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Research review paper

Better together: Strategies based on magnetic particles and quantum dots for improved biosensing

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ABSTRACT

Novel technologies and strategies for sensitive detection of biological responses in healthcare, food and environmental monitoring continue to be a priority. The present review focuses on bioassay development based on the simultaneous use of quantum dots and magnetic beads. Due to the outstanding characteristics of both particles for biosensing applications and the large number of publications using a combined approach, we aim to provide a comprehensive overview of the literature on different bioassays, the most recent advances and innovative strategies on the topic, together with an analysis of the main drawbacks encountered and potential solutions offered, with a special emphasis on the requirements that the transfer of technologies from the laboratory to the market will demand for future commercialization of biodevices. Several procedures used in immunoassays and nucleic acid-based bioassays for the detection of pathogens and biomarkers are discussed. The improvement of current approaches together with novel multiplex detection systems and nanomaterials-based research, including the use of multimodal nanoparticles, will contribute to simpler and more sensitive bioanalyses.

Contents

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1. Introduction

Novel technologies and strategies for sensitive measurement of biological processes in healthcare, food and environmental monitoring and defense continue to be a priority. In the field of biosensors and bioassays, where labelled specific factors are used to emit a detectable signal, the unique properties of nanomaterials have led to the development of new assays and new transduction mechanisms with increased performance and sensitivity (Bally and Vörös, 2009; Jianrong et al., 2004).

Among the nanomaterials used for biosensing and imaging applications, quantum dots (QDs) have produced one of the most successful stories since their discovery in 1983 (Brus, 1984, 1983). However, it took until 1998 to establish the advantages of QDs for biological applications and biosensing tools (Bruchez et al., 1998; Chan and Nie, 1998). QDs are inorganic nanocrystals of around 1–6 nm with unique optical and chemical properties but complicated surface chemistry (Resch-Genger et al., 2008). Their optical properties are controlled by the constituent material, particle size and size distribution and surface chemistry, and widely rely on method of particle synthesis. Although QDs can be prepared with atoms from groups II–VI, II–V or IV–VI of the periodic table and in many different alloyed versions, the most popularly used are cadmium (Cd)-based QDs (Wegner and Hildebrandt, 2015). Typical QDs are core-only (such as cadmium telluride, CdTe) or core-shell (composed of a core of a semiconductor with a smaller band gap material, enclosed within a shell of another semiconductor material with larger spectral band gap; for example, cadmium selenide core with a zinc sulfide shell, CdSe/ZnS) nanostructures, which can be functionalized with different coatings (Resch-Genger et al., 2008). QDs optical properties include a high quantum yield even in near-infrared wavelengths; narrow, symmetric and size-tunable fluorescence spectra, and extremely broad and intense absorption, enabling a unique flexibility in excitation that overcomes some of the limitations of organic dyes (Esteve-Turrillas and Abad-Fuentes, 2013; Resch-Genger et al., 2008). This kind of nanoparticles therefore presents an additional advantage for multiplexing approaches, since QDs with different sizes can be excited with a single wavelength of light, resulting in different emission peaks that can be measured simultaneously (Yang and Li, 2006). Their superior stability in comparison with other fluorescence imaging agents also allows longer investigation times for advanced in vitro and in vivo applications (Wegner and Hildebrandt, 2015). An in-depth description of the preparation, functionalization, properties and applications of QDs is beyond the scope of the present work, and has been extensively discussed in the scientific literature (for a more comprehensive review see Esteve-Turrillas and Abad-Fuentes, 2013; Resch-Genger et al., 2008; Wegner and Hildebrandt, 2015).

