Magneto Actuated Biosensors for Foodborne Pathogens and Infection Diseases Affecting Global Health

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Abstract Infectious diseases are responsible for hundreds of thousands of deaths and an enormous burden of morbidity worldwide. Although most of these major infectious diseases are treatable, the accurate and rapid identification of the pathogens remains a major issue to disease control, since the incidence of infectious disease would be reduced if appropriate diagnostic tests were more widely available in the developing world as well as in low-resource settings in the developed world. Strong research efforts are thus being focused on replacing standard diagnostic and monitoring methods, by affordable and sensitive tests able to be performed at the community and primary care level. The development of new methods that are needed includes solid-phase separation techniques. In this context, the integration of magnetic particles within bioassays and biosensing devices is very promising since they greatly improve the performance of the biological reaction. The diagnosis of complex samples with magnetic particles can be easily achieved without any purification or pretreatment steps often required for standard methods, simplifying the analytical procedures. The target can be thus specifically preconcentrated from complex matrixes by magnetic actuation, increasing the specificity and the sensitivity of the assay. This chapter addresses these promising features of the magnetic particles for the detection of biomarkers in emerging technologies related with the major problems facing the global health, including pathogenic bacteria occurring in food outbreaks, in order to ensure safety in food and water supplies in low resources settings, as well as major global infection disease in low-incomes countries, such as malaria or AIDS.

Keywords Magnetic particles · Magnetic actuation · Immunosensors · DNA biosensors · Global health · Foodborne pathogens · Malaria · AIDS

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1 Introduction

1.1 Global Health: A Challenge for Key Enabling Technologies

Converging technologies result from the synergistic merging of nanotechnology; biotechnology, information technology, and cognitive science (NBIC) [1]. Information and communication technology helped produce the profound transformation of daily life in the 20th Century. Biotechnology is transforming agriculture, medical diagnosis and treatment, human and animal reproduction while the impact of nanotechnology is under intensive assessment. Recently the EU identified nanotechnology, advanced materials, industrial biotechnology, among others, as 'cross-cutting' KETs (acronym of Key Enabling Technologies), which are technology bricks that enable a wide range of applications [2]. The convergence and integration of these profoundly transformative Key Enabling Technologies is the first major research initiative of the 21st Century due to their potential for solving societal challenges. Despite the impact of the KETs in daily life, the burden of infectious disease is still an issue, affecting global health.

Annually, just under 5 million people die from AIDS or complications related to AIDS and tuberculosis, 2.9 million from enteric infections and 1 million from malaria [3]. Globalisation and population movement have accelerated the spread of infectious disease outbreaks around the world that initially were geographically localised [4]. In most cases, early diagnosis and treatment can interrupt the transmission of the infectious agent and prevent the development of long-term complications.

These infectious diseases are treatable, and access to drugs has improved markedly over the past decade with the advent of drug-access campaigns, mass-treatment programmes and public resources promoting by the United Nations Millennium Development Goals towards Global Health. Global health is defined as 'the area of study, research and practice that places a priority on improving health and achieving equity in health for all people worldwide' [5]. It was also defined as 'public health without borders' [6]. Most global health centers are in high-income EU countries with strong links with low-income countries [7], due to the investing support of the European Parliament in R&D for global health. By supporting this essential and challenging area of European innovation, is also possible to strengthen the EU economy, providing a competitive advantage for European industry and research. Improving the health not just of Europeans but globally will have positive effects on health systems, employment, and global health security [6].

However, and despite this major initiative, the need for accurate identification of the agents affecting global health remains a major stumbling block to disease control, and the burden of infectious disease could be substantially reduced if appropriate diagnostic tests were more widely available.

1.2 Traditional Methods for the Detection of Food Borne Pathogens and Infection Diseases

Conventional microbiological culture techniques are currently the gold standard for isolation, detection, and identification of microorganisms, usually involving a morphological evaluation of the microorganism as well as tests for the organism's ability to grow in various media under a variety of conditions. These methods are time-consuming, consisting in the following steps: pre-enrichment, selective enrichment, selective plating, biochemical screening and serological confirmation. Although they are considered to be reliable, they are also laborious and might introduce sampling and enumeration errors at low bacteria concentration [8]. One of the main drawbacks of conventional culturing relies on the fact that some of the microorganisms are slow growing or extremely dangerous, requiring special safety facilities. In these instances, identification is determined by the serological detection of the immune response (antibodies) against the infectious agent. More recently, standard culture-based pathogen detection methods have been refined and even improved, with an eye towards reducing the time to detection. This is generally done by replacing the selective and differential plating step with more rapid immunological or molecular-based assays. Among these, the Immunological assays (IAs), DNA hybridisation, and polymerase chain reaction (PCR) methods should be highlighted. IAs rely on the specificity of the antigen antibody recognition, being suitable for the detection of whole range of agents affecting global health. In particular, Enzyme Linked ImmunoSorbent Assays (ELISAs), such as sandwich with direct and indirect enzymatic labelling are the most common formats used for the detection of pathogens. Therefore, IAs are advantageous for decreasing the assay reaction time in comparison with microbiological culturing techniques, providing also the possibility of being easily integrated in automated equipments, which consists an important advantage for many applications. Moreover, ELISA is widely used in clinical diagnostics for the detection of a broad range of biomarkers due to its relatively robustness, versatility and high-throughput. ELISA methods have been approved by regulatory agencies, being commercially available. Nevertheless, the efficiency of an immunoassay is strongly dependent on the antibodies affinity and specificity towards the target. The risk of antibody cross reactions consists of a disadvantage of immunological assays by increasing the possibility of false positive results or high background signals [9]. Moreover, the good performance of this assay depends on operator skills. For instance, in the case of foodborne pathogens, the limit of detection (LOD) are normally in the range of 10^4 and 10^5 CFU mL^{-1} and the assay time can take around 48 h, since a pre-enrichment step is commonly required in order to achieve the threshold limits for the presence of the bacteria on food samples [9].

The development of molecular diagnostic techniques represents a great advance in the diagnosis and follow-up of infectious diseases [10]. Nucleic acid-based detection may be more specific and sensitive than immunological detection. Nucleic acid amplification methods include end-point polymerase chain reaction (PCR) and

real-time PCR (qPCR) for single or multiplex detection. PCR allows the production of multiple copies of DNA from the amplification of a single copy or a few copies of a DNA template. Due to its high sensitivity, nucleic acid amplification has been widely used for the identification and detection of pathogens, being considered as an alternative to conventional microbiological culture techniques.

Furthermore, PCR can be easily coupled to enhance the sensitivity of nucleic acid-based assays, especially for slow growing or hazardous microorganisms. Reverse transcriptase polymerase chain reaction (RT-PCR) has also played an important role in diagnosing RNA-containing virus infections. As occurred in the immunological assays, PCR methodologies for foodborne pathogens usually require an enrichment step, being able to detect, for instance in the case of *Salmonella*, few CFUs in 25 g of food product. The fact that this methodology does not discriminate between live of dead bacteria are pointed out as the main limitations [11].

Hence, it was shown that traditional methodologies can be sensitive for food microbiological control and infection diseases. However, they are relatively complex, and therefore technically demanding and costly, requiring skilled personnel, regular maintenance or reliable electric supply. Therefore, new methodologies are needed for low-resource settings, accordingly to the *ASSURED* recommendation published by World Health Organization (WHO), this acronym being defined by Affordable, Sensitive, Specific, User-friendly, Rapid and Robust, Equipment free, and Deliverable to those who need it [3], as it will be discussed in the next section. Beside all these challenges in diagnostic tests in low-resource settings, the same technology would be very useful for point-of-care in small health centers in middle and high-income countries for getting the result back at the moment, as well as for following-up and prescribing the correct treatment without delay avoiding thus high test turnaround time.

1.3 Technical Challenges in Diagnostic Tests in Low-Resource Settings

Global health diagnostic tests must have low complexity without any lost in diagnostic accuracy in a format that is practical in low-resource settings. The complexity of a test includes the need for user interpretation, the level of training necessary, the number of manual manipulations, the number of user intervention steps required, and the instrumentation requirement [12]. In this direction, the FDA defines the characteristics that a simple test for the near-patient diagnostic should ideally have [13]. Interestingly, a quick reference instruction sheet that is written at no higher than a 7th grade reading level is recommended. As previously stated, the characteristics of new platforms for diagnostics to meet the United Nations Millennium Development Goals related with global health for reducing the burden of disease are summarized under the acronym ASSURED defined by the WHO

(Affordable; Sensitive; Specific; User-friendly; Rapid and Robust; Equipment-free; and, Delivered to those who need it).

