

Nanotechnology-based Diagnostics; Are we Facing the Biotechnology Evolution of the 21st Century?

Ikonomopoulos John*

Agricultural University of Athens, School of Animal Science and Aquaculture, Department of Anatomy – Physiology, Greece

Abstract

Undoubtedly the last third and even more so, the concluding decade of the previous century was a period during which the emergence and later on the broad application of Molecular Biology, revolutionized the field of Diagnostics. However the application of this type of technology despite its obvious success has also revealed some weak points. Nanotechnology seems to provide a very promising alternative that bypasses these weaknesses and perhaps supports the prospective for a new generation of diagnostic tests that will be easier to apply and hopefully equally efficient. The relevant evidences are elaborated in this article towards concluding whether we are facing the 21st century diagnostic revolution.

Introduction

Undoubtedly the last third and even more so, the concluding decade of the 20th century was a period during which the emergence and later on the broad application of Molecular Biology, revolutionized the field of Diagnostics. The ability to detect minute amounts of the targeted analytes within just a few hours was recognized in most cases as a truly welcomed technical improvement but in others, like for example the demonstration of the presence of mycobacteria in clinical samples, made all the difference in the world. The issues of evidence-based individualized treatment or the simultaneous detection of large numbers of point mutations associated with microbial drug resistance might seem vague a few years ago, but now they are already within the grasp of the scientific community.

Expectedly the application of this type of technology despite its obvious success has also revealed some weak points. Decreasing the minimum detection limit even down to a single cell dictated the need to apply vigorous precautions to avoid the carry-over effect (successive passage of amplicons from one reaction to the other) leading to false-positive results. Similarly, the poor quality of the targeted analyte or the performance of the sophisticated equipment that are usually required, can lead to the exact opposite event. And of course there is always the issue of cost that becomes significant especially when the applied technology relies on expert personnel, dedicated equipment and space.

During mainly the last decade, an increasing number of reports have been published describing an alternative approach for a great variety of applications including pathogen detection, incorporating nanotechnology. Semiconductor quantum dots (QDs) or nanocrystals can interact with biomolecules and can be accessed through one-to-one interactions between the target analyte and the signal generating particle. QDs can be used for multi-labeling or multi-target detection within the context of diagnostic applications designed to identify the genetic or the immunogenic footprint of a pathogen.

However does this type of technology have the potential to generate in this century, a diagnostic revolution similar to that recorded in the previous by the application of Molecular Biology?

Basic properties

The term nanoparticle is commonly used to describe particles with dimensions less than 100 nm. Nanoparticles contain a considerably higher percentage of atoms at their surface compared to bulk material

which causes an “unexpected behavior” otherwise referred to as the quantum effect. This influences their optical, electrical, chemical and physical characteristics. For example, when used in dispersions they have very strong diffusion tendency while at the same time they avoid deposition or floating. An interesting in terms of diagnostics optical feature of semiconductor nanoparticles is that the minimum amount of energy required for the release of an outer electron from the nucleus (bandgap) is size-dependent, which translates into a color shift from red to blue as the size of particles decreases.

Depending on the properties of the material one is interested to make use of, there are a number of metal or polymer nanoparticles from which to choose. In the last years, nanodiagnostics have focused more on colloidal gold and cadmium selenide quantum dots that seem to be very promising for the detection of pathogens.

Technical principles

Colloidal gold nanoparticles (AuNPs) range in size from 3-100 nm, and exhibit strong size-dependent optical resonance [1]. Photoactivation results to a huge enhancement of the electromagnetic field that causes scattering and absorption of light, which can be exploited to generate a color change (visual detection) or heat (construction of biosensors). In brief, the negatively charged AuNPs can be chemically directed to selective deposition of detectable by a change of color. The same result can be achieved by linkage of two or more properly modified for conjugation AuNPs to adjacent regions of a nucleotide target, which results to aggregation due to the decrease of the intraparticle distance [2]. This, and the ability to accurately measure the shift of light absorption generated by the conjugation of the nanoprobe to their targets has consisted the foundation of their

*Corresponding author: Ikonomopoulos John, Agricultural University of Athens, School of Animal Science and Aquaculture, Department of Anatomy – Physiology, Greece, E-mail: ikonomop@gmail.com

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use as biodetectors [3]. Notably the use of AuNPs within this context offers some unique advantages compared to the use of fluorescent dyes since they avoid photodecomposition, they are not toxic, and most significantly they demonstrate a very precise correlation of their chemical/physical properties to their optical characteristics, which if accurately measured can confer improved minimum detection limit and sensitivity [4].

QDs are semiconductor crystals with physical dimensions not larger than a few nanometers. Today cadmium selenide (CdSe) nanoparticles are already becoming increasingly popular, especially around the size range of 2-6 nm which makes them dimensionally more compatible with nucleic acid and proteins. When a photon of visible light hits such a semiconductor some of their electrons are excited into higher energy states. A photon of a frequency characteristic of that material is emitted when they return to their ground state. The ability of QDs to exhibit size-dependent fluoresce-emission wavelengths is the foundation of their use as biodetectors, which can be greatly improved by the incorporation in their outer surface of a "shell" such as ZnS or silica [5-7]. Notably the latter can be easily linked to bioconjugators such as avidin allowing the way of these nanoparticles into several already established diagnostic assays. In summary the QD shells can protect the core from oxidation, minimize or eliminate core-derived toxicity and increase its water solubility. CdSe may be size-tuned very accurately and therefore be used for the simultaneous detection of multiple targets with a single excitation wavelength.

