection of a 10-single nucleotide polymorphisms (SNP) panel as a genetic signature related to the breast cancer. The proposed device exploits the LFA where the anti-biotin-functionalized gold nanoparticles are deposited, in dry form, on the conjugate pad. Twenty 5′ amino-modified oligonucleotides (capture oligos) were immobilized on the spots allowing to capture via hybridization their complementarities tag sequences of the 20 allele-specific primers. In the presence of the corresponding alleles, red spots are formed on the paper obtaining an accurate genetic signature from each sample where detection and monitoring are accomplished either by naked eye or by scanning with a flatbed scanner.

Microfluidic paper based analytical device (µ-PAD) are considered potential POC diagnostic tools capable of simultaneously performing multiple functions, allow multiplex and quantitative analysis with low sample requirement and offering a distinct flow advantages over the traditional dipstick and LFAs.[94] Microfluidic channels can be created by patterning paper into hydrophilic channels delimited by hydrophobic barriers.[95] The width and length of the channels can be defined thanks to the patterning process and the thickness of the paper defines the height. By defining a channel geometry and on/off switches it is possible to control the flow rates and change delays for flow of aqueous fluids while the presence of a possible 3D fluidic network enables to different fluidic paths for the sequential delivery of multiple fluids without the need for peripheral equipment.

Since their introduction in 2007, several diagnostic µPAD devices have been developed to detect pathogens and biomarkers such as Ebola virus RNA, Salmonella, Hepatitic C antibodies, glucose and NO_2 in saliva.[96-101] µPADs provides flexible flow of sample and exploiting chemiluminescence, electrochemical and spectroscopic techniques it is possible to obtain quantitative detection.[102-103] One limitation of these diagnostic systems is related to their use at the POC in resource limited settings due to the easily degradation and inactivation of antibody and enzymes commonly used in biological assay where temperature and humidity are difficult to control[104] and the higher detection limits for some biological analyte concentrations.[105] However, in order to improve the sensitivity, new sensing motifs are introduced on µPAD by Liang et al, 2016 to develop aptamer-based fluorescent µPAD for a multiplex screening of cancer cells. The authors reported a paper device coated with graphene oxide and quantum dots (QDs), functionalized with DNA aptamers that are able to bind specific cancer cell lines and also to graphene through π-π stacking quenching the fluorescence of the QDs. The binding to their respective cancer cells allows the QDs to be

unquenched and fluoresce, where a different color of the QDs is associated to specific cell type that can be detected qualitatively with the naked eye. The use of fluorescence detector allows a quantitative measurements with low detection limits.[106] Another interesting approach to reach lower detection limits is represented by the use of electrochemistry technique. In clinical diagnostic, these detection methods require some form of chemical amplification that are often considered unattractive in μPADs due to the use of expensive reagents, to the fabrication of a complex device and the multistep carried out by the end-user.[107,108] Exploiting oxidase enzyme reactions (glucose oxidase, lactate oxidase, and uricase) and a single electrode type, an electrochemical based detection μPAD able to detect simultaneously glucose, lactate, and uric acid in biological samples has been developed. Thanks to photolithography and screen-printing technologies, microfluidic channels and electrodes can be created on filter paper showing the successful integration of paper-based microfluidics and electrochemical detection as an easy to-use, inexpensive, and portable alternative for POC monitoring.[109]

The fabrication of a reconfigurable three-dimensional (3D) “pop-up” electrochemical paper-based analytical device (pop-up-EPAD) for beta-hydroxybutyrate (BHB) detection was reported by Wang et al.[110] The authors developed a paper-based test strip for the detection of BHB, a biomarker for diabetic ketoacidosis, that operates using a commercial glucometer (Fig. 2D). Thanks to the reconfigurable 3D structure, the control of the timing steps and the fluidic path can be modified reducing the concentration of enzymes and the cost of the analysis.

