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Nanotechnologies for pathogen detection: Future alternatives?

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ABSTRACT

The development of multiplex and flexible tests allowing the simultaneous analysis of pathogens presenting a transfusional risk is a real challenge. Current miniaturized platforms have been particularly marked by microarrays. These microsystems allow the optical detection of hundreds of individual targets simultaneously. However, they suffer from a low sensitivity and their combination with a preliminary target amplification step such as PCR is necessary. The variable level of expression of the infectious genomes of interest and their large diversity complicate multiplex amplification. Finally simultaneous analysis of multiple blood-transmitted agents poses numerous difficulties in diagnosis that remain unresolved by currently available technologies.

Until recently, scientific and technological advances for pathogen detection have focused on target amplification and optical detection steps. Today, sample preparation is recognized as a critical area to improve. Nanotechnologies can reach the single-cell or molecular scale and consequently overcome several current technological obstacles. They offer new technological tools for improving sample preparation but also for avoiding target amplification and the current fluorescent labeling. The combination of nano-objects and nano-systems in current technologies offers new possibilities for potential applications in the detection of infectious agents.

1. Context of diagnostic microsystems

Miniaturized platforms for high-throughput genotyping are being evaluated in transfusion for applications in immunohematology whereas developments are proving to be more complex for their use in detecting infectious agents. Nevertheless, blood testing must be able to adapt to the demands of microbiological safety since the offensive engaged against blood-transmitted agents remains ongoing [1–8]. Today, nucleic acids amplification technologies are used in transfusion for the routine screening of HIV, HCV and HBV genomes in a number of developed countries. The limitations of first generation technologies become apparent in complex biological situations when a large spectrum of pathogens needs screening. Recent examples such as the West Nile pandemic in the USA in 2003, the Chikungunya virus on Reunion Island in 2006 and *Trypanosoma cruzi* (Chagas disease) in Guiana in 2007 underline the current difficulties in rapidly meeting safety requirements of the transfused population. Thus a more flexible blood testing platform would favor its participation in public health actions regarding risk evaluation and follow-up of the population on pertinent parameters.

Advances in microtechnologies over the last 20 years have led to the development of new miniaturized supports for the analysis of nucleic acids and proteins [9,10]. Chips or microarrays offer the advantage of being able to detect in parallel multiple targets permitting a conceptually new approach for pathogen diagnostics in blood testing [11–16]. DNA-chips represent also an efficient technology for the rapid detection of genetic variations of a given virus, as shown by the team of Dr Rios monitoring the genetic variations of the West Nile virus [17]. A prototype DNA-chip (bioMérieux, France) for Hepatitis B Virus (HBV) genotyping was evaluated recently and showed a sensitivity and specificity of 97.5% and 97.8%, respectively [18]. At the protein level, most of the chips proposed for use in microbiology are in fact immunoassays in a miniaturized format. They offer the advantage of parallelism and a reduced cost per analysis but the limited sensitivity of the assays represents a critical issue. Protein assays are above all used in research but recent studies predicted a strong development for use in diagnostics for the detection of infectious agents [19]. As reviewed recently by Uttamchandani et al., other microarray-based platforms have been proposed for pathogen detection and systems are commercialized

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for use in the biodefense arena [20]. Limitations for their applications in clinical diagnostics were quickly recognized due to technological and commercial constraints.

The simultaneous analysis of multiple blood transmitted agents remains a real challenge. Technological developments for molecular testing have focused on target amplification step and optical detection based on fluorescent labeling (Fig. 1). These technologies are expensive and not affordable for a number of developing countries for testing the most relevant infectious agents like HIV, HCV or HBV [21]. Inactivation techniques may come into routine to reduce the pathogen load and compete technological developments [22]. Recent advances in micro- and nano-technologies propose alternatives of the current paradigm. Sample preparation is now recognized as a key parameter to improve sensitivity and specificity performances and new amplification and detection approaches could challenge respectively those of target amplification and optical detection currently used. Integrated systems of all analytical steps are in development for pathogen detection.

2. Sample preparation: a critical point for future innovations

The nanosciences and nanotechnologies allow a better control of the organization of atoms and molecules to create nanostructures with new or improved properties. The nanobiotechnologies can reach the single-cell or molecular scale and consequently overcome certain technological obstacles. The physicochemical characteristics of **nanoparticles** make them ideal for use in diagnostics [23–28]. Sample preparation is a critical parameter to improve in order to work with miniaturized platforms. The challenge is to use a sample size that has clinical relevance for pathogen detection (hundreds of microliters) and reduce it down to miniaturized size. Nanoparticles and microfluidics could offer new strategic tools to extract the targets of interest present in the sample before direct or indirect detection in a miniaturized support.

