Nanomaterials-based devices for point-of-care diagnostics applications

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In this review, we discuss the capabilities of nanomaterials for point-of-care (PoC) diagnostics and explain how these materials can help to strengthen, miniaturize and improve the quality of such devices. Since the optical, electrochemical and other physical properties of nanomaterials are dictated by their composition, size and shape, these factors are critical in the design and function of nanomaterials-based PoC diagnostics.

Key learning points

(1) Needs and challenges of point-of-care diagnostics.
(2) Nanomaterials as interesting building blocks for biosensing.
(3) Overview of different detection methods offered by using nanomaterials.
(4) Advantages and drawbacks of nanomaterials-based sensing strategies.
(5) Opportunities offered by paper as cost-efficient biosensing platform.

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1. Introduction

The advancements made on medicine in the last years have been impressive. However, diseases not detected on time or not properly monitored are still the main causes of death in today's society. Ischemic heart diseases, respiratory infections, diabetes or bacterial infections as tuberculosis or diarrheal diseases are some of the examples that cause more deaths around the world but, if detected on time, could be prevented. Nowadays, we have the potential technologies and the tools to detect all of them, therefore, why so many people are still dying due to these diseases? Probably the main reasons are two. First the lack of equipment, especially in development countries where the costs are not affordable for all the population or even for the medical centers. Second, the time frame between the moment when the symptoms are appreciable by the patient and the time when diagnostic is accomplished by the specialist. How could these problems be solved? May we have a doctor anywhere for a single patient, ready at any time, accurate and fast, or is this a utopia? Now, instead of having a real doctor imagine having a little device, portable and easy-to-use, able to monitor several parameters and variables, like a portable laboratory[1]. With all the data collected this tiny device could decide in few minutes either by itself or by an immediate communication with a specialist/medical doctor, which action is required by the patient being everything done at home or even in the field, without requiring any kind of medical knowledge by the user.

This “futuristic” idea is exemplified in Fig.1: a mother is using a portable device to diagnose what virus has her daughter, send the data to the pharmacy and conclude that some chicken soup may make the child feel better (no need for any medication at all).

Nowadays some portable devices able to monitor and diagnose the condition of the user are already available. A well-known example is the glucose meter that, with few microliters of blood, is able to determine the glucose concentration in the sample and notify the diabetes patient if there is any action required as the injection of insulin or the intake of food.

It is noteworthy the capabilities of mobile phones to carry out these type of tasks[2] given the huge amount of population that has, at least, one mobile phone and the tools that these devices include as cameras, light sensors, power sources, movement detectors and wireless connection among others (and more to appear in the future). In this review we also will show how some parts of a mobile phone as the camera[3], the light sensor[4] or even the NFC (near-field communication) antenna[5] are used to monitor different variables in nanomaterials-based platforms with interest for future diagnostics.

1.1. Point-of-Care tests

Devices able to perform fast analysis and accurate diagnostics near the patient care are known as “bedside” or “point-of-care” (PoC) tests. The ideal PoC test should be user-friendly and as simple as possible so that any user, even without any type of medical or laboratory knowledge, will be able to use it and understand its response (as shown in the example of Fig. 1). Also, low-cost is an important and desirable quality of PoC to ensure that the device is easy to be acquired by everyone and anywhere. Because of this, one of the most popular materials employed as substrate for PoC devices is paper[6-8], which is cheap, abundant, recyclable and biosustainable. Other qualities that PoC test should have are robustness (the capacity to withstand changes on environmental conditions), selectivity (the ability to respond to a unique analyte or parameter, not to be affected by interferences) and sensitivity (the quality to discriminate between similar values).

There exist different methodologies to produce the signal on PoC devices (or in any other type of sensor and biosensor) and in most of the cases the chosen methodology will depend on the transducer employed. Optical and electrochemical-based PoC devices are probably the most popular, as they are the examples of pregnancy test and glucose meter respectively. With the emerging of nanotechnology both kind of devices are taking advantages of nanomaterials integrating these in different parts of existing sensing platforms or offering quite innovative detection systems.

1.2. Nanomaterials for Point-of-Care

Regarding the transducer, synthetic nanomaterials (with a diameter between 1 and 100 nm) offer a wide range of possibilities due to their size, shape and properties as can be biocompatibility, fluorescence, electrical and thermal conductivity, magnetism, etc. Nanomaterials can be classified according to their shape as: “0D” (spherical nanomaterials), “1D” (e.g. nanotubes, nanowires), “2D” (e.g. graphene or other well-known 2D materials), 3D (e.g. nanoprisms, nanoflowers etc.)[9]. In the following sections POC based on different kinds of nanomaterials and their advantages and drawbacks during applications in diagnostics will be reviewed.

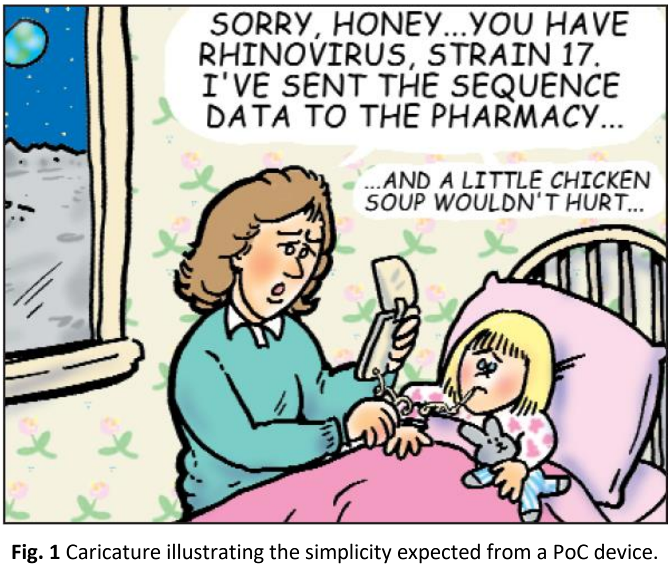

Fig. 1 Caricature illustrating the simplicity expected from a PoC device. Reprinted with permission from ref. 1, copyright 2002 American Association for the Advancement of Science.

