Biofunctionalised nanomaterials in medical diagnostics: Hopes and applications for the future

*Harriet D. Gliddon**

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Abbreviations
ELISA · LOD · PCR · PSA · LSPR

Introduction

The early detection of diseases can greatly increase the chances of successful treatment. Ultra-sensitive diagnostics are now becoming feasible due to the unique properties of nanomaterials, which offer novel mechanisms to detect disease biomarkers. Although nanomaterials themselves are not necessarily novel, our understanding of them has dramatically improved in recent years, allowing us to exploit their properties like never before.

Limitations of current diagnostics include poor sensitivity and inadequate response speeds. They are usually designed to detect simply the presence of a biomarker, rather than the level at which it is present or its degree of activity. We might gain more information about the needs of individual patients, as well as a greater understanding of disease progression, if we looked towards nanomaterials as a basis for novel in vitro diagnostics.

Where have we been going wrong?

For the past 30 years, molecular biology has provided the majority of the tools currently in use in medical diagnostics. As our understanding of biological processes deepens, the medical community is increasingly looking towards personalised healthcare as a means of improving health outcomes in the future. However, the point-of-care diagnostics and 'lab-on-a-chip' devices needed to make this a reality require substantial development.

Proteins are increasingly being used as disease biomarkers. The enzyme-linked immunosorbent assay (ELISA) is often seen as the standard technique for protein detection, but has a number of disadvantages associated with it, and is not easily applicable to lab-on-a-chip devices. Furthermore, although ELISAs can detect the *presence* of an enzyme, they cannot give any indication of the *activity* of an enzyme, a parameter which would be extremely useful to have knowledge of during disease tracking and when examining treatment efficacies. Novel diagnostic strategies based on nanomaterials offer increased sensitivity of tests, with the prospect of quantifying enzyme activity as well as presence.

The polymerase chain reaction (PCR) is widely regarded as the current gold standard of nucleic acid detection due

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to its high degree of sensitivity. However, this technology is not amenable to on-chip detection for point-of-care diagnostics. New approaches are emerging using carbon nanotubes, which simply require one drop of test solution in order to detect a particular nucleic acid sequence.

Our growing understanding of nanomaterials and their advantages over more traditional technologies means it is likely that they will be increasingly used as *in vitro* diagnostic tools in the future.

What makes nanomaterials so special?

Nanomaterials represent a class of materials that do not exceed 100 nm in one or more dimensions. This small size results in an increased surface-to-volume ratio, which offers greater sensitivity and lower limits of detection (LOD), meaning that only a few molecules of a particular analyte need to be present in solution in order to be detected.

Nanostructures also have unique optical and electrical properties, meaning they often behave very differently to the bulk material. This is a result of the confinement of electrons within the nanostructure. For example, a nanoparticle (a model '0D nanostructure') is defined as a small object that acts as a complete unit with regards to its physical and chemical properties. Gold nanoparticles have easily tuned physical properties, which make them ideal candidates for developing bioassays.

Carbon nanotubes are an example of a 1D nanostructure. They are essentially rolled up sheets of carbon that exhibit extraordinary electrical properties. These properties are very sensitive to chemical and biological species in the immediate environment, meaning they are often used to give an electrical output upon detection of a biological analyte.

In order to translate the use of nanostructures for diagnostic purposes, it is necessary to functionalise their surfaces using various biomolecules such as antibodies, peptides and nucleic acids. Functionalisation is the process of immobilising these biological molecules to the surface of a nanostructure, essentially coupling the molecular biology input to the nanostructures capacity for output signal.

Nanomaterials in diagnostics

Nanomaterials can be used in diagnostics either as labels or supports. As labels, they are in some way connected to a receptor specific to a specific analyte. They function to detect a binding event and give a particular output, such as fluorescence or a change in absorption wavelength.

When a nanostructure performs as a support, it is usually used to immobilise a receptor and so becomes the surface on which recognition events occur.