New properties are revealed into the nanoworld as the size of each of its components is reduced. For instance (Fig. 2), the reduction in size of the particles towards the nanometric scale increases considerably the surface available for attachment of the oligonucleotide or protein probes complementary to the target molecule and therefore increases the sensitivity of recognition.

The use of magnetic beads for the purification of DNA or proteins has grown over the last several years and is exploited in a number of commercially available kits. The reduction to nanobeads format optimizes the capture of analyte of interest. For example, Fuentes et al., used magnetic nanoparticles to detect traces of DNA. In this way the presence of two molecules of cDNA of HCV genome in 1 ml of solution could be revealed by PCR [29]. A large number of developments involve the use of magnetic beads for labeling and purification of the analyte [30,31] but also for a direct quantification as recently shown with a magnetic nanotag-based protein detection assay [32]. Considering these numerous advantages, magnetic nanoparticles are increasingly being used in biodetection for the conception of miniaturized devices.

3. New perspectives for pathogen detection

3.1. Signal amplification

Quantum dots (QD) are inorganic fluorophores offering significant advantages over organic fluorophores conventionally used to label nucleic acids or proteins for optical detection [24,33,34]. These biocompatible semiconductor crystals are composed of a nucleus and a shell allowing the binding of ligands and thus the attachment of this fluorescent marker to the target. They are stable and very luminescent fluorophores each offering a wide excitation spectrum and narrow emission spectrum with wavelength controlled by the size and nature of the nucleus. These remarkable properties are due to their nanometric size. They are also compatible with analyses of whole blood [24]. The QDs can be used in viral diagnostics as shown by Agrawal et al. in 2005 for the detection in real time of respiratory syncytial virus (RSV) [35]. The major interest for their diagnostic use relates to the possibility of multiplexing [33]. In practice, QDs remain difficult to synthesize, functionalize and integrate into miniaturized platforms [36].

Progress towards the ability to control the size and to functionalize the surface of nanoparticles has allowed the production of optically and chemically defined probes for the detection of biomolecules [26,28]. **Gold nanoparticles** are good markers for use in biosensors as numerous optical or electrochemical techniques can be adapted to detect them. A number of nanoparticle-based assays have been developed by C. Mirkin et al. for biomolecular detection, with DNA- or protein-functionalized gold nanoparticles used as the target-specific probes [24,37,38]. Work published in 2000 demonstrated the possibility of detecting hybridization on a chip of oligonucleotides labeled by gold nanoparticles with a simple scanner [39]. This "scanometric" detection is simple and selective and allows the discrimination of a point mutation on the strand of DNA. In addition, coupling this detection to a silver amplification method led to a level of sensitivity 100 times greater

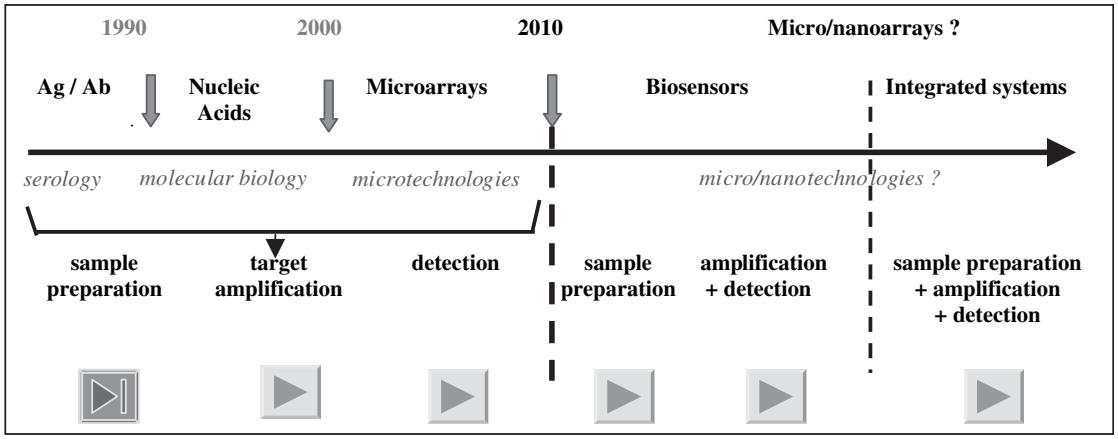

Fig. 1. Technological advances.