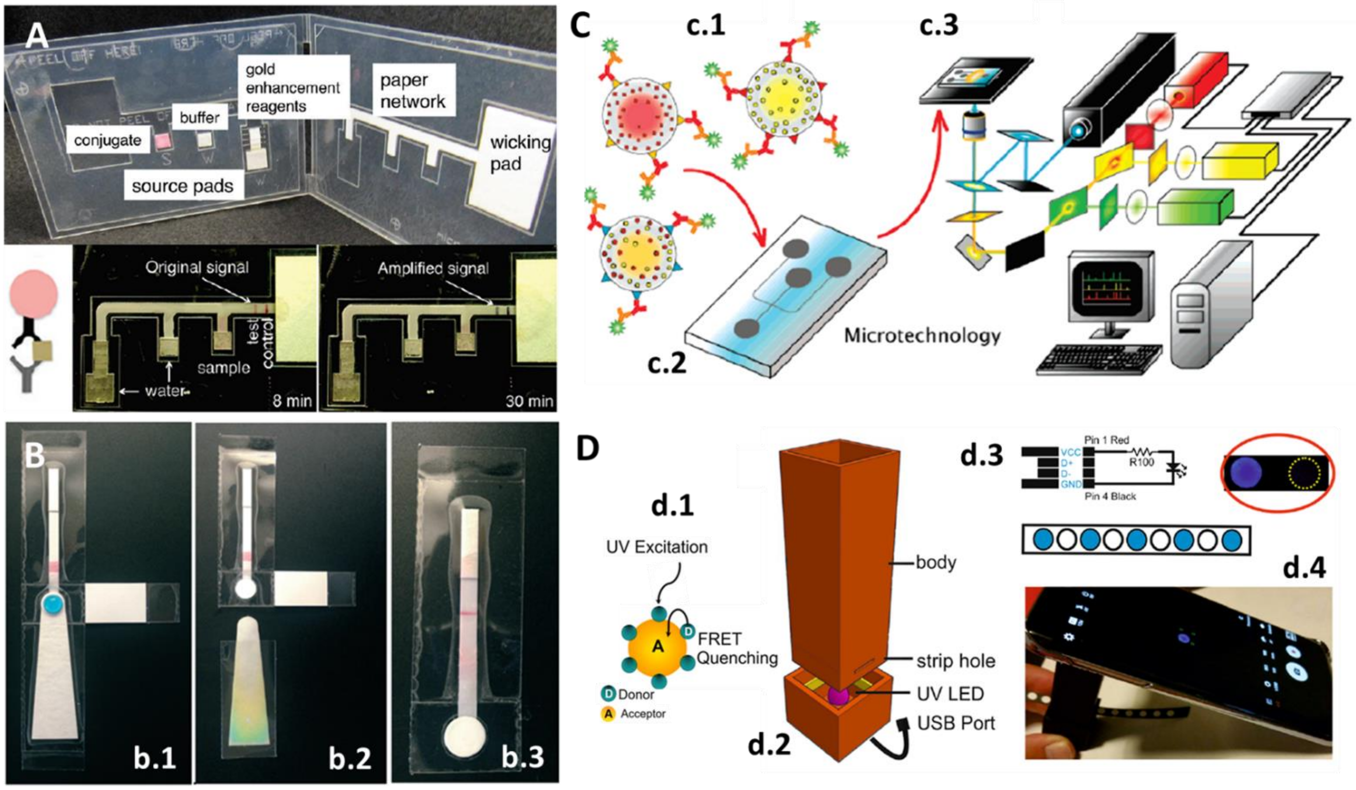

Fig. 2 (A) PoC colorimetric device in which the signal of a LFBs system is increased by means of a paper network consisting on paper pads storing colour enhancement reagent. Adapted with permission from ref. 17, copyright 2012 American Chemical Society. (B) LFBs system to extract, amplify and detect nucleic acids: (b.1) sample is added on sample port, (b.2) absorbent pad is removed after washing steps, (b.3) after the addition of amplification reagents, purified analytes flow across the LFBs. Adapted with permission from ref. 18, copyright 2016 Royal Society of Chemistry. (C) Multiplexed barcode system including QDs: (c.1) QDs of different colours encapsulated into microbeads with specific biomarkers for different targets, (c.2) microfluidics system, (c.3) detection platform. Adapted with permission from ref. 22, copyright 2007 American Chemical Society. (D) Mobile phone integrated PoC system: (d.1) graphene QDs are quenched in presence of acceptor compounds, (d.2) 3D-printed device with UV LED, (d.3) LED mechanism and paper substrate containing dried graphene QDs, (d.4) mobile phone measuring the quenching of QDs. Adapted with permission from ref. 3, copyright 2017 Nature Publishing Group.

2. Spherical nanomaterials (0D)

The most widely used nanomaterials are spherical ones, owing to their simple preparation and manipulation. When a spherical nanoparticle is attached to a biomarker, a molecule that reacts only under specific pathological conditions, the resulting functionalized nanosphere can serve as a biological label and, for instance, to signal the presence of a given analyte or pathogen. The detection mechanisms for such nanoparticles can be quite diverse[10]: measuring the light absorption of the nanoparticles when attached to the analyte (after a cleaning step), measuring the shift of peak wavelength due to the agglomeration of the nanoparticles, enhancement by secondary enzymatic reactions onto nanoparticles surface, by a quenching effect (an intensity decrease of a fluorescent signal), surface plasmon resonance (optical changes and radiative enhancements on a nanomaterial due to disturbance of the dielectric constant induced by the adsorption of a molecule), electrical or electrochemical changes (when the nanomaterial is conductive or can catalyze a reaction that is electrochemically detectable), electrical impedance spectroscopy (changes on the electrical resistance of a medium), etc.

Among all the 0D nanomaterials, gold nanoparticles (AuNPs) have been highly reported and discussed[11]. This nanomaterial stands out due to its high bioaffinity, strong colour (whose wavelength varies with small changes on its diameter or its surface) and even catalytic properties, thus it is commonly used for both optical and electrochemical PoC devices.

2.1. 0D optical-based PoC devices

Lateral flow biosensors (LFBs)[7] are optical-based PoC devices fabricated on paper substrates. Among the best-known examples are common pregnancy tests. LFBs evolved from thin-layer immunoaffinity chromatography[12], a method based on the formation of a "sandwich" comprising a primary biomarker (often an antibody) attached onto the paper substrate (cellulose or, to favor the attachment of the biomarkers, nitrocellulose), an analyte (proteins, cells, bacteria, molecules or even heavy metals) and a secondary biomarker, which is conjugated to the tag nanoparticle. The formation of this sandwich triggers the appearance of colour in the "test zone" (where the first biomarker has been deposited) for the positive sample and, if the sandwich is not assembled (lack of analyte in the sample), the test zone remains colourless (a negative sample). Latex beads were commonly used on LFBs and still are used, for most commercial pregnancy tests. However, the inclusion of AuNPs on LFBs[13], among other nanomaterials, improved the sensitivity of the method because of the strong colour of the nanomaterial which is due to the surface plasmon resonance effect, present in metallic nanoparticles but not on latex beads. Furthermore, the shrinking of the label size down to the nanoscale eases the flow and boosts the label/analyte ratio. These improvements enabled semi-quantitative assays, relating the color intensity with the analyte concentration, similarly as with a pH paper. By using a colorimetric reader or even a mobile phone[2] the quantification level can be improved.

Besides AuNPs, other nanomaterials as silver nanoparticles (AgNPs) have been used in LFBs; the wavelength variations provoked by size and shape modifications are bigger with AgNPs than with AuNPs, leading to color tonalities quite different between nanoparticles with less than 10 nm of difference in size. Thus, AgNPs permit the performance of multiplexed tests[14] (i.e. for the simultaneous detection of different analytes on the same device), in which a different colour is obtained in each test zone. Also fluorescent nanoparticles, like quantum dots[15] (QDs) or up-converting phosphor reporters[16] (UCPs; although these are expensive, since they are made using rare elements such as europium or yttrium), are making their way on LFBs. Fluorescent nanoparticles lead to greater sensitivity and specificity than do non-fluorescent nanomaterials, since in fluorescence methods only the signal coming from the nanomaterial is read. However, the response cannot be observed by the naked eye: they always require equipment to excite and read the resulting fluorescence signal.