By using 3D geometry, a 3D paper-based enzymatic fuel cell (EFC) for self-powered glucose monitoring in biological solutions was developed by Fischer et al.[111] The device is composed by layer of origami paper-based biofuel cell paper with an air-breathing cathode that exploit

used oxygen as the electron acceptor screen-printed and chitosan/glucose oxidase anode, providing self-powered alternative (Fig.1C). Another paper-based electrochemical sensing devices to detect the concentrations of various analytes such as heavy-metal ions and glucose in aqueous solutions, including body fluids, was demonstrated by Nie et al., showing a detection limit of glucose in the current µPED about 0.22 mM. The simplicity and portability of this µPED sensing device coupled to the excellent analytical performances and the possibility to integrate of high-density detection systems should make it suitable for not specialized medical personnel.[112]

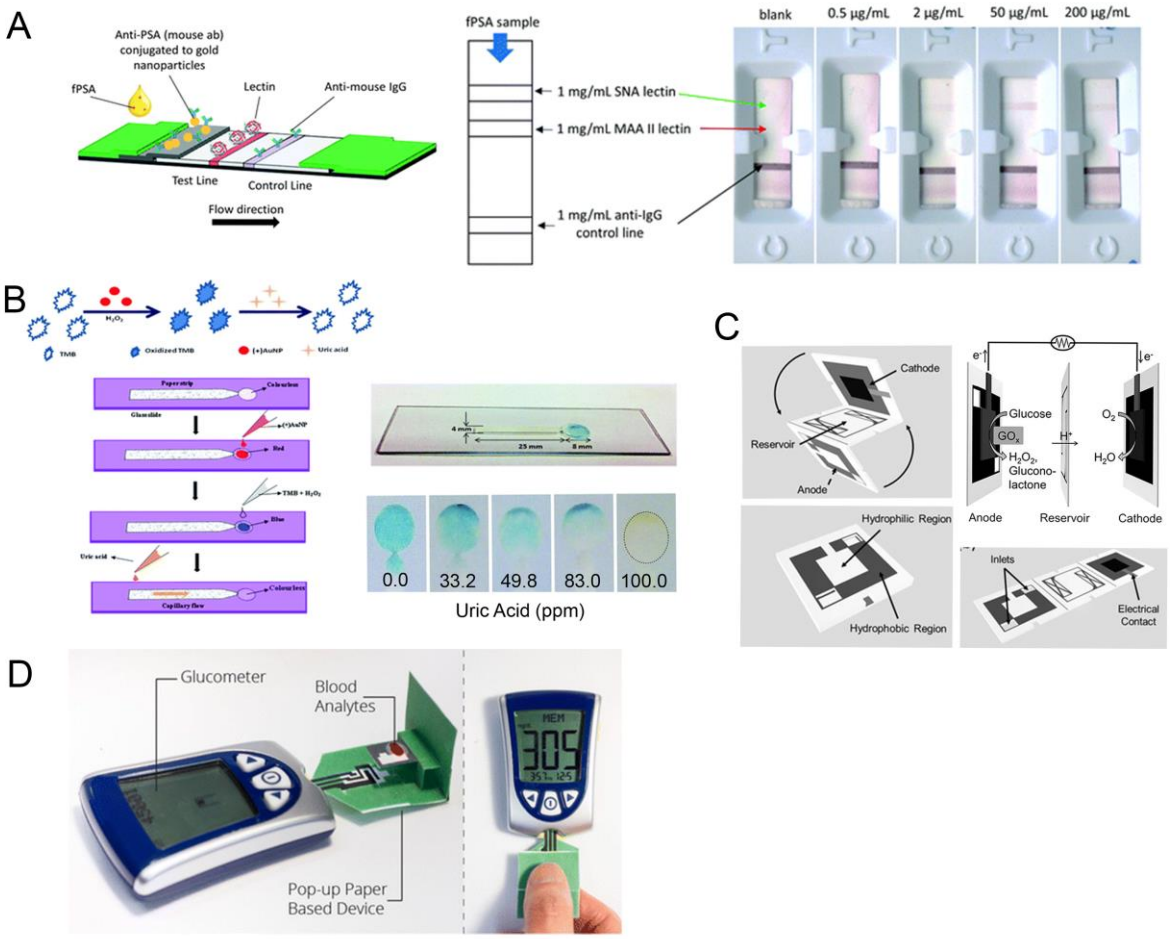

Fig. 2. Paper-based devices for diagnostic applications. (A) Lectin-based lateral flow assay for the detection of the prostate specific antigen. Reproduced from ref. 91 with permission

from Royal Society of Chemistry, copyright 2016. (B) A paper based microfluidic device for easy detection of uric acid. Reproduced from ref. 82 with permission from Royal Society of Chemistry, copyright 2015. (C) 3D paper-based enzymatic fuel cell for glucose monitoring. Reproduced from ref. 111 with permission from Elsevier, copyright 2016. (D) A "pop-up" electrochemical paper-based device for analysis of Beta-Hydroxybutyrate, a biomarker for diabetic ketoacidosis. Reproduced from ref. 110 with permission from American Chemical Society, copyright 2016.