Bio-detection

Nanoparticles have already been integrated in research and routine diagnostic applications [8,9] with several already available in the market such as that of the Quantum Dot Corporation (Hayward CA, USA) that modified an assay for detection of bodulin-toxin to the development of an easy-to-use pregnancy test. In order for nanoparticles to be used as biodetectors they have to be functionalized. Within this context both biomolecules and nanoparticles are modified to present one or more suitable chemical groups such as nucleophiles or sulfhydryls, which can be performed in various ways.

Gold nanoparticles incorporated into colourimetric assays have been used for the detection of genetic polymorphisms including point mutations allowing single-base discrimination without the need of precise temperature control [10,11]. More recently cadmium selenide (CdSe) quantum dots (QDs) coated with a peptide that HIV uses to enter human cells (TAT) allowed simultaneous screening of several cellular functions within each cell using QD-conjugates with various peptides. The same group applied an automated alternative that relied on the use of fluorescence energy transfer, which produced a high throughput detection system [12].

QDs have also been used for the detection of several bacterial species [13-16] even when present together in the same material (*biofilms*). This application has been used to provide in addition to differential detection of these pathogens, an evaluation of their viability [17]. Assays incorporating bioconjugated nanoparticles have allowed *in situ* pathogen quantification in less than 20 minutes with a minimum detection limit of a single cell [18]. More recently multi-focal binding of QDs was used for the simultaneous quantifiable and differential detection of two food-born pathogens i.e. *E.coli* O157:H7 and *Salmonella typhimurium* [19]. Similarly a rapid (1 hour), simple, high sensitive bacterial detection system was developed

using biotin-tagged bacteriophage and QD-nanocomplexes [20]. This method, which was found to be particularly useful for slow growing bacteria such as mycobacteria is already being developed for military applications for fast, accurate and portable evaluation of samples (*NCI and NIST, USA*). Finally, the same goal, i.e. development of in-field diagnostic tests, was targeted by Storhoff et al., who developed a spot-and-read colourimetric detection method for identifying nucleic acid sequences based on the distance-dependent optical properties of gold nanoparticles. The assay enabled detection of zeptomole quantities of nucleic acid targets without amplification and allowed direct detection and differentiation of methicillin-resistant *S. aureus* in DNA samples [21].

What is especially intriguing about the applications mentioned above in terms of their potential practical use is in addition to their low minimum detection limit and small dependency on dedicated equipment, that they can be developed for all-in-one assays, i.e. the same principles of detection applied with a variety of nanoprobe designed to detect peptide or nucleic acid. This approach was materialized by the author's group with an application developed to detect mycobacterial DNA [22] and the heparin-binding hemagglutinin [23]. The fact that this type of technology relies on the attachment of the functionalized nanoparticles onto several probing sites, sets the basis for the development of assays with increased sensitivity and simultaneous confirmation of the result. Furthermore for those cases that the minimum detection limit is adequately low to avoid pre-amplification of the target region, the resulting methodology is less prone than PCR to false results generated by the carry-over effect or the presence of amplification inhibitors. Furthermore given that even in the cases of multi-labeling the probing sites are much smaller in length than PCR amplicons, the chances of false negative results caused by fragmentation of the target region is again decreased.

Conclusion

The technical possibilities offered by the application of nanotechnology in the field of diagnostics seem practically numerous and by all means exciting. The prospective of easy-to-use assays that because of their small dependency on dedicated equipment or space can be applied even at resource-poor-settings allowing high analytical sensitivity can by all means set the basis of the 21st century biotechnology revolution. However the challenge is not small. Though as mentioned above the principle of target detection is simple, very often it incorporates several hybridization steps that can be laborious to perform. Furthermore, in order to optimize minimum detection limit, all the hybridization parameters need to be very carefully tuned, which again is not easy to achieve and poses considerable difficulties when the same method is applied in different laboratories. The relevant improvements recorded on PCR-based methodologies were facilitated by the fact that all the scientists involved shared a background of basic Biology. Unfortunately this does not apply to Nanotechnology. In this case Medical scientists and researchers of Physics, Chemistry, Molecular Biology and Microbiology are called upon to provide solutions to problems that are of very broad intra-disciplinary nature. A foundation may have to be constructed consisted of scientists that will be trained in both fields of Nanotechnology and Biology to facilitate the development of this technology to its full potential as well as its integration to practice.