There exist various signal enhancement strategies for nanoparticles on paper substrate[7], however they often require the user to apply several additional steps, making the PoC system less user-friendly increasing the human error factor. A way to simplify these steps was reported by Fu *et al.*[17]. They present a two-dimensional paper network combining LFBs with other paper pads used for storing enhancement reagents (as shown in Fig. 2A). In this PoC system, the user must add the sample to the conjugate pad, on which the analyte is captured by AuNPs, and water to the other pads and then close the system. At first, it works like a conventional LFB strip, AuNPs stop in the detection zone, but once the enhancement reagents reach the detection zone the colour of the AuNPs changes to dark purple as their size increases. Against the white background, dark purple grants a higher contrast compared to the original red. The limit of detection (LOD) achieved with this enhancement strategy was four times lower than with standard LFBs. Rodriguez *et al*.[18] also propose an interesting LFBs able to isolate, amplify and detect nucleic acids, all-in-one, equipment-free and much faster than conventional methods. Their system comprises a LFB strip equipped with some additional removable parts (Fig. 2B): a pad for washing the sample but retaining the purified DNA (Fig. 2b.1); a tab to prevent evaporation of isothermal amplification reagents (Fig 2b.2); and some hydrophobic tape barriers to prevent DNA and other solvents from flowing prematurely to the LFB strip (Fig. 2b.3). Besides LFBs strips format, other possibilities to store nanomaterials on paper are reported, but with the same signaling mechanism as on previous mentioned immunoassays. One example is the prototype proposed by Pauli *et al*.[19], a lab-on-a-syringe used to collect urine and pump it to paper pads stored in serially connected cartridges. The first cartridge contains AuNPs for the capture of the analyte, a cancer biomarker, and the second one the detection pad. The detection pad consists on nitrocellulose paper with detection antibodies inside a wax ring to focus the flow to pass through the antibodies. As on AuNPs-based LFBs, the inner part of the ring will turn more reddish as higher the amount of analyte is. Contrary to LFBs, the lab-on-a-syringe requires the user to control the flow, being it less user-friendly than a paper strip, however it permits modifying the incubation times for AuNPs with analyte, which can lead to improved sensitivities. Also, a filter can be coupled to the syringe in order to reduce matrix effects. It is also remarkable the application of nanopaper, also known as bacteria cellulose paper as it is produced by bacteria. As reported by Morales-Narváez *et al*[20], 0D nanoparticles can be stored and even produced on this colorless substrate resulting in a plasmonic or fluorescent paper with great potential as an alternative to enzyme-linked immunosorbent assay (ELISA) plates. This work demonstrates that PoC devices fabricated with nanopaper can take other forms besides ELISA plates as they are cuvettes or simple spots on a piece of paper. Plasmonic resonance, fluorescence and quenching effects are some of the measurements that can be done these systems by using nanomaterials as AgNPs, AuNPs or QDs. Similarly to nanopaper, hydrogel can be used to store nanomaterials for their use in PoC applications as Yetisen *et al.*[21] did with AgNPs and a phenylboronic acid-functionalized hydrogel. Their system can filter urine samples retaining glucose. Then, a laser can measure within five minutes the diffraction provoked by the interaction of AgNPs and glucose. Surprisingly the system is reusable, unlike paper-based systems.

As previously mentioned, QDs are small 0D nanomaterials with fluorescent properties. Klostranec *et al.*[22] took advantage of the intense signal of QDs to construct a multiplex system able to detect different blood infectious diseases related targets by using a "barcode" system (Fig. 2C). Different colored QDs were encapsulated into microbeads (Fig. 2c.1), each bead being conjugated with antibodies specific to a different target, and the particles then were mixed inside a microfluidic system (Fig. 2c.2) with the sample. The incubation inside the microfluidics was controlled electrokinetically. Data collection was performed with a software and a detection platform (Fig. 2c.3) which as claimed by authors could be miniaturized for PoC

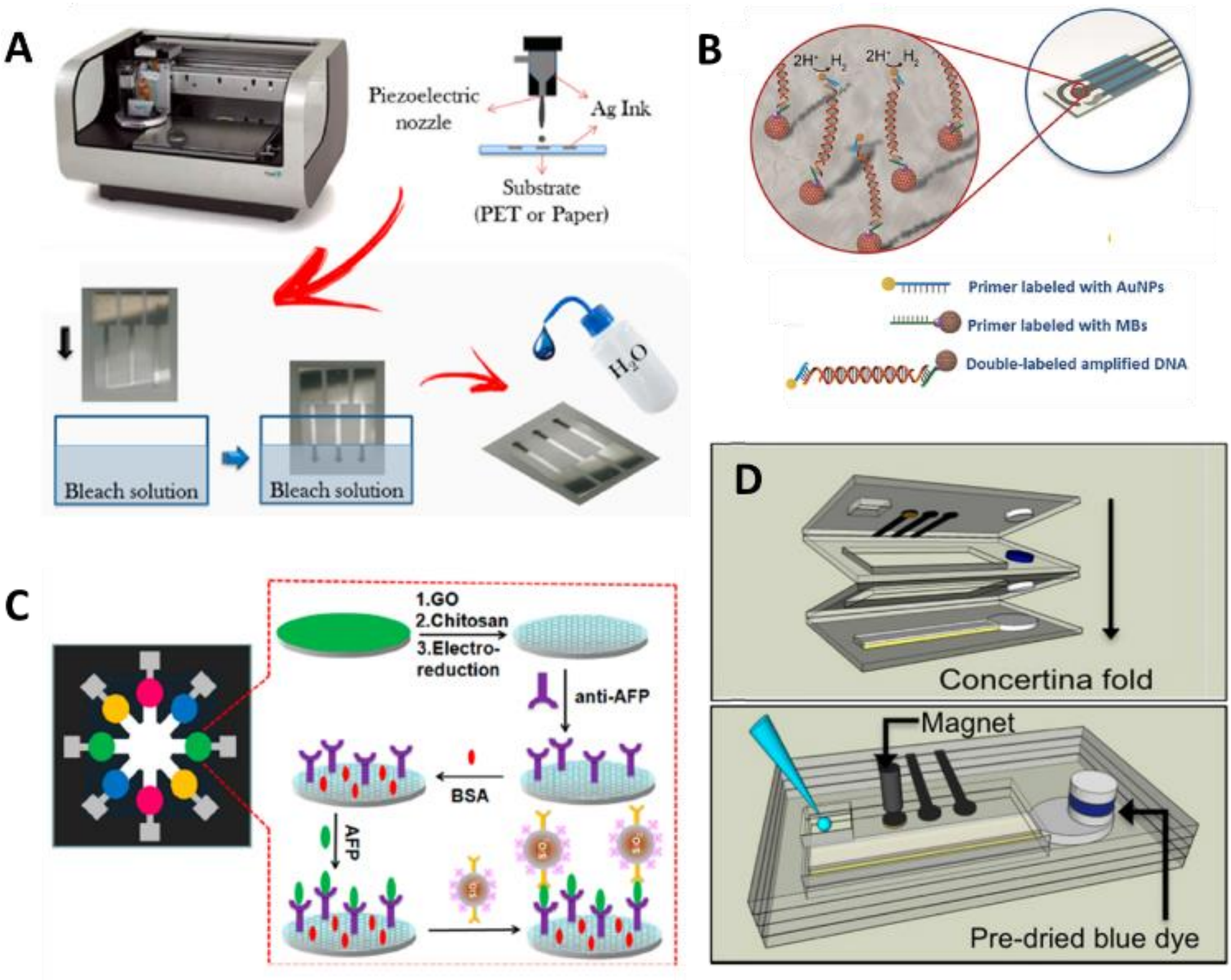

Fig. 3 (A) Ag/AgCl reference electrodes fabricated from AgNPs using inkjet technology and bleach treatment. Adapted with permission from ref. 23, copyright 2014 American Chemical Society. (B) Combination of AuNPs and MBs for the detection of DNA on a SPCE. Adapted with permission from ref 25, copyright 2015 John Wiley and Sons. (C) Paper PoC device for the electrochemical measurement of eight samples. Adapted with permission from ref. 30, copyright 2013 American Chemical Society. (D) Paper origami PoC for the electrochemical detection of AgNPs. Unfolded (up) and folded (down). Adapted with permission from ref. 31, copyright 2016 American Chemical Society.

diagnosis in the future was developed. Their platform can identify independently the wavelength of each QDs type, normalize it and, like a barcode reading, estimate the amount of each target.