The development of novel diagnostic platforms to meet the WHO requirements should therefore be focused on: (A) Cost-effective emerging technologies such as biosensors, lateral flow and agglutination tests, appropriate for application at community and primary-care level as well as in low resource settings; (SS) novel diagnostic targets and disease biomarker development, to achieve specificity (no false positives) and sensitivity (no false negatives); (U) analytical simplification in order to minimise pipetting, washing steps and manipulation of reagents to provide analytical tools requiring minimal training for final users; (R) robust tests, portable and stable at room temperature, able to provide rapid results and to enable taking actions immediately such as treatment at first visit; (E) bench-top and high cost instrumentation should be avoided and visual detection should be prioritised; (D) biomarkers should be selected from the major problems facing global health, including pathogenic bacteria occurring in food outbreaks, in order to ensure safety in food and water supplies in low resource settings, as well as major global infection disease such as AIDS and malaria, dengue, influenza and tuberculosis in low-income countries. To meet the demands, the dominant format currently in use is lateral-flow immunochromatographic immunoassay strip tests. Despite the poor sensitivity levels and the high reported LODs [14] generally observed for this technology, many methodological improvements has been done [15–18]. As complexity increases ranging from agglutination [19, 20], lateral flow [16], ELISA [21–23] and PCR [24], sensitivity and specificity also increase but so do cost and turnaround time, as well as the need of instrumentation [25]. The main challenge in bioanalysis is thus to provide low-cost yet simple methods wit any loss in the analytical performance and test accuracy [3].

As previously discussed, the amplification of target nucleic-acid sequences using techniques such as PCR can improve test sensitivity up to 100-fold over antigen detection tests, such as ELISA. However, the first generation of nucleic-acid amplification technologies requires instrumentation for temperature cycling [12]. Single-temperature or isothermal amplification has been developed and can be adapted to a point-of-care format [26, 27]. Improved efficiency of detection systems must also be achieved, using novel hybrid bionanomaterials or signal amplification strategies [28–30]. These bionanomaterials, including nanostructured carbon materials, inorganic nanoparticles (i.e., semiconducting, noble metal and magnetic nanoparticles), among others, appears to be keys in multiplex detection enhancing the biological reactions, providing high selectivity and improving the LODs [31–34].

A prominent development trend in recent years has been to miniaturize or integrate existing diagnostics into a biosensors devices or lab-on-a-chip [30, 35, 36]. These strategies potentially solve many issues by lowering test complexity in a platform that would be practical at the point of care. Cost is also reduced by using lower reagent volumes that would be housed and stored in a kit format. Microfluidic systems provide several advantages such as portability, lower reagent consumption, rapidity and possibility for automation. Usually, these systems are combined with agarose gel electrophoresis for DNA analysis, but they can also be coupled with

other platforms [37]. The cost of production of microfabricated devices, requiring in most cases bench-top equipment for the readout, still constitutes a bottleneck and may put them out of range for end users in the developing world.

Biosensors are analytical devices, incorporating a bioreceptor in contact with a transducer. Despite the massive use of glucose biosensors with electrochemical transduction, examples of other commercial devices for applications including diagnosis of infection diseases are currently very limited. Although instrumentation may be required, it should be designed to be low maintenance, battery operated, and low cost [13].

Besides the progress in emerging technologies for diagnosis, the study of novel biomarkers for the early detection of infectious diseases is a worldwide challenge [38]. The WHO has defined a biomarker as 'almost any measurement reflecting an interaction between a biological system and a potential hazard, which may be chemical, physical, or biological' [39]. The measured response may be functional and physiological, biochemical at the cellular level, or a molecular interaction [40]. The identification of novel biomarkers represents a challenge not only for the improvement of early diagnostics, but also for patient monitoring and for evaluation of the efficiency of a therapeutic strategy. Biomarkers are also very promising candidates in achieving customisation of healthcare for personalised medicine.

To summarize, some of the new diagnostic tests that are needed are already on the market, but have not been adequately validated. In other cases, diagnostic targets and biomarkers have been identified, and it seems likely that an adequate test could be developed using existing technology. In these two cases, feasibility is high [3]. In other cases, the biomarkers have not yet been identified and, feasibility is thus low.

2 Magnetic Particles in ASSURED Diagnostic

Novel development in diagnosis that is needed involves preconcentration procedures on solid supports which can be easily integrated with emerging technologies. Microorganism and biomarkers in complex samples can be thus preconcentrated while the interfering matrix is removed at the same time, increasing the sensitivity and the specificity of the test. One of the most prominent materials to meet this challenge is magnetic particles (MPs) [41]. MPs can be tailored to specifically bind the biomarkers and concentrate them from the complex specimen under magnetic actuation, avoiding interference before testing [42–44].

Magnetic particles have been commercially available for many years. Nowadays several companies offer a wide range of products based on MPs, such as Adembeads® [45], Dynabeads® [46], BioMag® [47], SiMAG® [48], MACS® [49], among many others, which are widely used in laboratories to extract and preconcentrate desired biological components, such as cells, proteins, organelles or DNA, from a liquid. As shown in Fig. 1 they consist of an inorganic core of magnetic

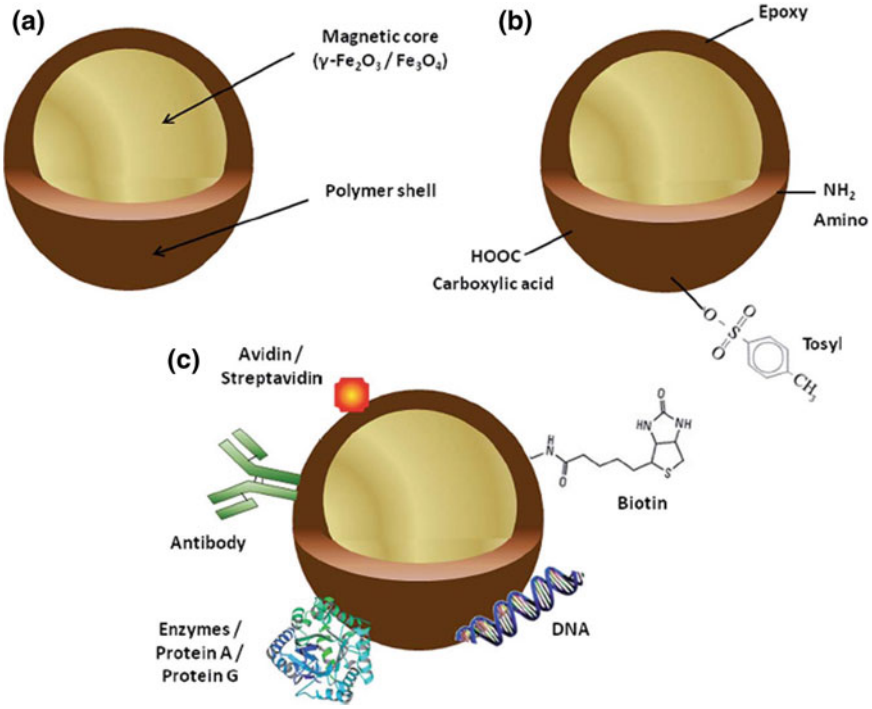

Fig. 1 Schematic representation of magnetic particles (**a**), activated with functional groups (**b**) and conjugated to biological molecules (**c**)

materials such as iron, nickel, cobalt, neodymium–iron–boron, samarium–cobalt or magnetite coated with polymer to confer stability (such as polystyrene, dextran, polyacrylic acid, or silica), which can be modified with functional groups to make subsequent conjugations easier. Although there are commercially available MPs already functionalised with biomolecules for a variety of bioanalytical and biotechnology applications, they can be modified with specific receptors, using surface chemical groups such as tosyl, amine, carboxyl or epoxy, for the identification/isolation of biomarkers including whole organisms, proteins and peptides, antibodies, DNA, among others [50]. Hence, magnetic particles can be tailored-modified with a whole range of ligands, including peptides, small molecules, proteins, antibodies, and nucleic acids.

Magnetic particles can have any size from a few nanometers up to a few micrometres. Nano-sized particles (5–50 nm) are usually composed of a single magnetic core with a polymer shell around it. Larger particles (30 nm–10 mm) are composed of multiple magnetic cores inside a polymer matrix. These particles can be used for efficient transport, faster assay kinetics, improved binding specificity and as labels for detection [51].