References

1. Alivisatos AP (2008) Birth of a nanosciencebuilding block. *ACS Nano* 2: 1514-1516.
2. Jain PK, Lee KS, El-sayed IH, El-sayed MA (2006) Calculated adsorption and scattering of gold nanoparticles of different size, shape and composition: Applications in biological imaging and biomedicine. *J Phys Chem B* 110: 7238-7248.
3. Pissuwan D, Cortie CH, Valenzuela SM, Cortie MB (2010) Functionalised gold nanoparticles for controlling pathogenic bacteria. *Trends Biotechnol* 28: 207-213.
4. Michalet X, Pinaud FF, Bentolila LA, Tsay JM, Doose S, et al. (2005) Quantum Dots for live cells, in vivo imaging, and diagnostics. *Science* 307: 538-544.
5. Alivisatos AP (1996) Semiconductor clusters, nanocrystals and quantum dots. *Science* 271: 933-937.
6. Dabbousi BO, Rodriguez-Viejo J, Mikulec FV, Heine JR, Mattoussi H, et al. (1997) (CdSe)ZnS Core-Shell Quantum Dots: Synthesis and characterization of a size series of highly luminescent nanocrystallites. *J. Phys. Chem* 101: 9463-9475.
7. Parak WJ, Gerion D, Pellegrino T, Zanchet D, Micheel C, et al. (2003) Biological Applications of Colloidal Nanocrystals. *Nanotechnology* 14: 15-27.
8. Parungo CP, Ohnishi S, Kim SW, Kim S, Laurence RG, et al. (2005) Intraoperative identification of esophageal sentinel lymph nodes with near-infrared fluorescence imaging. *J Thorac Cardiovasc Surg* 129:844-850.
9. Kalogianni DP, Koraki T, Christopoulos TK, Ioannou PC (2006) Nanoparticle-based DNA biosensor for visual detection of genetically modified organisms. *Biosens Bioelectron* 21: 1069-1076.
10. Du BA, Li ZP, Liu CH (2006) One-step homogeneous detection of DNA hybridization with gold nanoparticle probes by using a linear light-scattering technique. *Angew Chem Int Ed Engl* 45: 8022-8025.
11. Pang L, Li J, Jiang J, Shen G, Yu R (2006) DNA point mutation detection based on DNA ligase reaction and nano-Au amplification: A piezoelectric approach. *Anal Biochem* 358: 99-103.
12. Delehanty JB, Medintz IL, Pons T, Brunel FM, Dawson PE, et al. (2006) Self-assembled quantum dot-peptide bioconjugates for selective intracellular delivery. *Bioconjug Chem*. 17: 920-927.
13. Hahn MA, Keng PC, Krauss TD (2008) Flow cytometric analysis to detect pathogens in bacterial cell mixtures using semiconductor quantum dots. *Anal Chem* 80 : 864-872.
14. Liandris E, Gazouli M, Andreadou M, Comor M, Abazovic N, et al. (2009) Direct detection of unamplified DNA from pathogenic mycobacteria using DNA-derivatized gold nanoparticles. *J Microbiol Methods* 78: 260-264.
15. Zahavy E, Heleg-Shabtai V, Zafrani Y, Marciano D, Yitzhaki S (2010) Application of fluorescent nanocrystals (q-dots) for the detection of pathogenic bacteria by flow-cytometry. *J Fluoresc* 20: 389-399.
16. Pissuwan D, Cortie CH, Valenzuela SM, Cortie MB (2010) Functionalised gold nanoparticles for controlling pathogenic bacteria. *Trends Biotechnol* 28: 207-213.
17. Kloepper JA, Mielke RE, Wong MS, Nealson KH, Stucky G, et al. (2003) Quantum Dots as Strain- and Metabolism-Specific Microbiological Labels. *Appl Environ Microbiol* 69:4205-4213.
18. Zhao X, Hilliard LR, Mechery SJ, Wang Y, Bagwe RP, et al. (2004) A rapid bioassay for single bacterial cell quantitation using bioconjugated nanoparticles. *Proc Natl Acad Sci* 101: 15027-15032.
19. Yang L, Li Y (2006) Simultaneous detection of *Escherichia coli* O157:H7 and *Salmonella typhimurium* using quantum dots as fluorescence labels. *Analyst* 131:394-401.
20. Edgar R, McKinstry M, Hwang J, Oppenheim AB, Fekete RA, et al. (2006) High-sensitivity bacterial detection using biotin-tagged phage and quantum-dot nanocomplexes. *Proc Natl Acad Sci* 103: 4841-4845.
21. Storhoff JJ, Lucas AD, Garimella V, Bao YP, Müller UR (2004) Homogeneous detection of unamplified genomic DNA sequences based on colorimetric scatter of gold nanoparticle probes. *Nat Biotechnol* 22: 883-887.
22. Gazouli M, Liandris E, Andreadou M, Sechi LA, Masala S, et al. (2010) Specific detection of unamplified mycobacterial DNA using fluorescent semiconductor quantum dots and magnetic beads. *Journal of Clinical Microbiology* 48: 2830-2835.
23. Liandris E, Gazouli M, Andreadou M, Sechi LA, Rosu V, et al. (2011) Detection of Pathogenic Mycobacteria Based on Functionalized Quantum Dots Coupled with Immunomagnetic Separation. *PLoS One* 6.