Another interesting work involving QDs was reported by Álvarez-Diduk *et al.*[3]. As shown in Fig. 2D they took advantage of the fluorescence quenching effect occurred on graphene QDs in presence of some polyphenolic compounds (Fig. 2d.1). They fabricated a 3D-printed dark chamber (Fig. 2d.2) including an UV LED powered by a mobile phone to excite the QDs (Fig. 2d.4). Using wax-printed traced spots onto a paper strip, QDs were physisorpted inside the spots obtaining an ELISA plate-like system (Fig. 2d.3). When the polyphenolic compounds are dropped onto the spots, fluorescence is quenched and captured by the mobile phone camera (Fig. 2d.4). The images can be analyzed directly on the mobile phone, with an app, or later with computer software.

2.2. 0D electrochemical-based PoC devices

Electrochemical measurements allow to identify electrical phenomena related to a chemical change. The function of nanomaterials in this kind of measurements is not limited to analyte labelling only. Nowadays nanoparticles are commonly used in commercial inks for fabrication of electrodes. 0D nanomaterials as AuNPs or AgNPs and 1D nanomaterials as carbon nanotubes (CNTs) are the most common ones. As an example, in the work of da Silva *et al.*[23] they used commercial AgNPs to fabricate a miniaturized and portable Ag/AgCl reference electrode, of known and stable potential. They printed the electrode on two different flexible substrates, paper and polyethylene terephthalate (PET), using a high resolution piezoelectric inkjet materials printer (Fig. 3A). Once printed, AgNPs ink was cured at 120ºC and treated with bleach (sodium hypochlorite, NaClO) to produce the Ag/AgCl mixture to serve as pseudo reference electrode.

Silver reduction reaction is catalyzed by the presence of AuNPs which in turn given their high affinity to biological molecules are extensively reported as transducers in electrochemical assays[24]. Another reaction which is often applied in electrochemistry and catalyzed by AuNPs is the hydrogen evolution reaction, the formation of H_2 gas by the reduction of H^+ ions. This reaction was measured by de la Escosura-Muñiz *et al.*[25] who, combining DNA amplification strategies, magnetic beads (MBs) and AuNPs, developed a system on screen printed carbon electrodes (SPCE) that discriminates dog DNA from Leishmania parasite DNA, hosted inside the cells. MBs and AuNPs are linked to the amplified DNA and, using a magnet, the conjugate is placed onto the working electrode of the SPCE so the signal can be measured efficiently (Fig. 3B). Iridium oxide nanoparticles (IrO_2NPs) are other 0D nanomaterials used due to their catalytic properties towards water oxidation reaction[26], the production of oxygen from water, and their application in impedimetric sensors[27-28]. Impedance is a technique used to measure frequency changes on the dielectric medium close to the nanoparticles. So, by binding biomarkers on IrO_2NPs it is possible to detect variations on the conductivity of the medium depending on capturing of a biomarker to a target analyte.

Regarding AuNPs-based LFBs, in analogy to optical detection, some researchers are trying to integrate electrochemical sensing strategies into the paper strips to achieve lower detection limits and higher sensitivity in comparison to colorimetric lecture[29]. However, to do that it is necessary to cut the detection zone of the strips and dissolve it with acid, so the detection is performed with external electrodes on the dissolved nanoparticles. The addition of this extra step enhances the quantification on LFBs but the methodology still needs to be improved either by including the step into an automated PoC device or by finding an alternative to dissolve a part of the strip. A more user-friendly paper-based PoC tool was designed by Wu *et al.*[30] which permits the electrochemical analysis of eight samples sequentially (Fig. 3C). This device comprises eight electrodes pre-treated with antibodies specific to the target analyte. The samples are added to the electrodes and then, SiO_2 nanoparticles are dispensed on the electrodes (note that SiO_2 nanoparticles are inexpensive and easy to load with various compounds such as dyes, proteins or enzymes). In this case, the SiO_2 nanoparticles are loaded with antibodies specific to the analyte, to perform a sandwich assay, and with horseradish peroxidase, the enzyme that triggers the electrochemical reaction. The electrodes are washed and then a reactive solution is added to the core of the device, from where it then flows to the electrodes, thereby activating the electrochemical reaction. Another interesting paper PoC device was made by Cunningham *et al.*[31], an origami-styled system that, as proof-of-concept, was used for detection of AgNPs by oxidizing them using a gold-made working electrode.

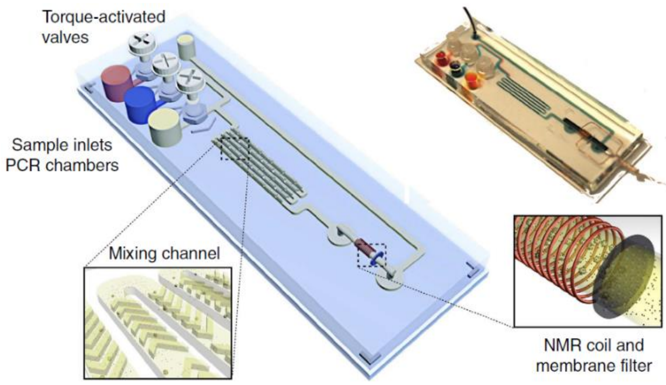

Fig. 4 NMR-based microfluidic PoC device. Reprinted with permission from ref. 32, copyright 2013 Nature Publishing Group.

The device consists of four folded paper layers (Fig. 3D): first layer containing electrodes, inlet and outlet; second and third layers containing a paper circuit delimited by wax and a blue dye that works as indicator of the stoppage of the flow; and the forth layer which contains a "sink" to redirect all the flow there.

2.3. 0D magnetic PoC devices

MBs and magnetic nanoparticles (MNPs) are often used as support in PoC devices, usually in washing and pre-concentration/amplification steps or in the precipitation of the analyte and other nanoparticles for example onto an electrode[25]. Some researchers are also taking advantage of the magnetic properties of MNPs and use these as transducers[32,33] by means of nuclear magnetic resonance (NMR). This technique is highly sensitive due to the low back-ground signal since non-magnetic substances should not interfere. In addition, NMR is able to detect all the tags present in the detection zone, while in optical and electrochemical sensors it is not always possible (in optical sensors, generally only the tags on the substrate surface are visible; in electrochemical sensors, it is often required that the tags are in contact with the electrode to be able to generate a signal). In addition, the detection time is faster than electrochemical assays. However, NMR systems are still expensive and may not be affordable to all the possible users.

Liong *et al.*[32] propose a microfluidics PoC device (Fig. 4) to detect amplified nucleic acids from a bacterium related to tuberculosis. The device is able to perform DNA amplification, MNPs-DNA incubation, washing and NMR detection. The device has three inlets for DNA, MNPs and a washing buffer; some mixing channels for the incubation steps and a NMR coil that counts the amount of MNPs, proportional to the initial value of DNA concentration. As main drawback in this device, although the NMR detection is fast, the amplification and the incubation steps can elongate the duration of the assay more than two hours. Anyhow, it is faster than other tuberculosis detection methods based on cell culture and microscopy. Chung *et al.*[33] developed another NMR-based microfluidics PoC device which they use for the detection of a biomarker in urine. Despite the matrix is complex, the noise signal is low

