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Harnessing Nanobiotechnology as a Diagnostic Tool to Improve Medical Laboratory Detection of Pathogenic Microorganisms

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ABSTRACT

A multitude of pathogenic microorganisms cause serious threats to human health and wellbeing. Rapid and accurate diagnosis is a critically important measure towards lowering the incalculable toll of morbidity and mortality from infectious diseases worldwide each year. The provision of early information enables a treating physician to prescribe an effective therapeutic intervention, thereby avoiding long-term clinical complications. From a public health perspective this also reduces the possibility of an infectious, undiagnosed patient unknowingly transmitting a given disease to others with whom they are in contact. Harnessing nanobiotechnology in the medical laboratory detection of microbial pathogens is advancing the diagnosis of infectious diseases by making use of very inexpensive materials to achieve highly sensitive and specific test results. Using nanoparticles, ultrafine molecules with high surface to volume ratio, enables attachment to the surface of targeted molecules, rendering them easily detectable by their optical and magnetic properties. Nanobiotechnology-based diagnostic techniques produce data in a much shorter time than do conventional methodologies, thus facilitating prompt case treatment. There are a number of technical limitations that need to be resolved, while proper ethical and legal guidelines have to be established. However, if these initial issues can be overcome, the attractive combination of high sensitivity, specificity, reduced cost, portability and reusability of nanobiotechnology is set to drive a new revolution in the diagnosis and treatment of infectious diseases.

Keywords: *nanobiotechnology; gold nanoparticle; magnetite nanoparticle; quantum dot; electromechanical system; cantilever array; microorganism; pathogen; diagnosis.*

Abbreviations: *AuNP, gold nanoparticle; Bio-MEMS, biomedical microelectromechanical system; ELISA, enzyme-linked immunosorbent assay; HIV, human immunodeficiency virus; ICP-MS, inductively coupled plasma mass spectrometry; MB, magnetic bead; MERS-CoV, Middle East respiratory syndrome-related coronavirus; NASBA, nucleic acid sequence-based amplification; PDA, polydopamine;QCM, quartz crystal microbalance; QD, quantum dot; RSV, respiratory syncytial virus; SARS-CoV, severe acute respiratory syndrome coronavirus; SPR, surface plasmon resonance.*

INTRODUCTION

Infectious diseases are typically caused by pathogenic microorganisms belonging to five main types: bacteria, viruses, fungi, protozoa and parasitic worms. These share the characteristics of multiplying rapidly, displaying host survival adaptations and undergoing phenotypic changes through genetic mutation [1]. Several different viruses such as Dengue, Ebola, HIV, SARS-CoV, MERS-CoV, Zika and, currently, SARS-CoV-2 have emerged since the middle of the twentieth century to pose a great public health threat [2]. Infections caused by these pathogens have seen a continuous global increase in associated morbidity and mortality.

Facilitated by the ongoing rise in international travel the rate of transmission of pathogenic microorganisms has escalated, leading to their spread around the world. In light of this, better means of diagnosis need to be developed to enable quicker, more appropriate treatment of infected patients. Novel diagnostic techniques should drive early detection, provide high sensitivity and allow potential point-of-care testing

[3]. This brief review outlines the application of nanobiotechnology to produce innovative nanoparticle-based rapid, sensitive diagnostic tests against a range of pathogenic microbes for use in medical microbiology laboratories.

NANOBIOTECHNOLOGY

The multidisciplinary concept of nanotechnology – the design, characterization and production of materials on an atomic, molecular and supra molecular scale for industrial purposes – was introduced in the late 1950s, although it took considerably longer to come to realization [4]. The study of nano science has advanced the development of many fields due to the unique physicochemical properties of nanoparticles from optical, mechanical, magnetic, catalytic and electrical perspectives [5]. In recent decades biomedical applications including tissue engineering, drug delivery, bio imaging and nano diagnostics have progressed significantly by harnessing nanobiotechnology, the discipline at the intersection of biological research and various fields of nanotechnology (Figure 1).