4. Smartphone-based devices

Thanks to the recent advancements in technology, operating systems, internal memory and high-quality camera lenses, smartphones are able to replace laptops and desktop computers. Furthermore, the widespread distribution and the connectivity pave the way for a new generation of biosensors reshaping the concept of mobile health. Smartphone-based biosensors have a great potential as point-of-care and point-of-need platforms for healthcare providing portable, cost-effective, and easy-to-operate systems that can be used by unspecialized personnel. Different approaches have been reported in literature, exploiting smartphone both as an instrumental interface connected to the analytical device via Bluetooth or Wifi and as an ultrasensitive detector.[113-118]

The use of a smartphone as an interface for the analytical tool results much easier than its implementation into an integrated biosensor and several systems have already been commercialized. In this context, many devices have been developed for the control of physical parameters (e.g. temperature, ECG, pressure).[119,120] As it concerns the measure of biomarkers exploiting analytical devices connected wirelessly to smartphone, glucometers

and ethilometers are the most commonly proposed and they have already been launched on the market.[121] Integrating the smartphone with the biosensor leads to a major complexity in the design of the biosensor itself, but it results much more useful in terms of ease of use and compactness of the system. Indeed, the several smartphone's facilities could be exploited in order to obtain a complete analytical system suitable for carrying out the entire analysis (measurements, data collection, data elaboration, communication of the results). Optical and electrochemical principle of detection are the most suitable for the implementation of the biosensor with the smartphone. In the frame of optical detection, the proposed systems take advantage of the peculiar and high performances of the smartphone's camera. Several biosensors were reported based on colorimetric,[122-126] photoluminescent[127-132] (Fig.2C) and bio-chemiluminescent (Fig.3D, 3E)[133-138] based detection. Bioluminescence and chemiluminescence are particularly suitable for smartphone-based biosensors, with respect to photoluminescence, thanks to the simplicity of the accessories required. Indeed, photoluminescence needs excitation source or specific sample geometry. For a further simplification of the analysis procedures, thermochemiluminescence (TCL) labels coupled with smartphone-based detection[139] could find an optimal combination between the ultrasensistive detection offered by luminescent techniques and the possibility to operate in a reagent-less mode. TCL is a CL–based detection technique that requires no reagents, which would simplify the microfluidic network in smartphone-based analytical devices. Roda et al.[140] reported a one-step competitive immunoassay for VPA detection based on vertical flow immunoassay (VFIA) format, employing silica nanoparticles doped with a TCL 1,2-dioxetane derivative as a label (Fig. 2B). The VFIA sensor was a stack of paper-based layers functionalized with reagents stored in a stable form, allowing to complete the test for the quantification of valproic acid in blood

or saliva in 12 min simply upon sample addition. By 3D printing, simple 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.

One of the most attractive format for smartphone-based diagnostic device is LFIA, as confirmed by several publications, since it represents an ideal analytical platform for everyone thanks to low-cost and ease-of-use (Fig.3A).[141-144]

Relying on the use of the photocamera, smartphone-based imaging biosensors were also proposed for microscopy applications.[145] This system was successfully applied for the detection of microbial contamination[146] and blood-cell characterization.[147] As another example, D'Ambrosio et al.[148] developed a smartphone-based brightfield video microscope to automatically detect and quantify Loa microfilariae in whole-blood samples from a finger-prick without any previous sample preparation or labelling steps. One more recent study developed a field-portable bright-field microscope based on smartphone for label-free detection of *S. haematobium* eggs in urine samples in rural areas of Ghana, Africa.[149]

On the other side, the electrochemical detection is very competitive since several ways to integrate a peripheral module to a phone have been developed all with distinct trade-offs in terms of available power, data rate, and compatibility with different makes and models. For electrochemical biosensors specifically, the main functionalities that can be offloaded to the smartphone are power, two-way data transmission, stimulus generation, and signal quantization using the variety of electrical interfaces available on modern cell-phones.[150] Consequently, there are numerous reports on smartphone-based systems combined with different electrochemical detection techniques, which are principally cyclic voltammetry (CV) and chronoamperograms, that stand out for the simplicity of interpreting the results.[151]

Lillehoj et al. (2013) presented a compact smartphone platform with chronoamperometry for rapid quantitative detection of Plasmodium falciparum histidine-rich protein 2 (PfHRP2), an important biomarker for malaria.[152] The phone can communicate with the embedded circuit by audio jack, control the fluid and biosensing on the microfluidic chip, and finally display results on the screen for immediate assessment. Then, Salomon et al. (2014) reported a multichannel amperometric platform, which provided a high-throughput screening method for serologic diagnosis of infectious diseases[153], while Nemiroski et al.[154], reported about the potentiometric measurement for Na^+ in urine for clinical analysis.