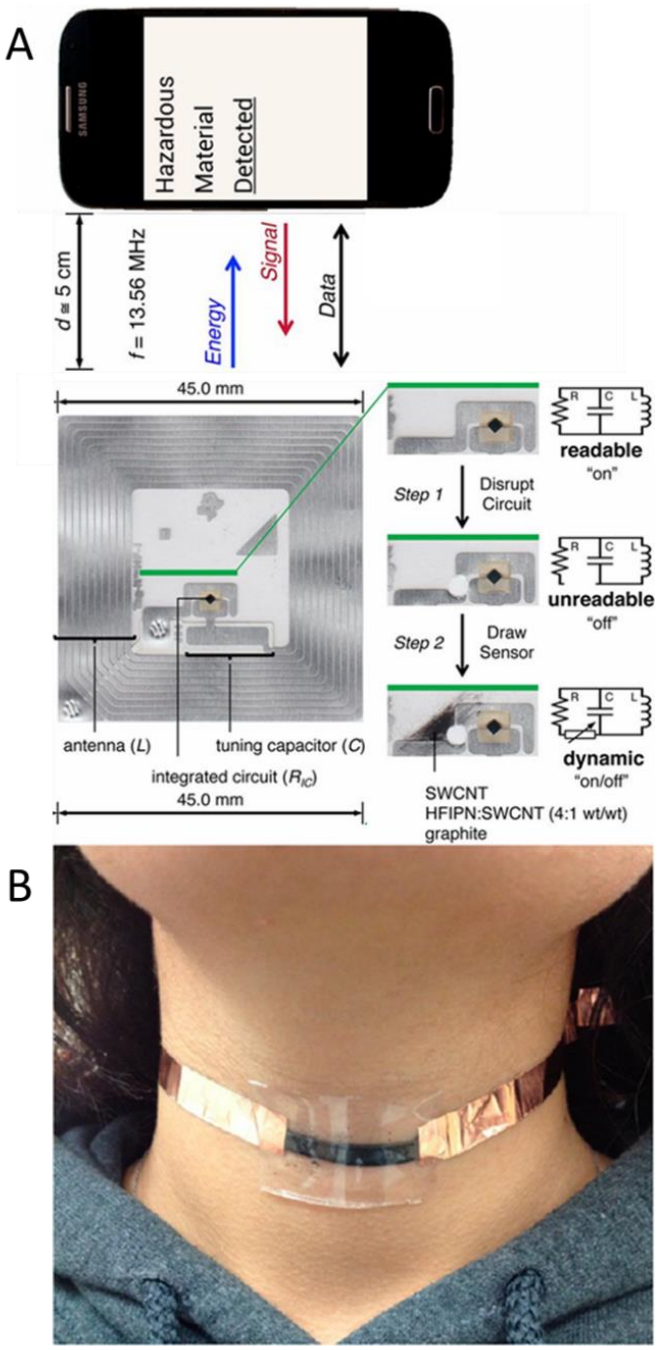

Fig. 5 (A) NFC tag circuit modified with SWCNTs to work as on/off logic gate in the presence of different analytes. Adapted with permission from ref. 5, copyright 2014 National Academy of Sciences. (B) Chewing gum containing CNTs adapted for motion monitoring. Reprinted with permission from ref. 38, copyright 2015 American Chemical Society.

due, as previously mentioned, to non-magnetic that substances do not interfere on the readout. Their device is compared to a reported colorimetric dipstick method obtaining a LOD 8 times lower.

3. One-dimensional nanomaterials (1D)

1D nanomaterials are those materials which growth is oriented in one dimension, on a linear way. The thickness could be as small as just one nanometer, as single-walled carbon nanotubes (SWCNTs), but the length could be a million times larger, hundreds of micrometers. The shape of these nanomaterials has an important effect on their application: the length and the diameter define the absorption wavelengths. Moreover, length and thickness also grant mechanical strength, useful in the creation of larger nanostructured materials. Nonetheless, the growth of 1D nanomaterials is not simple and often requires meticulous synthetic routes that in turn would determine their homogeneity

Nanowires are the simplest 1D nanomaterial, that can be created from other 0D nanomaterials or directly, being cooper, nickel, silver, gold, platinum and silicone the most used elements. In the work of Mostafalu and Sonkusale[34], they introduced nanowires (made from platinum, nickel and cooper) into paper substrate to create electrodes for electrocardiogram monitoring by means of tissue-electrode impedance which work in dry conditions. This approach that doesn't require the addition of electrolytic gel between the electrodes and the skin is advantageous since gels use to get dry quickly and are degraded with movement. The high surface area of nanowires provides good quality of response in the electrodes, from 100 to 1 KΩ in impedimetric measurements. Besides medical application, Mostafalu and Sonkusale demonstrated that the same paper electrodes worked properly as well as cathode in an acidic battery.

In the recent years CNTs and SWCNTs have been widely used on electrochemical applications[35-37] since these nanomaterials are highly conductive, both electrical and thermal. CNTs are easy to functionalize as well, not only with biological compounds, but also with other nanomaterials (especially with metallic oxide nanoparticles) provoking an enhancement of their electrical properties, among others[35]. CNTs are also included in several commercial inks for the fabrication of screen printed electrodes (as well as AgNPs are commonly used for the fabrication of reference electrodes, CNTs are used for the fabrication of counter and working electrodes[36]). In comparison to metallic nanowires, CNTs contribute to the fabrication of larger nanostructured materials with new properties as high flexibility, elasticity and very high lightness, since they are hollow inside. Also, CNTs are more robust than nanowires against temperature variations due to the fact that the thermal expansion coefficient of carbon bonds is much lower than in metals. However, it is important to consider that many reactions which could be applied on their surface will not be reversible, making the lifetime of CNTs shorter than other materials applied in sensing. Then, as an example of use of these materials, it should be mentioned the work of Nemiroski *et al.*[37] where they integrated electrodes made with CNTs into a mobile phone system by means of the audio jack of the device (the audio jack has the advantage that can send and receive information at the same time).

In a very smart way and for the first time, Azzarelli et al.[5] used

SWCNT to apply NFC technology for sensing. NFC technology is able to detect an antenna without requiring it to have an electrical power supply. This technology nowadays is present in most of the smartphone models, hotel doors lock systems, metro stations, toys and even in mail stamps, so NFC tags are becoming every day cheaper and easier to fabricate and