Metallic nanoparticles often serve as effective labels as they can be easily detected by optical or electrical approaches. In addition, their local surface plasmon resonance (LSPR) means that probing by absorption spectroscopy is possible. They often possess high absorption coefficients, and therefore they exhibit a high degree of sensitivity in optical detection-based methods.

Carbon nanotubes are commonly used as a support during biomarker detection. Binding of analytes to receptors immobilised on carbon nanotubes significantly changes the resistance of the carbon nanotube. This change in resistance is extremely sensitive and easily quantifiable [2].

Low is the new high

In the last year, novel research has been reported showing that prostate-specific antigen (PSA), a commonly used biomarker for prostate cancer, was detected using nanotechnology at a concentration nine orders of magnitude less than is needed to detect it using the traditional ELISA [3]. The process of detecting molecules at these levels has been termed 'inverse sensitivity', as lower

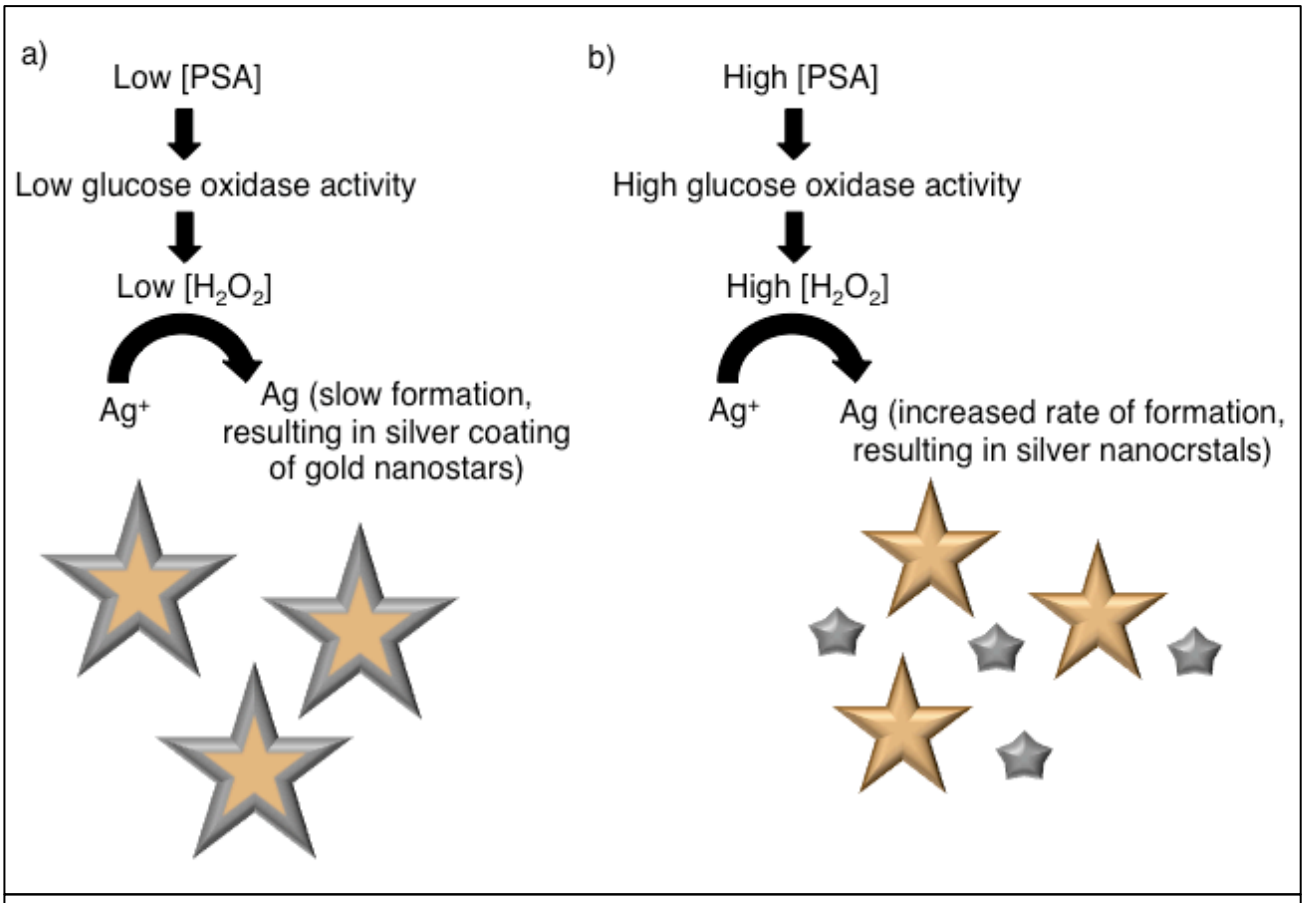

Figure 1. PSA concentration determines rate of silver ion reduction, leading to either silver coated nanostars or silver nanocrystals. (a) At low concentrations of PSA, less glucose oxidase is active. This results in slow reduction of silver ions and silver coating of gold nanostars. (b) When PSA concentrations are high, silver reduction occurs at a faster rate, leading to the formation of silver nanocrystals.

levels of the biomarker resulted in a higher signal level.

The enzyme glucose oxidase was used as a label in a traditional enzyme-linked immunoassay. Glucose oxidase generates hydrogen peroxide, which reduces silver ions resulting in the formation of a silver coating of the gold nanostars (Figure 1). This is detected as a blueshift in LSPR. However, when PSA concentrations are high, a larger amount of glucose oxidase is present. The resultant increased rate of silver crystal formation meant they began to nucleate and silver nanocrystals formed instead of the silver coating of the gold nanostars.

At low PSA concentrations, the silver coating of the gold nanostars was prevalent, but as PSA concentrations increased, nucleation of silver crystals began to dominate. Each state was easily quantifiable and concentrations of PSA as low as 10^{-18} g ml^{-1} were detected.

Detecting lower concentrations of biomarkers will pave the way for earlier diagnoses and improved patient outcomes.