As evidenced by the large literature, the use of smartphones in the development of biosensors for precision medicine applications is destined to have an increasing weight. To date, most of the devices presented are only a proof of concept, few smartphone-based biosensors are actually on the market even if the economic interest around this field is rapidly expanding. The potential prospects are huge and the real-time self-measurement of target analytes could become a reality in the near future leading to a significant breakthrough in the field of personalized medicine.

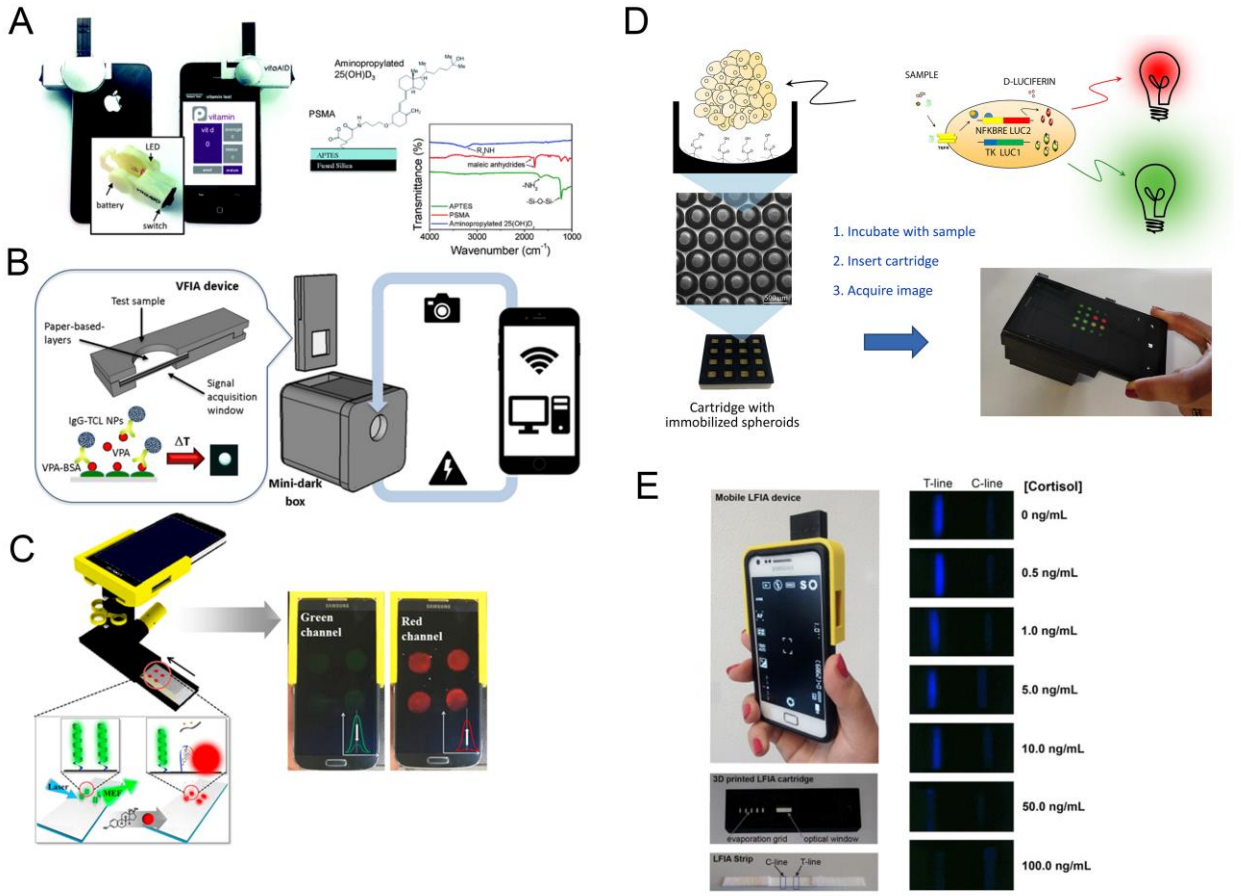

Fig. 3. Smartphone-based devices. (A) Smartphone platform for the quantification of vitamin D levels. Reproduced from ref. 143 with permission from Royal Society of Chemistry, copyright 2014. (B) Smartphone-based thermochemiluminescent immunosensor for valproic acid detection. Reproduced from ref. 140 with permission from Elsevier, copyright 2019. (C) Smartphone imaging-based label-free and dual-wavelength fluorescent biosensor for biomolecules detection. Reproduced from ref. 132 with permission from Elsevier, copyright 2019. (D) Smartphone-based multicolor bioluminescent 3D spheroid biosensors for monitoring inflammatory activity. Reproduced from ref. 137 with permission from Elsevier, copyright 2019. (E) Smartphone-based chemiluminescence lateral flow immunoassay for

salivary cortisol detection. Reproduced from ref. 142 with permission from Elsevier, copyright 2015.

5. Wearable biosensors

Wearable and implantable technologies will play an important role in advancing precision medicine by enabling measurement of clinically-relevant parameters in real time monitoring an individual's health state.[155,156] Wearable biosensors have a great potential to provide continuous, physiological information via dynamic, non-invasive measurements of biomarkers (e.g. metabolites, hormones, bacteria) in biofluids, such as tears, saliva, urine, sweat and interstitial fluids.[10] In particular, recent applications have focused on electrochemical and optical biosensors combined with the most advanced microfluidic technologies and the use of flexible and innovative materials.

In the literature various examples of wearable biosensors are reported and the number of publications concerning this application field is constantly increasing. One of the first example was reported by Mannoor et al., in 2012 who developed a biosensor graphene-based for a wireless bacteria detection on tooth enamel.[157]

Another work was reported by Kim et al.[158], who demonstrated an instrumented mouthguard capable of non-invasively monitoring salivary uric acid levels. The enzyme (uricase)-modified screen printed electrode system has been integrated onto a mouthguard platform along with anatomically-miniaturized instrumentation electronics featuring a potentiostat, microcontroller, and a Bluetooth Low Energy transceiver (Fig.4A). The mouthguard biosensor system offers high sensitivity, selectivity, and stability towards uric acid detection

in human saliva, covering the concentration ranges for both healthy people and hyperuricemia patients.