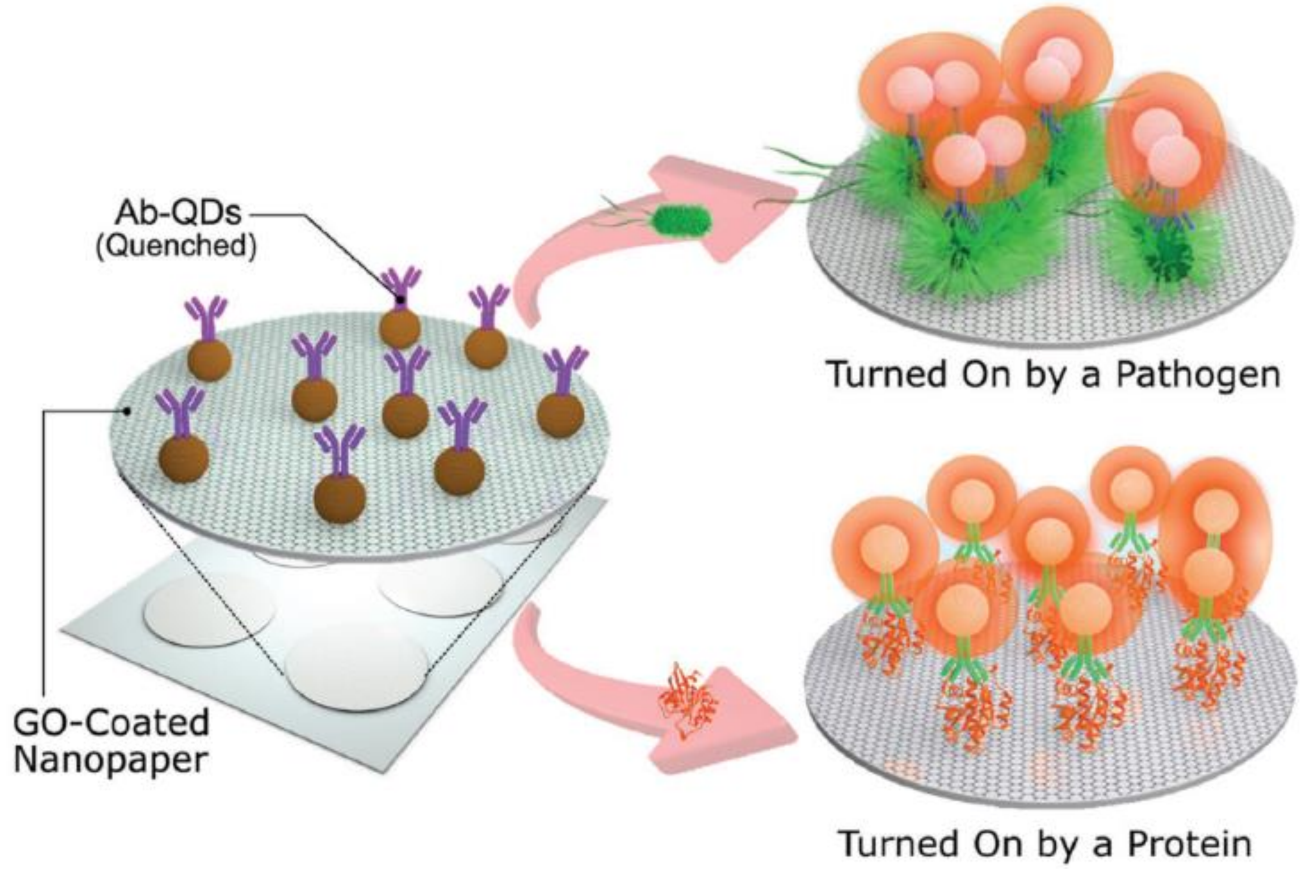

Fig. 6 Nanopaper coated with GO. QDs can be stored inside remaining quenched but when the analyte, e.g. bacteria or proteins, is additioned the fluorescence is released. Adapted with permission from ref 40, copyright 2017 John Wiley and Sons.

modify. So, they tuned an NFC tag by replacing part of the circuit with SWCNTs which, by means of a chemiresistive reaction, alter the conductivity in the circuit depending of the presence of different compounds in the air and, consequently, make it act as an on/off logic gate inside the NFC tag (Fig. 5a). This technique was applied for the detection of compounds in the air, but for future PoC applications it could be used to detect analytes on the breath or probably even in body fluids as blood or sweat.

One more surprising inclusion of CNTs was done by Darabi *et al.* into chewing gum[38]. They washed a chewing gum with water and ethanol and mixed it with a solution containing CNTs; the mixture was stretched and folded several times in one direction to favor the orientation of CNTs. The sensor worked properly for the detection of humidity in the medium (dryness of the mouth can be caused by certain medications or illnesses, by damage to the salivary glands or by hormonal changes) by measuring electrical resistance on the gum. Also as motion sensor as shown in Fig. 5b, able to detect not only body (neck in the figure) movements, also the breaths of the user. This device could be really interesting for PoC diagnostics (biting problems, dry mouth, pulsations or even could take advantage of chemical reactions to detect different targets). However, there are two important drawbacks that preclude the introduction of the device into the mouth: the response is measured using electrical circuits and CNTs are currently classified as cytotoxic nanomaterials.

4. Single-layer nanomaterials (2D)

The compounds which enter in 2D-nanomaterials classification are those who expand themselves in two directions, composed by a monolayer of atoms or by an ultrathin layer of a few. Albeit there exist several inorganic 2D nanomaterials, graphene has been by far the most used 2D material in the last years. Although graphene is considered a material composed by one single layer of atoms it is quite hard to find it with this nature, being usually found in groups of graphene layers. Depending on the number of layers the electrical, optical and mechanical properties of graphene may differ. Other important parameters that affect to the graphene behavior are its oxidation level, the number of structural defects, the purity degree, etc.[39] Values that can be controlled by the synthetic route.

Graphene can be combined with QDs working as quencher (silencing the fluorescent signal of QDs when both are approached). This property was harnessed for the fabrication of LFBs by Morales-Narváez *et al.*[15]. On their system they dispensed two lines of QDs on paper substrate, as test and control, being the first line capable of capturing some bacteria (by means of antibodies attached on QDs). After adding the sample on the LFBs it is added a solution of graphene oxide (GO), an oxidized form of graphene containing epoxy bridges, carboxyl and hydroxyl groups. GO will turn off the fluorescence of QDs in the control line and, if there are no bacteria in the

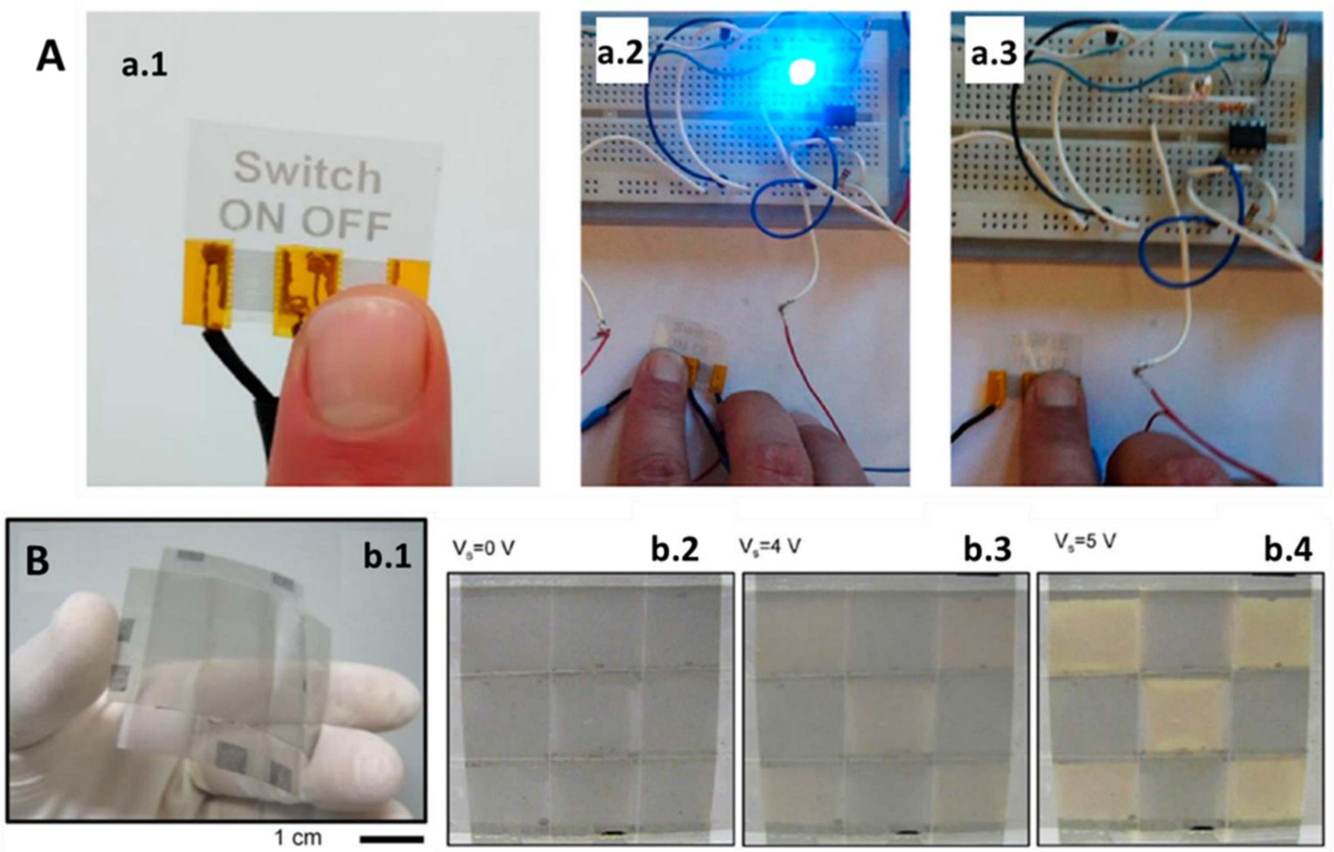

Fig. 7 (A) GO tactile device: (a.1) GO switch works by skin contact turning a LED from (a.2) ON to (a.3) OFF status. Adapted with permission from ref. 43, copyright 2016 American Chemical Society. (B) Electrochromic device composed by (b.1) flexible graphene electrodes whose transmittance is increased when applying current: (b.2) 0 V and (b.3-4) chess pattern visible at higher voltage. Adapted with permission from ref. 44, copyright 2014 Nature Publishing Group.