In the last decade, extensive research has been done on the integration of micro- and nanomaterials in magneto actuated platforms. In particular, superparamagnetic particles are highly attractive to be used in magneto-actuated devices due to their capability to magnetise under an applied magnetic field. Thus, the particles can be separated easily from the liquid phase with a small magnet, but can be redispersed immediately after the magnet is removed [52]. They confer a number of benefits, including easy separation and suitability for automation. When coated with recognition molecules, MPs are ideal for efficient capture and separation of the target. Unwanted sample constituents may be washed away, following a simple magnetic separation step.

In particular, antibody-coated superparamagnetic particles are used for the immunomagnetic separation (IMS) of proteins, viruses, bacteria and cells. Immunomagnetic separation has been proven to be a very efficient method for separating target from complex samples including food or biological samples such as whole blood. Several procedures may be used for subsequent final detection, raging from conventional culturing, microscopy, impedance technology, ELISA, latex agglutination or DNA hybridisation involving amplification techniques. Since IMS can be used in conjunction with different readout technologies, it is expected that several automated analytical procedures will make use of this potent technique in the near future [53, 54].

To summarize, the integration of MPs can simplify the analytical procedure, avoiding the use of classical centrifugation or chromatography separation strategies, since no pre-enrichment, purification or pretreatment steps, which are normally used in standard analytical methods, are required. Moreover, their use as solid support in bioassays has been shown to greatly improve the performance of the biological reactions.

3 Electrochemical Biosensors

The development of biosensors is a growing area, in response to the demand for rapid real-time, simple, selective and low cost techniques for analysis. Biosensors are compact analytical devices, incorporating a biological sensing element, either closely connected to, or integrated within, a transducer system. The combination of the biological receptor compounds (antibody, enzyme, nucleic acid) and the physical or physico-chemical transducer producing, in most cases, “real-time” observation of a specific biological event (e.g. antibody–antigen interaction) [55]. Depending on the method of signal transduction, biosensors can also be divided into different groups: electrochemical, optical, thermometric, piezoelectric or magnetic [56]. They allow the detection of a broad spectrum of analytes in complex sample matrices, and have shown great promise in areas such as clinical diagnostics, food analysis and environmental monitoring [57, 58]. The sensitivity of each of the sensor systems may

vary depending on the transducer's properties, and the biological recognizing elements. An ideal biosensing device for rapid diagnostic should be fully automated, inexpensive and routinely used both in the field and the laboratory. Optical transducers are particularly attractive as they can allow direct "label-free" and "real-time" detection, but they lack of sensitivity. The phenomena of surface plasmon resonance (SPR), has shown good biosensing potential and many commercial SPR systems are now available. The Pharmacia BIAcore™ (a commercial surface plasmon resonance system) is by far the most reported method for biosensing of molecular contaminants in food and it is based on optical transducing [59, 60]. The detection of food pathogens by SPR, however, do not reach the required LOD to allow food safety without performing a preenrichment step [61].

Electrochemically based transduction devices are more robust, easy to use, portable, and inexpensive analytical systems [62–64]. Furthermore, electrochemical biosensors can operate in turbid media and offer comparable instrumental sensitivity. The electrochemical measurement system is highly sensitive, cheap, and has already been available in portable formats. Even the advanced pulsed, voltammetric and galvanostatic techniques are available in hand-held instruments from several companies: PalmSens and EmStat (Palm Instruments), mStat (DropSens), PG581 (Uniscan Instruments), 910 PSTAT mini (Metrohm), as well as other prototypes designed in laboratories [65]. As the measuring element, the screen-printed electrodes (SPE) are widely applied due to easy and reproducible fabrication at both laboratory and mass production scales [66, 67]. The suppliers of SPEs include companies such as BVT Technologies, DropSens and The Gwent Group. However, researchers can print the sensing patterns themselves using commercial inks and pastes or even using custom mixtures containing carbon nanotubes [68] and metal nanoparticles [69] for enhanced response.

Rigid conducting graphite-epoxy composites (GEC) based on graphite microparticles have been extensively used in our laboratories as electrochemical transducer and shown to be suitable for electrochemical (bio)sensing due to their unique physical and electrochemical properties [70]. Carbon composites result from the combination of carbon with one or more dissimilar materials. Each component maintains its original characteristics while conferring upon the composite distinctive chemical, mechanical, and physical properties. The user's ability to integrate various materials is one of their main advantages.

An ideal material for electrochemical biosensing should allow the effective immobilization of bioreceptor on its surface, a robust biological reaction between the target and the bioreceptor, a negligible non-specific adsorption of the label, and a sensitive detection of the biological event. GECs fulfill all these requirements. Other advantages of GEC-based biosensing devices over more traditional carbon-based materials are: higher sensitivity, robustness, and rigidity in addition to greater simplicity of preparation. Additionally, the GEC surface can be regenerated by a simple polishing procedure. Unlike carbon paste and glassy carbon, the malleability of the GEC material before the curing step permits different

configurations with respect to shape and size which are then fixed after the curing step. Moreover, the surface of the composite can be easily modified by dry and wet adsorption of the bioreceptor (DNAs, oligonucleotides, proteins, antibodies), yielding a reproducible and stable layer of bioreceptor on the transducer surface [71] that can be used in electrochemical detection.

An additional interesting property of GECs is their biocompatibility. This feature allows not only adsorption but also integration of the bioreceptor into the bulk of the GEC without subsequent loss of the receptor's biological properties, thus generating a rigid and renewable transducing material for biosensing, namely, a graphite-epoxy biocomposite (GEB). With the bioreceptor integrated within its bulk, the biocomposite acts as a reservoir for the biomolecule while retaining all the interesting electrochemical and physical features previously described for GECs. The main advantage of GEBs is that they can be easily prepared by adding the bioreceptor to the composite formulation using dry-chemistry techniques, thereby avoiding tedious, expensive, and time-consuming surface immobilization procedures. Moreover, the surface of GEB electrodes can be easily modified with DNA, oligonucleotides, proteins, antibodies for electrochemical detection.

The use of affinity proteins such as avidin, protein A or protein G, in the biocomposite provides a robust platform for the oriented immobilization of DNA or immunospecies that improves the performance of the electrochemical biosensing devices by ensuring exposure of the bioreceptor to the complementary sites of the target molecule [72, 73]. After its use, the electrode surface can be renewed by a simple polishing procedure, thus allowing multiple uses—a further advantage of these materials with respect to surface-modified approaches such as classical biosensors and other common biological assays.

The integration of gold nanoparticles in a graphite-epoxy composite (nanoAu-GEC) has been also proposed as an alternative to continuous gold surfaces films as this strategy avoids the need for stringent control of surface coverage parameters during immobilization of thiolated oligos or antibodies. In this transducer, islands of chemisorbing material (AuNPs) surrounded by a rigid, non-chemisorbing, conducting GEC are obtained [74]. The spatial resolution of the immobilized thiolated DNA can be easily controlled by varying the percentage of gold nanoparticles in the composition of the composite. Moreover, as with GEBs, the surface of nanoAu-GEC electrodes can be easily modified with DNAs, oligonucleotides, proteins, antibodies, etc., for electrochemical detection.

To summarize, electrochemical immunosensors and genosensors can meet the demands summarized under the acronym ASSURED defined by the WHO, offering considerable promise for obtaining information in a faster, simpler and cheaper manner compared to traditional methods. Such devices possess great potential for numerous applications, ranging from decentralized clinical testing, to environmental monitoring, food safety and forensic investigations.

4 Magnetic Immobilisation Coupled with a Magneto-Actuated Electrode for Electrochemical Biosensing

As previously discussed, one of the most promising materials in bioanalysis is biologically modified magnetic particles, the use of which is based on the concept of magnetic bioseparation. Magnetic particles offer several novel attractive possibilities in biomedicine and bioanalysis since they can be coated with biological molecules and manipulated by an external magnetic field gradient. As such, the biomaterial, i.e., specific cells, proteins, or DNA, can be selectively bound to the magnetic particles and then separated from its biological matrix by applying an external magnetic field. The integration of MPs and electrochemical biosensing strategies improves analytical performance. Instead of direct modification of the electrode surface, both the biological reactions (immobilization, hybridization, enzymatic labeling, or affinity reactions) and the washing steps can be successfully performed on the MPs. After the modifications, the particles are easily captured by applying a magnetic field onto the surface of the GEC electrodes, which contain a small magnet (m-GEC) designed in our laboratories.