Reasons for optimism

It is widely accepted that the future of healthcare will be increasingly based on personalised medicine. Treatments will be tailored according to the needs of the patient on the basis of a particular profile of biomarkers. If this is to become a reality, it is essential that point-of-care diagnostics are improved. Miniaturising diagn ostic tools, improving reaction speeds, increasing portability, reducing amounts of reagents, and the use of smaller sample sizes may be achieved by using

nanomaterials as a basis for these diagnostics. In addition, nanotechnology is amenable to multiplexing, meaning that 'one pot diagnostics' is a feasible prospect for the future.

What's the catch?

While the foundations for the used of nanomaterials in molecular diagnostics have been laid, few nanomaterial-based techniques have been translated to application in the field.

Various barriers are responsible for this current lack of translation. Although excellent results have been shown using simple laboratory-based solutions, the matrix effects of using complex samples such as blood or urine means considerable optimisation is first necessary in order to calibrate and optimise certain methods.

Another barrier is the cost of using nanomaterials. Although relatively small amounts of nanomaterial is needed for these devices, the initial start-up costs are not insignificant.

Other limiting factors of the use of nanomaterials in molecular diagnostics include the possibility of nonspecific adsorption/binding, aggregation, size variability, solubility and stability issues.

Concluding remarks

The use of nanomaterials in molecular *in vitro* diagnostics has huge potential, both in terms of modern personalised medicine, but also in global health applications [1]. The prospect of lab-on-a-chip diagnostics is appealing as it would lead to reduced costs and labour requirements.

The field of nanodiagnostics is a highly active area of innovation with research aiming to drive down cost, reaction times and improve sensitivity and flexibility.

Nanotechnology is an exciting, multidisciplinary field of research offering new hope for the detection of biomarkers at earlier points of disease onset, leading to improved prognoses and enhanced patient care. Use of nanomaterials will enable point-of-care diagnosis and treatment, allowing clinicians to track and treat diseases with increased efficiency.

References

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