Google[x], a team within Google of Mountain View, California, sensing the potential of this research field, developed a 'smart' contact lens that may eventually provide noninvasive blood glucose monitoring for diabetics, and correct vision in individuals with presbyopia. The system comprises a lens made of conventional lens hydrogel material with a tiny wireless chip, a miniaturized glucose sensor and a tiny battery embedded between two layers in the periphery of the lens, avoiding the iris and pupil. A pin-hole in the lens allows tear fluid to seep into the sensor, generating blood glucose readings that can be transmitted to a smartphone device and, potentially, directly to a physician.[159] Another biofluid that has gained much attention is sweat. This matrix is readily obtainable for chemical sensing applications since sweat glands are distributed across the entire body. The first biosensors for monitoring biomarkers in sweat were based on the use of patches to be applied to the skin for the detection of several target analytes. As an example, Koh et al.[160], developed a system that can directly and reliably harvest sweat from pores on the surface of the skin. The device routes this sweat to different channels and reservoirs for multiparametric sensing of markers of interest, with options for wireless interfaces to external devices for image capture and analysis. The devices can mount at multiple locations on the body without chemical or physical irritation by use of biocompatible adhesives and soft device mechanics, including flexible and stretchable properties, and watertight interfaces. These devices measure total sweat loss, pH, lactate, chloride, and glucose concentrations by colorimetric detection using wireless data transmission.

Gao et al.[161], presented a mechanically flexible and fully integrated sensor array for multiplexed in situ perspiration analysis, which simultaneously and selectively measures

sweat metabolites (such as glucose and lactate) and electrolytes (such as sodium and potassium ions), as well as the skin temperature (to calibrate the response of the sensors). This work combined plastic-based sensors that interface with the skin with silicon integrated circuits consolidated on a flexible circuit board for complex signal processing. The wearable system was used to measure the detailed sweat profile of human subjects engaged in prolonged indoor and outdoor physical activities, and to make a real-time assessment of the physiological state of the subjects. Another approach to analyse biomarkers in sweat is the temporary tattoo (Fig.4B).[162] The sensor represents the first example of an easy-to-wear flexible tattoo-based epidermal diagnostic device combining reverse iontophoretic extraction of interstitial glucose and an enzyme-based amperometric biosensor. The iontophoretic-biosensing tattoo platform is reduced to practice by applying the device on human subjects and monitoring variations in glycemic levels due to food consumption. This preliminary investigation indicates that the tattoo-based iontophoresis-sensor platform holds considerable promise for efficient diabetes management and can be extended toward noninvasive monitoring of other physiologically relevant analytes present in the interstitial fluid.