The preparation of the m-GEC electrode consists on different steps that are schematically explained in Fig. 2. To a female electric connector with a metal end of 2 mm diameter (i), a metal screw (6 mm d) is fitted (ii). After that, a copper disk with a diameter of 5.9 mm is placed on the female electric connector end (iii) with a welder using Sn wire. A cylindrical PVC tube (iv) (6 mm id, 8 mm od, 21.5 mm long) is then place over the female electric connector. A gap with a depth of 3 mm is thus obtained in the end of the body electrode, which is then filled with a thin layer of the graphite-epoxy composite (GEC) paste (v). The 3 mm diameter neodymium magnet is then placed in the center (vi). The electrode body gap is then completely filled with the soft GEC paste (vii). The electrode is then cured at 90 °C for 3 days until the paste become completely rigid. The electrodes can be stored in a dried place at room temperature.

This magneto-actuated electrode constitutes a versatile platform for electrochemical biosensing (both genosensors and immunosensors) for a broad range of application, including the detection of food contaminants such as pesticides, [75] antibiotic residues, [76] bacteria, [77, 78] food additives, [79] allergens, [80] or diseases biomarkers, such as malaria [81] or CD4 cells for AIDS monitoring [82]. In all cases, the electrochemical readout is achieved using horseradish peroxidase (HRP) as electrochemical reporter. Enzyme labelling has been transfer from non-isotopic classical methods to electrochemical biosensing. In electrochemical genosensing, the DNA duplex can be labeled with either strept(avidin)-HRP or antiDIG-HRP conjugates, depending on the tag of the DNA signaling probe (biotin or digoxigenin, respectively). Although a second incubation step is usually required for labelling, higher sensitivity and specificity have been reported for the enzyme

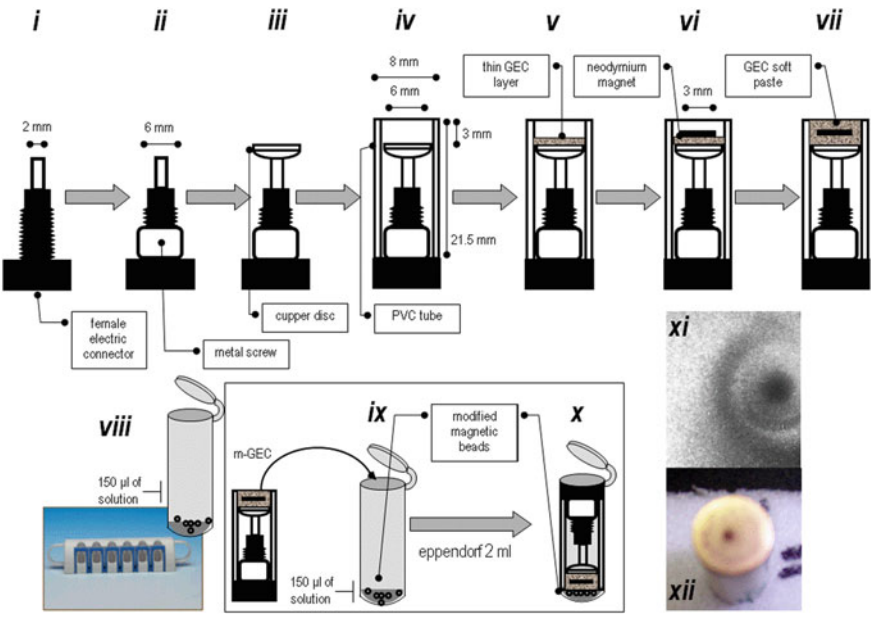

Fig. 2 Schematic representation of the construction of m-GEC (steps *i–vii*) and manipulation of the m-GEC electrodes, comprising the immobilization of DNA on magnetic beads (*viii*) following by capturing the modified beads on m-GEC electrode (*ix* and *x*). Scanning electron microphotographs showing the captured magnetic beads on the surface of m-GEC magneto sensor (500 µm, 15 kV) and a photograph showing the aspect of the sensor with the immobilized beads are also shown (*xi* and *xii*, respectively) (Number of magnetic beads: 6.2×10^6)

labelling method compared with the other reported methods [83, 84]. In electrochemical immunosensing, the enzymatic tag depends on the format of the immunoassay. In competitive immunoassays for small haptenic molecules, it is usually a conjugate obtained with HRP and the hapten. In other immunological formats, such as in sandwich assays or indirect approaches, the enzymatic label is typically a conjugate obtained with HRP covalently linked to the Fc part of the specific antibody. In all cases, amperometric determination is finally based on HRP activity following the addition of H_2O_2 and using hydroquinone as mediator. The modified electrode is immersed in the electrochemical cell containing hydroquinone and, under continuous magnetic stirring, a potential of –0.100 V vs. Ag/AgCl is applied. When a stable baseline is reached, H_2O_2 is added into the electrochemical cell (to a concentration able to saturate the total amount of enzyme employed in the labeling procedure) and the current is measured until steady state is reached (normally after 1 min of H_2O_2 addition).

5 Electrochemical Biosensors of Agents Affecting Food Safety

The World Health Organization (WHO) have considered Food Safety as the main topic of World Health´s Day in 2015 [85, 86]. It is estimated that two million deaths occur every year worldwide from contaminated food or drinking water, of each 200 are related to foodborne diseases, caused by harmful bacteria, viruses or parasites [87]. The emergence of foodborne infectious diseases in humans worldwide is attributed to several causes, such as the loss of biodiversity due to an intensive agriculture, food industry and land changes, together with the evolution of drug resistance [88]. Moreover, climatic factors related to the increase of the average global temperature and consequently to the raise of CO_2 concentrations, as well as precipitation changes have implications for food production, since they can potentiate a higher growth and survival of pathogenic microorganisms leading to the occurrence of food safety hazards [87, 89]. The socioeconomic status impact on food safety has also been studied, showing that low income individuals are more exposed to foodborne illness related to a poor hygiene and proper food handling practices and nutrition [90]. In Europe, over 320,000 human cases are reported each year being mostly related to the presence of pathogenic microorganisms in meat products, as well as fruits and vegetables, being *Salmonella* spp., *Escherichia coli* and *Listeria monocytogenes* the most common reported pathogens [91]. *S. enterica* sv. *Enteritidis* and *Typhimurium* are a frequent cause of foodborne outbreaks among *Salmonella* serovars [92]. One of the most recent cases was reported in August 2014, a multi-country outbreak reported in Austria, France and Luxembourg associated with eggs from Germany [86, 93].

One of the most serious outbreaks in Europe related to *E. coli* was reported in Germany, with a total of 3126 cases of diarrheal disease, including 17 deaths in Germany and additional extension to other countries as Norway, USA, Canada and Switzerland. It was caused by a Shiga toxin-producing E. coli (STEC) strain found in meat and fenugreek sprouts. This *E. coli* strain belongs to O104:H4 serotype, having the ability to excrete a shiga toxin which is lethal to humans [94, 95]. L. monocytogenes is a Gram positive pathogen that can survive in different environments, such as low temperatures and pH values or high salt concentrations. In the years of 2008 to 2012 an increase of number of listeriosis cases reported in the EU was registered with a total of 198 deaths in 2012 [96, 97].

"Make food safe" becomes a vital task worldwide, as a result, preventive approaches like Good Agricultural Practices (GAPs), Good Manufacturing Practices (GMPs), Hazard Analysis and Critical Control Point (HACCP) and the food code (Codex Alimentarius) have been implemented, which can considerably reduce the survival of pathogens during the process of handling, preparation and storage processing. These approaches include measures for the introduction of methods for decontamination, disinfection and cleaning, the implementation of analytical methods for screening feed and feed ingredients, to provide more inspection and control at industries and farms, as well as the implementation of communication strategies for

consumers and the food industry [98, 99]. Examples of regulatory agencies or centers worldwide are the WHO, the US Centers for Disease Control and Prevention (CDC), the US Food and Drug Administration (FDA), the Public Health Agency of Canada (PHAC), the European Food Safety Authority (EFSA), the European Centre for Disease Prevention and Control (ECDC), OzFoodNet, PulseNet International (PNI), National Institute of Public Health, Japan, among many others. Identification and detection of foodborne bacteria is in general required for routine surveillance and monitoring, evaluation of the most common food sources responsible for specific foodborne, during regulatory actions or from investigation of a foodborne outbreak. A wide range of methods are available for foodborne bacteria identification and detection, in connection with these programs, for the prevention and identification of problems related to health and safety. The choice of the method is a key factor for the detection of foodborne pathogens and the intended use of the method, for instance whether for a qualitative or semi-quantitative screening, quantitative and/or confirmatory analysis, must be clearly defined [100]. Additionally, an ideal method should be rapid, providing results in a few hours, easy handling, accurate, applicable to several food matrices and foodborne bacteria [98–100]. Electrochemical biosensors are good candidates to meet these demands. The latest development that combines the use of MPs for preconcentration and electrochemical detection is of particular interest due to the considerable improvement achieved on the analytical features such as assay time and limit of detection. In this case, an immunomagnetic (IMS) or phagomagnetic (PMS) separation is integrated in electrochemical magneto genosensing and immunosensing approaches. Figure 3 summarized the scheme of three different strategies based on IMS/PMS separation coupled with electrochemical genosensing and immunosensing taking *Salmonella* as a model of food pathogen. In these approaches, magnetic particles have the dual function of (i) pre-concentrating bacteria from the complex matrix, using different biorecognition reactions (immunomagnetic (IMS) and phagomagnetic (PMS) separations) and (ii) improving the analytical features of both electrochemical genosensing and immunosensing of bacteria.