sample, the test line. However, in presence of bacteria conjugated onto graphene surfaces by coating pure graphene

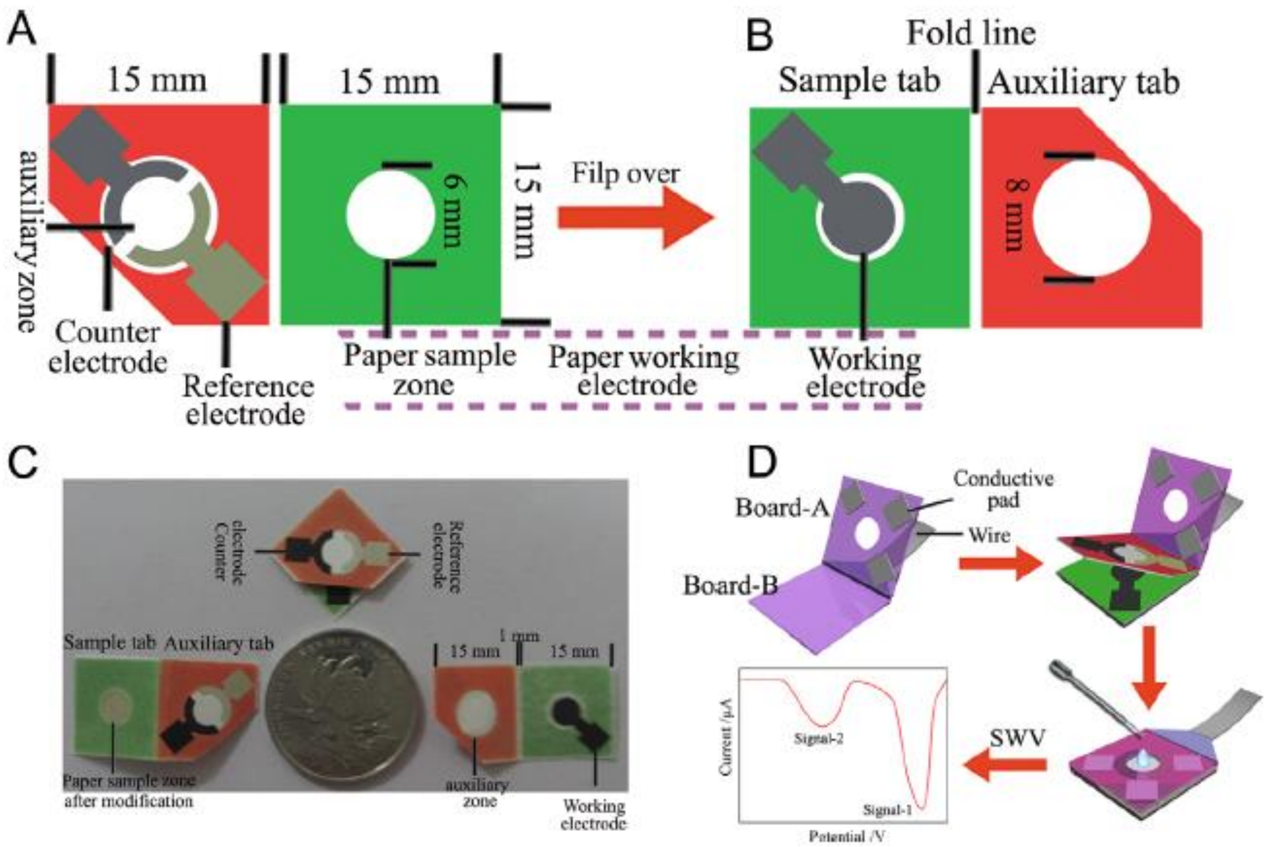

Fig. 8 Multiplex electrochemical origami paper device: (A) front part, silver reference electrode and carbon counter electrode; (B) back part, carbon working electrode; (C) size comparison of the device and (D) its application for electrochemical measurements. Adapted with permission from ref. 45, copyright 2014 Elsevier.

captured on the test line it will produce a gap between GO and QDs letting the fluorescence to emit. In comparison to traditional LFBs this method prevents the formation of false positives during the assay since a negative sample will always turn off both lines (in case that something external provokes the test line not to be quenched, control line will be also affected, obtaining an invalid strip but not a false positive). However, the assay time (more than 1 h) is longer than for standard LFBs (usually of 5-10 min) due to the extra step of GO addition and drying, including the waiting time for the bacteria to flow across the strip.

Cheeveewattanagul *et al.*[40] coated nanopaper with GO and introduced on this composite a suspension of antibodies attached to QDs. Again, the quenching effect keeps the fluorescence of QDs silenced. It is when adding an analyte (e.g. bacteria or proteins; for which the antibodies are selective) that a gap between the QDs and GO is produced, negating the quenching effect and releasing the fluorescence (Fig. 6). This technique is quite advantageous since does not require washing steps, is portable and fast, a promising alternative to ELISA tests.

Owing to its electrical properties, graphene can be used for the fabrication of working electrodes[41,42]. Antibodies can be with polymers or by exploiting the chemistry of carboxylic or hydroxyl groups in GO. Moreover, since graphene is planar, the antibodies can all be oriented perpendicularly to the graphene layer to increase the probability of capturing the analyte[41]. Going further, graphene electrodes are enough sensitive to allow label-free sensing, small changes (i.e. electrochemical[41,42] or impedimetric[39,43] alterations) on the electrode surface are easy to detect. On the other hand, as drawback, it demands a highly meticulous control on the reproducibility of graphene synthesis, especially in regard to the number of layers and structural defects.

Baptista-Pires *et al*.[43] designed a new solvent-free method for printing GO on different substrates using wax-printed patterns, printed on nitrocellulose membranes, and vacuum filtration. GO remains on the non-wax-printed areas and by pressure is transferred to the target substrate. To demonstrate the possibilities of this technique a touch sensor based on the resistance changes provoked by finger contact on the GO printed circuit was printed. This GO touch sensor is shown in Fig. 7A, connected to a LED and a power source (Fig. 7a.1): the sensor works as a simple switch, turning on/off the LED (fig. 7a.2 and 7a.3 respectively). This technology could be used to replace current touchscreens, which use controversial elements like indium and rare-Earth metals[2], and in wearable PoC applications that require flexible devices (e.g. skin tattoo sensors[34]) or those based on contact-sensing (e.g. pressure or motion sensors[38]).