Wearable biosensors are expected to become more and more present in our everyday life and in the future they will be able to monitor a wide range of biomarkers, including proteins and nucleic acids. The interest attracted by wearable biosensors is also reflected in the market where first systems, mainly aimed at the detection of glucose, have already been proposed[163-170] and is thus expected to rapidly grow.

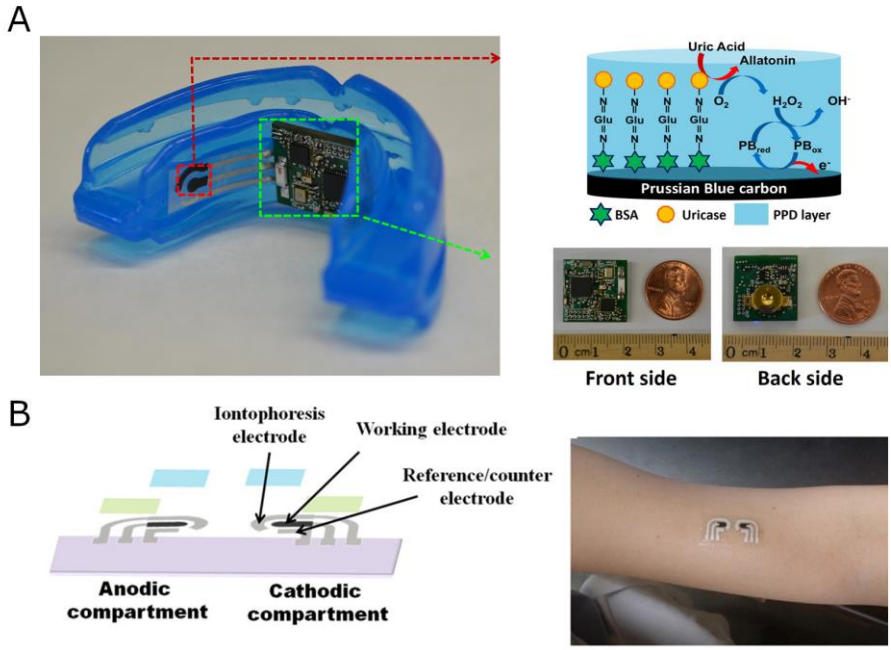

Fig. 4. Wearable biosensors. (A) Wearable salivary uric acid mouthguard biosensor with integrated wireless electronics. Reproduced from ref. 158 with permission from Elsevier, copyright 2015. (B) Wareble tattoo-based non invasive glucose monitoring. Reproduced from ref. 162 with permission from American Chemical Society, copyright 2015. (https://pubs.acs.org/doi/10.1021/ac504300n)

7. Outlook and conclusions

Since the appearance of the first portable glucose monitoring device for diabetic patients with the consequent possibility of promptly intervening with the appropriate therapy, many steps have been taken in the development of analytical tools for precision medicine. In particular, platforms and analytical approaches now exploit innovative, ecological and low-

cost materials (i.e. nanomaterials, paper-based devices) and detectors that can be used by everyone, like smartphones. These devices are designed with the aim of monitoring the health status in real time, thus reducing the number of invasive interventions required. Furthermore, the analytes of interest include not only biomarkers related to the presence of specific pathologies, but also genetic and environmental parameters since precision medicine consider the patient's genetic makeup, the environment in which he lives and his habits of daily life. However, these advances reported in the scientific literature have not yet been translated into the market where the examples of devices for personalized medicine are still very limited. Furthermore the main need is still the discovery of new more specific clinical biomarker of diagnostic and prognostic use and the data achieved from proteomics studies on a big set of data using mass spectrometry and related technologies will be immediately translate for the development of new rapid test based on different portable formats to fit the medical need.

In the coming years, therefore, a great increase in the availability of these bioanalytics tools in commerce and widespread distribution in everyday life is expected. This will open up the possibility of revolutionizing the world of clinical diagnostics, making it more accessible to everyone, significantly reducing time and costs for pathologies diagnosis and making treatment more effective and timely.

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