5.1 Immuno (IMS) and Phagomagnetic Separation (PMS) Coupled with Electrochemical Genosensing on Magneto Actuated Electrodes

The first approach, summarized as a "IMS/double-tagging PCR/m-GEC electrochemical genosensing" [101] was based on a double biorecognition of bacteria, in this case immunological followed by genetic biorecognition. The procedure consisted briefly of the following steps, as depicted in Fig. 3: (i) Immunomagnetic separation of the bacteria from food samples; (ii) Lysis of the bacteria and DNA separation; (iii) DNA amplification of Salmonella IS200 insertion sequence by double-tagging PCR; (iv) immobilization of the doubly-tagged amplicon in which the biotin tag of the dsDNA amplicon is immobilized on the streptavidin MPs;

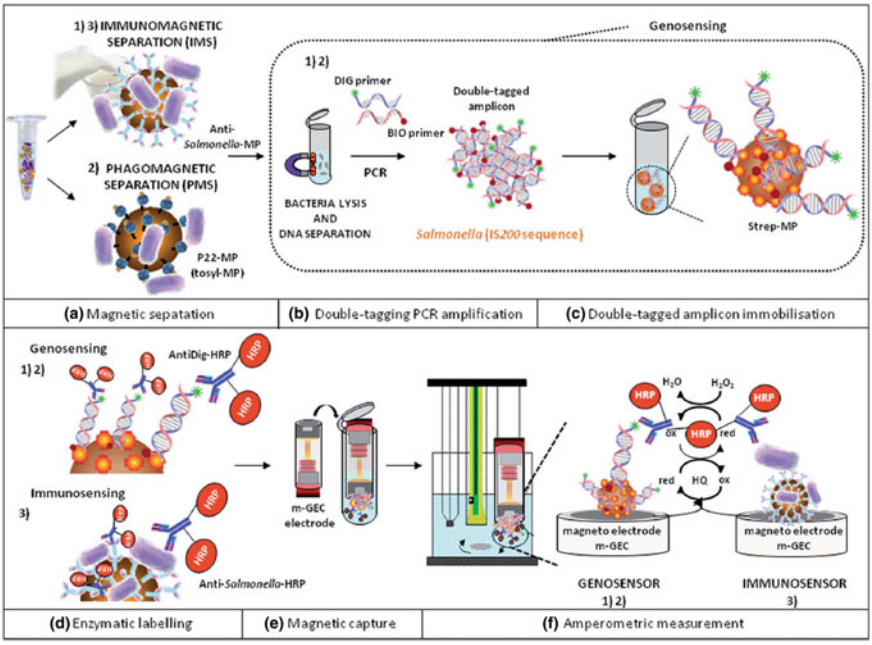

Fig. 3 Schematic representation of the electrochemical strategies for *Salmonella* spp. detection: (*1*) "IMS/double-tagging PCR/m-GEC electrochemical genosensing" [101] (*2*) "PMS/double-tagging PCR/m-GEC electrochemical genosensing" [103] and (*3*) "IMS/m-GEC electrochemical immunosensing" [109]

(v) enzymatic labelling using as enzyme label the antibody anti-DIG-HRP capable of reacting the other tag extreme of the dsDNA amplicon; (vi) magnetic capture of the modified magnetic particles; and (vii) amperometric determination [101].

In this approach, the bacteria can be captured and preconcentrated from food samples by IMS using both commercial magnetic microparticles [101] or tailored magnetic micro and nanoparticles with the specific antibody against Salmonella [102]. No significant differences were observed in the efficiency of the IMS using magnetic micro or nanoparticles, with the exception of the binding pattern, as shown in Fig. 4. After the lysis of the captured bacteria by IMS, further amplification of the genetic material by PCR with a double-tagging set of primers is performed to confirm the identity of the bacteria. Both steps (IMS and double-tagging PCR) are rapid alternatives to the time consuming classical selective enrichment and biochemical/serological tests.

The double-tagging PCR is performed with a set of two labeled PCR primers—one with biotin and the other with digoxigenin [77]. During PCR, not only amplification of the bacterial genome is achieved but also double-labeling of the amplicon ends with: (i) the biotinylated capture primer, to achieve immobilization on the streptavidin-modified MPs, and (ii) the digoxigenin signaling primer, to achieve electrochemical detection. The "IMS/double-tagging PCR/m-GEC

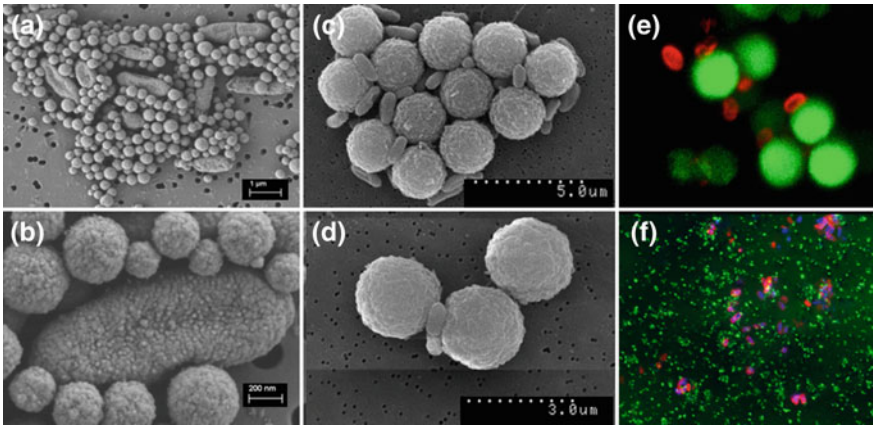

Fig. 4 Microscopic characterization of tailored magnetic nanoparticles (**a** and **b**) and microparticles (**c** and **d**) by scanning electron microscopy for *Salmonella* concentrations of 10^4 and 10^6 CFU mL^{-1}. The confocal microscopy study for micro (**e**) and nanoparticles (**f**) is also shown

electrochemical genosensing" approach was demonstrated for the sensitive detection of *Salmonella* artificially inoculated into skim milk samples. A limit of detection of 1 CFU mL^{-1} is obtained in 3.5 h without any pretreatment, in LB broth and in milk diluted 1/10 in LB. When the skim milk is pre-enriched for 6 h, the method is able to feasibly detect as low as 0.04 CFU mL^{-1} (1 CFU in 25 g of milk) with a signal-to-background ratio of 20 [101]. Interestedly, the specificity of this approach is conferred by both the antibody in the IMS and the set of primer during the double-tagging PCR, in this case for detecting *Salmonella* spp. The same approach could be also designed for detecting different *Salmonella* or *E. coli* serotypes by selecting a specific pair of primers or antibody.

The second strategy, "PMS/double-tagging PCR/m-GEC electrochemical genosensing" [103] was based on the use of bacteriophages, which offer several analytical advantages as biorecognition elements for the magnetic separation of pathogenic bacteria. The phage capabilities as a biorecognition element were explored by using the model phage nanoparticle P22 towards *Salmonella*. P22 bacteriophages were immobilised on tosyl-activated magnetic microparticles and carboxyl magnetic nanoparticles in an oriented way. The bacteria were then captured and pre-concentrated by the phage-modified magnetic particles through the phage–host interaction (Fig. 5). To confirm the identity of the bacteria, further double-tagging PCR amplification of the captured bacterial DNA and electrochemical magneto genosensing of the amplicon were performed, as schematically shown in Fig. 3.