Additionally to the mentioned characteristics, graphene also is an electrochromic material, it can be tuned to change its colour in a reversible way depending on the current supplied. Polat *et al*.[44] developed different electrochromic flexible devices using this property. In one of their devices they attached two plastic substrates coated by one side with graphene electrodes and a liquid electrolyte in between. When applying some voltage across the electrodes, graphene turns translucent due the voltage is enough to penetrate the graphene layers creating structural defects that fade its characteristic black colour. Their final device is shown in Fig. 7B made by various graphene electrodes. The device is flexible (fig. 7b.1) and graphene can resist a curvature of 1 cm radius without being damaged. By applying different voltage on some of the electrodes they create a chess pattern as transmittance is increased on the layers in which the current is increased: 0V (fig. 7b.2), 4V (fig. 7b.3), 5V (fig. 7b.4). Thanks to this technology, electrochemical signaling can be converted into optical responses *in situ*, thus enabling wearable PoC devices that are more user-friendly.

5. Other nanomaterials (3D)

There exist an extensive variety of 3D materials both in shape as in size, which leads to different possibilities in the final sensing application. Non-spherical nanoparticles exhibit properties quite similar to 0D nanomaterials, however the change of shape often drifts into absorbance changes (i.e. the colour of a solution of gold nanospheres may be different from a solution of nanocubes or nanotriangles, even when the chemical composition and size are the same). Taking advantage of this fact, plasmonic-based PoC sensors can be fabricated as the one reported by Fu *et al.*[4]. Their device is portable and can be coupled into a mobile phone, using its light sensor, included on most of modern mobile phones to improve photography quality or control the screen brightness. The device consists on a plate, where sample is added, and a LED that illuminates the sample in the plate and reaches the light sensor. To demonstrate the capabilities of their device they use it to measure the plasmonic changes occurred on triangular silver nanoprisms in absence of a cancer biomarker, indirect detection method, by provoking a shape transformation on the nanoparticles with hydrogen peroxide. Li *et al.*[45] used another 3D silver nanomaterial too, nanoporous silver which can be used as signal enhancer, label and metallic ions carrier. Their system consists in a multiplex electrochemical origami paper device (Fig. 8) that uses nanoporous silver loaded with different metallic ions as label for tumor sensing. Silver electrode is used as reference (Fig. 8A) and screen printed carbon electrodes as counter and working electrodes (Fig.8B). The paper device can be folded (fig. 8C) and integrated into an electrical circuit (fig. 8D).

Some non-spherical nanoparticles exhibit electrochromic

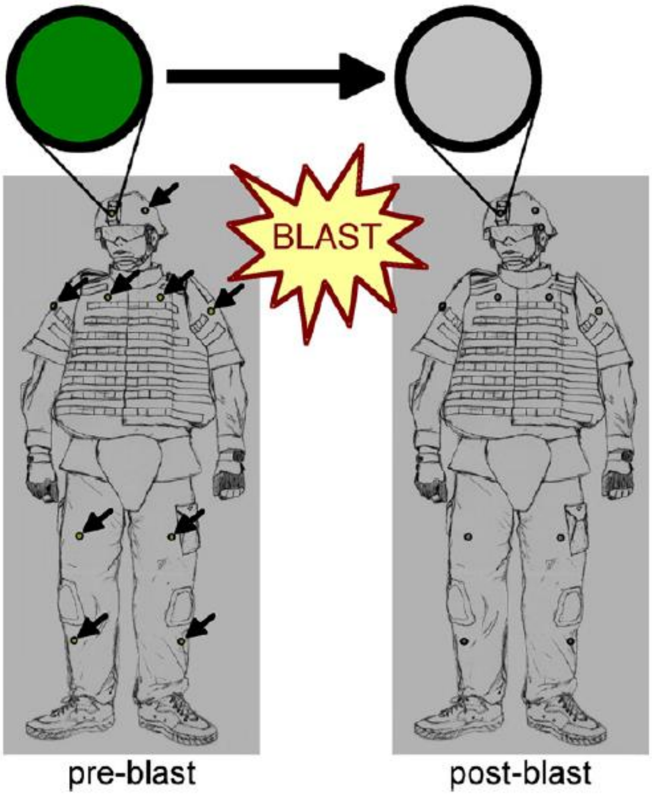

Fig. 9 PC-based wearable that changes colour in response to the shock wa

Table 1 Summary of nanomaterial types: examples, corresponding PoC detection principles, advantages and disadvantages.

properties, as WO_3 nanoparticles, used by Marques *et al.*[46] on paper substrate. The nanoparticles change from yellow to blue colour in presence of electrochemically active bacteria. Thus, this is a simple sensor able to work as ELISA assays but without requiring long-time steps or the use of delicate reagents (i.e. biological reagents have short expiration dates, while nanomaterials can last long periods of time even stored at room temperature). Photonic crystals (PC) are other example of electrochromic materials often composed by other nanoparticles that are assembled following a crystalline pattern. These nanomaterials have demonstrated good adaptability in different types of sensors due to their resistance to being bent[47], being used in microfluidic systems, both in polymeric channels[48] or in paper[49], permitting label-free sensing (colour change occurs when the analyte attaches or passes near the PC). Cullen *et al*.[50] converted PC into simple PoC wearable stickers that can be settled on clothes and warn its user, in war zones, about possible aftermath related to expansive wave of a blast (Fig. 9). PC are broken when brought under high pressure as consequence of an explosion thus changing the colour, a notification to the user that the blast could have induced non-visible injuries as internal traumatisms or brain damage.

6. Conclusions

Day by day PoC devices are becoming indispensable tools for diagnostics required not only by medical specialists at hospitals or doctor's office but from anyone even at home or outdoors. Despite most of the discussed devices are still demonstrated as proof-of-concept it is clearly shown that nanomaterials are bringing several advantages while being integrated within PoC systems.

Table 1 summarizes the different nanomaterial types, with the most common examples of each classification, the possible detection methods that the final PoC could employ and some of the strengths and weaknesses for each case. Depending on the properties of nanomaterials and the way how these are integrated within PoC devices various detection technologies are used being optical and electrochemical ones the most reported.

While electrical techniques employing nanomaterials as electrodes or electrocatalysts are well known for bringing higher sensitivity and lower detection limits optical techniques are emerging once again given their simplicity, easy integration within paper/plastic platforms including easy and efficient coupling with smartphone.

The synergy of nanomaterials with a variety of biosensing systems and communication technologies is excepting to bring innovative PoC systems that may go out of the research labs to fulfil the ASSURED (Affordable, Sensitive, Specific, User-friendly, Rapid & Robust, Equipment-free and Deliverable to end-users) criteria by WHO[6] for applications in diagnostics.

Conflicts of interest

There are no conflicts of interest to declare.

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Nanomaterial type	**Examples of nanomaterials**	**PoC detection principle**	**Advantages**	**Disadvantages**
0D	• AuNPs • AgNPs • QDs • UPCs • Magnetic nanoparticles	• Optical • Fluorescent • Electrochemical • Magnetic	• Simple synthetic procedures • Small size • Easy to bioconjugate • Adaptable into other nanomaterials	• No special structural properties • Some 0D nanomaterials can easily agglomerate
1D	• Nanowires • CNTs	• Electrochemical • Motion	• Highly conductive • Orientable • Structurally resistant	• Complex synthesis • Difficult to control their shape
2D	• Graphene	• Electrochemical • Fluorescence • Tactile	• Easy to modify • Flexible • Fluorescence quencher	• Difficult to separate a single graphene sheet • Structural defects are common
3D	• Non-spherical nanoparticles • PCs	• Optical • Electrochemical	• Can combine the properties of other nanomaterials • Tunable optical properties	• Like 0D nanomaterials, 3D nanomaterials can easily agglomerate • Usually, not as small as 0D nanomaterials

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