Although the unique optical properties of QDs make them a powerful platform in biology and biochemistry, including imaging and sensing purposes, their usage might be limited by two main drawbacks. The first one is their potential toxicity, which has especially delayed the progress towards clinical applications (Yong and Swihart, 2012). QDs cytotoxicity has been demonstrated in several in vitro studies, mainly resulting from the release of Cd ions due to degradation, the presence of certain surface-covering molecules, the intracellular distribution and the generation of reactive oxygen species (Chen et al., 2012; Yong and Swihart, 2012). The strategy used for the synthesis of QDs, their hydrodynamic diameter and their surface chemistry are the main factors that determine the cellular interactions. On the other hand, in vivo studies about QD toxicity are still scarce and their results are somehow contradictory. Although they accumulate in organs with high blood flow and induce immune responses, no pathological effects have been observed in small animal studies at the concentrations used for imaging applications (Botelho et al., 2016; Chen et al., 2012; Dobrovolskaia and McNeil, 2007; Hauck et al., 2010; Sahu et al., 2014; Su et al., 2011). More extensive long-term studies of QD toxicity and pharmacokinetics are lacking. Meanwhile, novel QD formulations based on indium phosphide or silicon, which eliminate local cytotoxicity caused by the release of Cd ions, are under development in order to improve their optical properties and become an acceptable alternative (Erogbogbo et al., 2011a, 2011b; Yong et al., 2009). Alternatively, QD coating for example with silica or polyethylene glycol (Zhelev et al., 2006; Painuly et al., 2013) or improved synthetic procedures resulting in more photostable nanocrystals (He et al., 2011; Chen et al., 2013a) may translate in diminished cytotoxicity. In the case of biosensing applications, the measurements are performed ex vivo in samples not containing living cells and require small amounts of QDs, therefore the concerns about cytotoxicity are limited. However, it may pose some difficulties for the approval of regulatory agencies, especially in the cases where the biodevice is expected to be used by the general public.

The second main issue is the strong blinking effect - i.e. random and intermittent light emission that makes individual particles to go dark (non-radiant) only for nanoseconds or remain dark for minutes at a time, or some interval in between - , which limits the applicability of QDs for single molecule fluorescence studies (Nirmal et al., 1996; Rombach-Riegraf et al., 2013). Although several hypotheses have been proposed to explain the blinking phenomenon, the most accepted one is the charging/discharging of the nanocrystal core when lower photoluminescence intensities correlate with shorter photoluminescence lifetimes (Galland et al., 2011). Some strategies have been proposed in order to reduce this undesired effect, including the introduction of 'anti-blinking agents' in the solution environment, such as β-mercaptoethanol, which bind to QD surfaces and increase the radiative lifetime; the functionalization of QDs with oligo(phenylene vinylene) (Fomenko and Nesbitt, 2008; Hammer et al., 2006; Hohng and Ha, 2004); or the site-directed binding of QDs to cysteine residues by the presence of thiol groups, which works probably because the coordinate covalent bond close to the nanocrystal reduces the number of electron traps and therefore increases the radiative pathways (Rombach-Riegraf et al., 2013).Other approaches rely on modifying or tuning internal core/shell structures, instead of introducing surface modifications or surface-mediated interactions, such as the use of optimized synthetic procedures with a slow shell growth rate that eliminate the luminescence photodarkening (Chen et al., 2013b), or QD heterostructuring by interfacial alloying, thick or "giant" shells, and specific type-II electronic structures (Hollingsworth, 2013)

In addition to QDs, magnetic micro- and nanoparticles have acquired unprecedented utility for a wide variety of applications in separation techniques and bioassays. Magnetic beads allow linking to different molecules, including monoclonal antibodies, DNA or other receptors, and coating with streptavidin, protein A, etc., thus ensuring specific interactions with the targets of interest and easy recovery of the material by the use of an external magnet. By providing a solid support for biorecognition, magnetic particles, which commonly consist of magnetic elements such as iron, nickel and cobalt and their chemical compounds and can therefore be manipulated using magnetic field gradients, are able to accelerate the binding kinetics and facilitate analyses in shorter times. Magnetic particles allow the attachment of both ligands and receptors on their surface, thus restricting the conformational freedom and reducing the recognition kinetics when compared with freely diffusing species (Baudry et al., 2006). Magnetic separation is exceptionally efficient because most biological materials are not susceptible to magnetic fields (Hatch and Stelter, 2001). Among other advantages, magnetic beads can be easily separated from the reaction mixture with a magnet and immediately re-dispersed after removal of the magnetic field; they have a large surface area that allows immobilization of large numbers of biomolecules, leading to increased sensitivity without affecting biomolecular activity; and several detection techniques can be applied, ranging from fluorescence to electrochemical, chemiluminescence and colorimetric methods (Wei et al., 2012). Magnetic particles have been successfully applied for targeted drug delivery, bioseparation, biodetection, and labelling and sorting of cells (Labiadh et al., 2015). As an example, immunosensors and other immunoassays, reviewed in detail in following sections, based on magnetic beads and fluorescence detection have shown promising results for sensitive