In the detailed strategies, magnetic separation based on different affinity biorecognition principles was evaluated, i.e. immunomagnetic and phagomagnetic separation. Although similar analytical performance were obtained (LOD of 1 CFU mL^{-1} in 3 h assay time), the use of bacteriophages as a biorecognition element offers additional advantages. It must be highlighted that for the first time

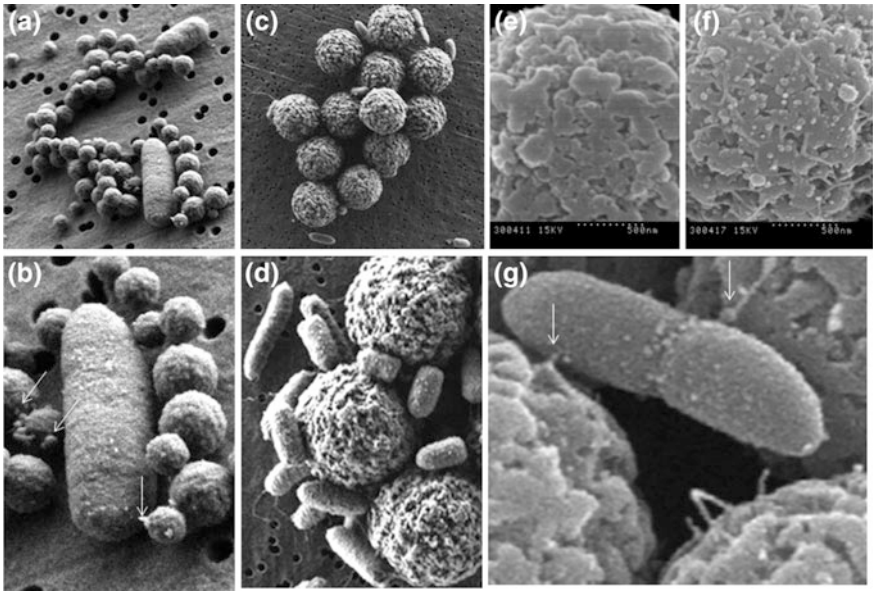

Fig. 5 Evaluation of the PMS by SEM at a Salmonella concentration of 3.2×10^6 CFU mL^{-1} using carboxyl magnetic nanoparticles (**a** and **b**) and tosylactivated magnetic microparticles (**c**–**g**). *Panels* **b** and **g** show the Salmonella cells attached to the magnetic nano and microparticles through tail spikes of the bacteriophages, signaled by arrows. *Panels* **f**shows the P22 bacteriophage immobilised on magnetic microparticles (2000 PFU/MP) while *panel* **e** shows the magnetic microparticle without any modification as a negative control. In all cases, identical acceleration voltage (15 kV) was used

non-modified bacteriophages were covalently coupled to magnetic particles, as shown in Fig. 5. Improved LODs (1 CFU mL^{-1}) were obtained in both cases when compared with the IMS and PMS followed by conventional gel electrophoresis (10^2 and 10^3 CFU mL^{-1}, respectively), as well as a significant reduction of the assay time when compared with IMS and PMS followed by the microbiological culture method (3 h vs. 18–24 h). The accuracy of the magnetic separation step coupled with microbiological culture is not measurable since agglomeration of particles often occurs and several target bacteria bound to the same particle give rise to only one colony forming unit (CFU) on the plating media, as shown in Fig. 5, panel A and C for magnetic nano and microparticles, respectively. Therefore, by coupling IMS or PMS with double-tagged PCR amplification and electrochemical magneto genosensing quantitative methods were achieved, due to the fact that a single cell is detected and these methods are not affected by the formation of aggregates. The double-tagging PCR also allows amplification of the analytical signal by amplifying the bacterial genome in a rapid way, instead of multiplication of the bacteria number by growing via traditional culturing methods. The magnetic separation and the double-tagging PCR provide specificity, as well as versatility to the assay, with the selection of different capture antibodies, bacteriophages or tagged primers.

Therefore, the models described can be widened to other bacterial targets affecting food safety and global health.

To summarize, bacteriophages are promising candidates to be used as a biorecognition element for the detection of pathogenic microorganisms. They provide many advantageous features such as outstanding selectivity, high sensitivity, and stability, which are three ideal attributes for any biorecognition probe that makes them suitable for in situ monitoring of food and environmental contaminants [104, 105]. Compared to antibodies, phages have distinct advantages as recognition receptors. On one hand, they are less fragile and less sensitive to environmental stress such as pH and temperature fluctuation reducing the environmental limitations, and on the other, their production besides being animal-free can be less complicated and less expensive than antibody production [106, 107].

The detection of *Salmonella* was demonstrated using both magnetic micro and nanoparticles modified with the bacteriophage P22 [108]. Although the covalent immobilization of P22 bacteriophages was successfully performed on both magnetic carriers achieving excellent coupling efficiencies, magnetic microparticles showed improved performance in terms of sensitivity and specificity, as well as lower matrix effect. These results could be related with the higher surface area per volume ratio given by their smaller size which could also increase the nonspecific adsorption, raising thus the influence of the matrix components during the assay.

5.2 *Immunomagnetic Separation (IMS) Coupled with Electrochemical Immunosensing on Magneto Actuated Electrodes*

In the third strategy, summarized as "IMS/m-GEC electrochemical immunosensing", a very simple and rapid method for the detection of *Salmonella* in milk is performed in which the detection of bacteria was achieved by a double immunological recognition (Fig. 3) [109]. In this approach, the bacteria were captured and pre-concentrated from milk samples with magnetic particles by immunological reaction with a specific antibody against *Salmonella*. A second polyclonal antibody labeled with peroxidase was used as serological confirmation, with electrochemical detection based on a magneto-electrode. Among the different procedures, better performances have been obtained using one-step immunological reactions. The "immunomagnetic separation step (IMS)/m-GEC electrochemical immunosensing" approach was employed, for the first time, in the detection of *Salmonella* artificially inoculated into skimmed-milk samples. A limit of detection of 7.5 10^3 CFU ml^{-1} in milk was obtained in 50 min without any pre-treatment. If the skimmed-milk is pre-enriched for 6 h, the method can detect as low as 1.4 CFU mL^{-1}, while following pre-enrichment for 8 h as few as 0.108 CFU mL^{-1} (2.7 CFU in 25 g of milk) are detected, thus complying with legislative criteria. IMS and detection with a second specific antibody can effectively replace "selective enrichment/differential

plating" and "biochemical/serological testing" assays, respectively. Moreover, the assay time is considerably reduced, from 4 to 5 days to 50 min.

5.3 *Simultaneous Electrochemical Magneto Genosensing of Foodborne Bacteria Based on Triple-Tagging Multiplex Amplification*

Over the past years, a new challenge has been attracting researchers in this field, the design of novel biosensors with multiplexing capabilities, where the integration of nanomaterials plays an important role. These novel bionanomaterials appears to be keys in bacteria multiplex detection in biosensors [31–34].

The simultaneous detection of *Salmonella enterica*, *Listeria monocytogenes* and *Escherichia coli* based on triple-tagging multiplex PCR and electrochemical magneto genosensing on silica magnetic particles was also reported [110]. A set of tagging primers were selected for the specific amplification of yfiR (375 bp), hlyA (234 bp) and eaeA (151 bp), being one of the primers for each set labelled with fluorescein, biotin and digoxigenin coding for *S. enterica*, *L. monocytogenes* and *E. coli*, respectively. Afterwards, electrochemical magneto genosensing of the bacteria was achieved by using silica magnetic particles as a carrier and three different electrochemical reporters, specific for each pathogen. For the first time, silica magnetic particles were used as a platform for DNA immobilization followed by electrochemical genosensing of *S. enterica*, *L. monocytogenes* and *E.coli*, based on triple-tagged amplicons. Interestingly, the silica magnetic particles showed differential adsorption properties, based on the negative charge density, for longer dsDNA amplicon incorporating the tagged-primers over shorter ssDNA tagged-primers, showing to be not only a robust platform for the electrochemical detection of PCR products but also a promising magnetic carrier for fluorescence or other detection approaches. This method was able to clearly distinguish among the pathogenic bacteria tested within 50 min, with detection limits ranging from 12 to 46 pg μL^{-1}.

6 Electrochemical Biosensors of Infection Agents Affecting Global Health

6.1 *Electrochemical Magneto-Actuated Biosensor for CD4 Count in AIDS Diagnosis and Monitoring*

According to last WHO report in 2013, at the end of this year, around 35 million people were living with HIV. Developing countries in Africa and Asia are the most affected by this disease, for example, sub-Saharan Africa concentrates almost 70 %

of the global HIV-infected people, with 25 million people living with HIV in this region alone [111]. An additional problem that these regions face is that only 5.9 of 12.9 million people who need antiretroviral therapy have access to this treatment [112]. HIV is a retrovirus which infects primarily $CD4^+$ T lymphocytes. Progression to AIDS occurs as a result of chronic depletion of CD4 cells, when the count falls below 200 cells mL^{-1} of blood, at a functional level where opportunistic infections and malignancies cannot be controlled [113, 114]. HIV infection is commonly diagnosed through a blood test detecting antibodies against HIV, followed by a confirmatory assay [115]. The serological tests for detection of HIV antibodies are generally classified as screening and confirmatory, being ELISA and Western blot, respectively. Moreover, a variety of simple, instrument-free, rapid tests including agglutination, immunofiltration, immunochromatographic and dipstick test, for example, OraQuick® Advance Rapid HIV-1/2, Reveal™ G-2 Rapid HIV-1 Antibody, Uni-Gold Recombigen® HIV, and Multispot HIV-1/HIV-2 Rapid Test are commercially available [116], all of the meeting the requirements of ASSURED given by the WHO.

However, after diagnosis, disease progression should be monitored through viral load based on viral nucleic acid detection or through the enumeration of CD4 cells by flow cytometry. Nucleic acid amplification test are laborious strategies, requiring dedicated equipment and trained technicians. In addition, flow cytometry requires complex and expensive equipment that requires regular maintenance and well trained personnel not only for data analysis, but also for the result interpretation. Currently, there are few cheaper alternative to the flow cytometer, mostly based on fluorescent labeling, requiring thus costly imaging equipment to achieve detection or manual counting by light microscopy [23, 117, 118].

Although RDTs are commercially available for diagnosis of HIV infection, CD4 cell counting is not available in the areas mostly affected [119–121], this control being imperative for assessing the progression towards AIDS [122]. As previously stated, the HIV virus infects the cells of the immune system, primarily $CD4^+$ T lymphocytes decreasing CD4 levels from the normal values (ranging from 500 to 1,200 cells μL^{-1}), which weakens the immune system and causes the progression to AIDS and death from cancer or opportunistic infections. When the number of the CD4 cells falls below 200 cells μL^{-1} of blood, it is considered to have progressed to AIDS [123]. AIDS it is also diagnosed with the emergence of one or more opportunistic illnesses regardless the CD4 count. Without treatment, people who progress to AIDS typically survive about 3 years. However, life-expectancy without treatment falls to about 1 year with the presence of opportunistic illness. Under antiretroviral treatment (ART) while maintaining a low viral load, a patient may enjoy a near normal life span without progression to AIDS. The $CD4^+$ T cell count is thus a critical parameter in monitoring HIV disease, since lower numbers of circulating $CD4^+$ T cells imply a more advanced stage of HIV disease and less competent defense mechanisms. In HIV infected patients, the $CD4^+$ T cell count is useful not only for assessing the degree of immune deterioration and speed of progression towards AIDS, but also for initiating ART, for deciding the timing for prophylaxis of opportunistic infections and, finally, for monitoring the efficacy of

the treatment [119]. The new recommendations encourage all countries to initiate the treatment in HIV infected adults with CD4 cell count down to 500 cells μL^{-1} when their immune systems are still strong, regardless of the presence or absence of clinical symptoms. Unfortunately, the areas mostly affected by the HIV epidemic are resource-limited countries, wherein the CD4 count is not available due to laboratory requirements and cost of the assay [124].

Although there are many commercially available possibilities for Point-of-Care HIV diagnosis, there is still the need for novel affordable alternatives to flow cytometry for CD4 cell count in order to monitor the AIDS disease and the treatment in low resource settings.

Recent advances involve integration of MPs into bioassays for both diagnosis of HIV infection, as well as for the progression and follow-up of AIDS. For instance, a magneto-actuated electrochemical biosensor for CD4 count in whole blood was reported [82]. The $CD4^+$ T lymphocytes were isolated, preconcentrated and labeled from 100 μL of whole blood by immunomagnetic separation with magnetic particles modified with antiCD3 antibodies (Fig. 6).

The captured cells were labeled with a biotinylated antiCD4 antibody, followed by the reaction with the electrochemical reporter streptavidin-peroxidase conjugate, as schematically detailed in Fig. 7. The limit of detection for the CD4 counting magneto biosensor in whole blood was as low as 44 cells μL^{-1} while the logistic range was found to be from 89 to 912 cells μL^{-1}, which spans the whole medical interest range for CD4 counts in AIDS patients. The electrochemical detection together with the immunomagnetic separation confers high sensitivity, resulting in a rapid, inexpensive, robust, user-friendly method for CD4 counting. This approach is

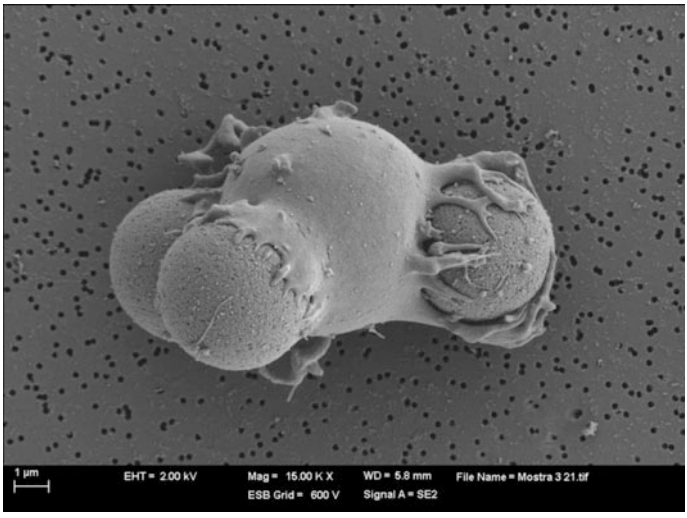

Fig. 6 Evaluation of the $CD4^+$ T lymphocytes immobilized on antiCD3 magnetic particles by SEM. Acceleration voltage (15 kV) was used

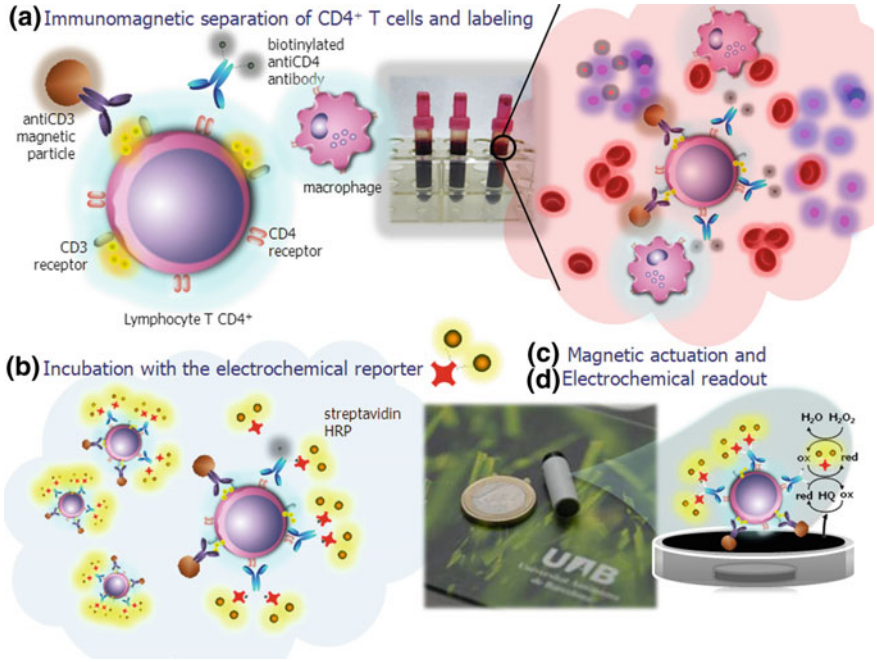

Fig. 7 Schematic representation of the CD4 counting magneto biosensor. **a** The $CD4^+$ T lymphocytes are captured from whole blood by the CD3-MPs and labeled in one step with antiCD4-biotin, **b** The incubation with the electrochemical reporter streptavidin-HRP is then performed. Finally, and after **c** the magnetic actuation, **d** the electrochemical readout is achieved the electrochemical readout is achieved

a promising alternative for costly standard flow cytometry and suitable as diagnostic tool at decentralized practitioner sites in low resource settings, especially in less developed countries.

6.2 *Electrochemical Magneto-Actuated Biosensor for CD4 Count in AIDS Diagnosis and Monitoring*

According to the WHO report, about 200 million people contracted malaria [125, 126] in 2012 and nearly 630,000 died of the disease [127]. Africa is the most affected area, counting one death every minute, most of them in children. Malaria is considered one of the major tropical parasitic disease and it is among the three most deadly communicable diseases [128].

In the absence of diagnostic tests, patients in low-resource settings are often treated based on clinical symptoms and local prevalence of disease. Whereas this approach captures most patients requiring treatment, it also unnecessarily treats

patients who do not require treatment. Equally important, this latter group of patients is not being treated for their specific disease due to misdiagnosis. This syndromic management of disease may also increase drug resistance. Recently, strains of *Plasmodium falciparum* resistant to chloroquine have spread rapidly. Taking into account that other malarial treatments are significantly more expensive the correct diagnosis of infected individuals seems to be a cheaper strategy, rather than treating all those with similar symptoms [3]. Moreover, the most ethical policy is to ensure that the new generation of drugs are only used for true cases of malaria to avoid the appearance of resistant strains [129].

Light microscopy is considered the gold standard method for malaria diagnosis; a drop of blood from a finger prick is fixed with methanol on a glass slide and stained with dyes to visualise the parasite. Unfortunately, limited infrastructure in low-resources settings results in extremely poor performance of microscopy as a diagnostic tool for malaria, which shows an accuracy of only 70–75 %. Moreover, microscopic diagnosis requires highly trained and experienced staff, being thus in some instances not suitable for routine use at the community level. Modern methods for malaria diagnosis include fluorescent microscopy, flow cytometry, automated blood cell analyzers, antibody detection, molecular methods, and laser desorption mass spectrometry. The main disadvantage, in most of the cases, is their high cost [130].

Only a few examples of the integration of MPs for malaria diagnosis have been reported. For instance, MPs were used for the selective preconcentration of the protein biomarker HRPII (histidine-rich protein II) for *Plasmodium falciparum*. A rapid and simple magneto immunoassay, which can be coupled with both optical or electrochemical readout for the detection of HRPII, was reported (Fig. 8) [82]. The method involved covalent immobilisation of anti-HRP2 IgM monoclonal antibody on MNPs (Fig. 8, panel A), followed by reaction with an anti-HRP2 IgG antibody labeled with peroxidase (Fig. 8, panel B), which could be used as electrochemical or optical reporter (Fig. 8, panel C). In the magneto immunosensor, the MNPs were used to preconcentrate the biomarker from the clinical sample, to eliminate interference from the matrix and to immobilise the biomarker in close contact to the electrode surface, improving thus the limit of detection.

7 Final Remarks

In this chapter, recent advances in foodborne pathogens and infection diseases affecting global health have been discussed, with special focus on electrochemical biosensing devices with magneto actuated platforms. The most prominent format is the integration of a magnetic capture step prior to detection, to achieve the preconcentration of the biomarker from the complex interferences present in the samples. In general, the most common approach involves the integration of immunomagnetic separation prior the electrochemical readout, based on commercial or tailored-modified magnetic micro or nanoparticles, with a specific antibody.

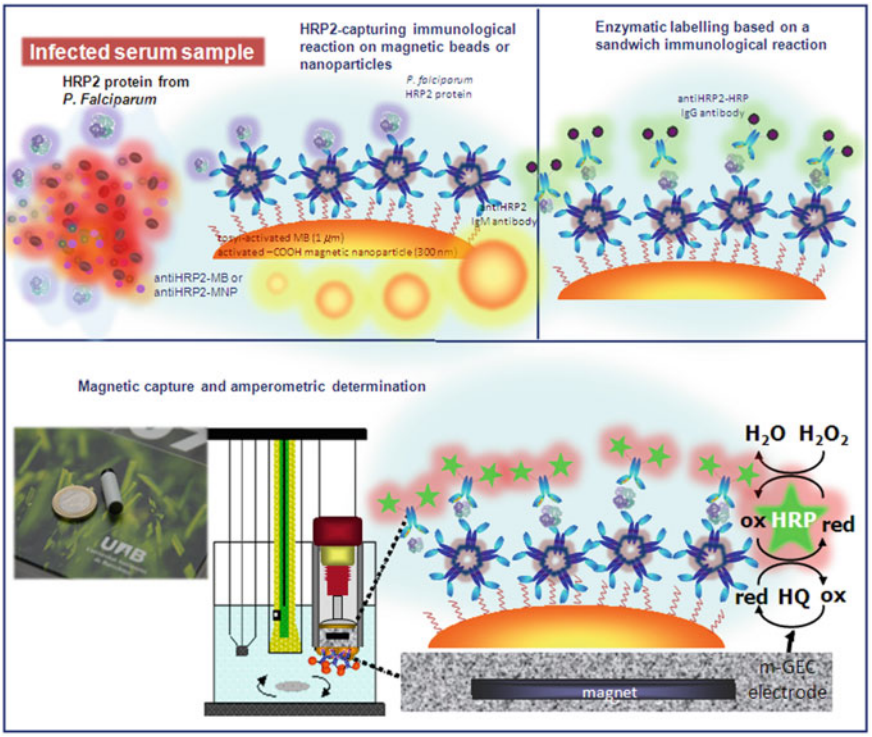

Fig. 8 Schematic representation of the sandwich immunoassay for Malaria detection performed on magnetic micro and nanoparticles with electrochemical readout

Magnetic capture was demonstrated to be compatible with different readout strategies, ranging from conventional methods such as culturing, microscopy and mass spectrometry, or emerging technologies such as lateral flow and biosensing devices, among others.

In some instances, the biomarker is preconcentrated and then released for further readout, although the detection of the biomarker is mostly performed while it is still attached to the MPs. In most formats, a label to achieve the readout is used, being a second antibody conjugated with enzymes, fluorophores, or nanomaterials (such as QDs or Au-NPs). Although the detection of biomarkers is in most instances performed by immunological recognition, there are some examples of the integration of MP in devices for DNA determination. Here, the MPs are used as a means of preconcentration of native DNA or RNA, or amplicons coming from PCR.

Although the MPs are mostly integrated in a preconcentration step prior to the readout, they can also be used as a carrier to achieve movement of the biomarkers along a microfluidic device through different reaction chambers, in order to achieve incubation and washing under magnetic actuation [131–133]. Unfortunately, there

are still few examples of total integration into a chip from sample introduction to readout, requiring in all cases bench-top equipments to achieve detection. Other limitations of these chips are low sensitivity limited by the small sample volume, irreproducibility in microfabrication and high cost of scaling down. One of the most promising approaches for rapid diagnostic in low resource settings are lateral flow with qualitative visual detection.

Recently, the integration of MPs into lateral flow design was demonstrated, improving the sensitivity and providing quantitative results when required [134]. The equipment is inexpensive if compared with traditional ELISA readers. In these methods, the MPs are used not only for preconcentration, but also for the readout based on the magnetic moment of the superparamagnetic particles attached to the biomarker. Another approach based on the integration of MP is the magneto biosensor, mostly using electrochemical detection. In this format, MPs are used not only as a way to preconcentrate the sample, but also to immobilise the biomarker on the surface of the transducer, increasing thus the sensitivity of the assay when compared with conventional surface modification of the biosensor.

Comparing the performance of magnetic carriers, similar analytical performance was observed for the immunomagnetic separations of bacteria with both magnetic micro and nanoparticles. However, magnetic nanoparticles showed increased matrix effect together with aggregation and required longer time for magnetic actuation, as the actuation time and also the matrix effect are influenced by the size of the particles. In general, integration of magnetic particles with different approaches demonstrated improvement in analytical performance in terms of specificity and sensitivity. Their use as solid supports in bioassays has shown to greatly improve the performance of the biological reactions, due to several factors: (i) an increased surface area which improves the efficiency of the reactions, (ii) faster assay kinetics achieved because the particles are in suspension and the analytical target does not have to migrate very far, and (iii) a minimised matrix effect due to the improved washing and separation steps.

The integration of magnetic particles can thus simplify the analytical procedure, avoiding the use of classical centrifugation or chromatography separation strategies, since no pre-enrichment, purification or pretreatment steps, which are normally used in standard analytical methods, are required. In here, the preconcentration and purification is achieved by simply applying an external magnet. Biomarkers can be specifically isolated and preconcentrated from complex biological or food matrixes by magnetic actuation, increasing the specificity of the assay. MPs have been shown to be a robust and versatile material for the detection of a whole range of biomarkers including mammalian cells, whole viruses, bacteria, proteins, antibodies and DNA related with infectious diseases affecting global health. The integration of MPs into emerging technologies shows very promising features, although there is still a long way to achieve point of care devices following the ASSURED recommendations given by WHO (Affordable, Sensitive, Specific, User-friendly, Rapid and Robust, Equipment free, and Deliverable to those who need it).

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