Figure 1. *Applications through which nanobiotechnology is revolutionizing biomedical sciences [8].*

The application of what is essentially a miniaturized form of bio technology to diagnostic medical microbiology has been researched extensively with a view to the requirements of clinical diagnostics for early detection of infectious disease-causing agents or markers of infection, high sensitivity and point-of-care testing [3, 6]. Nano particles of assorted materials and size have shown considerable potential as platforms for detecting disease markers, precancerous cells, fragments of virus, specific proteins, antibodies and other indicators of disease [7]. Nano biotechnologies enable diagnosis at single cell and molecular levels. It is therefore predicted that the use of nanobiotechnology in clinical diagnosis will become a major aspect of the future development of nano medicine [8]. Already, nano diagnostic platforms have the ability to generate rapid and reliable results on easy-to-use portable devices from routinely collected patient samples like blood, sputum and urine [3].

NANOPARTICLES

A nanoparticle is usually defined as a microscopic particle of matter that is either between 1–100 nano metres (nm) in diameter or has at least one dimension less than 100 nm. This size is of a similar scale to that of most biological molecules and structures; hence, nanomaterials can be used for both *in vitro* and *in vivo* medical research tools and translational clinical applications [7] (Table 1).

Being ultrafine, nano particles have a high surface area to volume ratio [5]. This characteristic makes them a very suitable scaffold to which to attach a large number of target molecules in order to optimize detection, thereby enhancing the sensitivity of diagnostic results. Nanoparticles possess certain size-dependent properties, particularly with respect to optical and magnetic parameters, that can be manipulated to achieve a detectable signal. The primary event in most nanoparticle-based assays is the binding of a nanoparticle label or probe to the target's surface that will produce a measurable signal that is characteristic of that bio molecule [7].

There is a wide range of nanoparticles that vary according to material, size and shape – the most common of which are sphere, dot, prism, rod and star. Nanoparticles can be synthesized from metals or polymers [9]. Among metallic nanoparticles those made of gold are used extensively in bioassays. The optical properties of gold nanoparticles (AuNPs) and quantum dots (QDs),

such as absorption coefficient, refractive index and colour, may be explained by a phenomenon called surface plasmon resonance [9], as discussed below. New nano diagnostic tools include carbon nanotubes, nanoshells, gold nanoparticles, cantilevers and QDs [7] (Table 1). Of these different nanostructures, QDs are the most frequently used and show the greatest promise for diagnostic applications. These are semiconductor nano crystals that are characterized by strong light absorbance and which can be used as fluorescent labels for bio molecules.

Modality	Potential Applications
Cantilevers	• High-throughput screening • Disease protein biomarker detection • DNA mutation detection (SNPs) • Gene expression detection
Carbon Nanotubes	• DNA mutation detection • Disease protein biomarker detection
Dendrimers	• Image contrast agents
Nanocrystals	• Improved formulation for poorly soluble drugs
Nanoparticles	• Targeted drug delivery, permeation enhancers • MRI and ultrasound image contrast agents • Reporters of apoptosis, angiogenesis, etc.
Nanoshells	• Tumor-specific imaging
Nanowires	• High-throughput screening • Disease protein biomarker detection • DNA mutation detection (SNPs) • Gene expression detection
Quantum Dots	• Optical detection of genes and proteins in animal models and cell assays • Tumor and lymph node visualization

Table 1. *Different types of nano biotechnological device and their use for specific purposes [7].*

The following sections describe the development and application of nanoparticle-based bioassays in the diagnosis of infectious diseases by using named examples to demonstrate each technique – dengue, tuberculosis, respiratory syncytial virus, malaria and HIV/AIDS.

Diagnosis of Dengue and Tuberculosis Using Gold Nanoparticles

Transmitted between humans by the bite of infectious *Aedes* mosquitoes Dengue virus is the cause of the world's most rapidly spreading vector-borne disease. Dengue is a major epidemic disease in tropical and subtropical countries, posing a public health threat to billions of people. Symptoms range from a mild influenza-like illness with undifferentiated fever to the life-threatening manifestations of dengue haemorrhagic fever and dengue shock syndrome [10]. Currently, despite intensive research there is no regulatory authority-approved specific therapy or protective vaccine against Dengue virus. As a consequence, rapid, accurate and sensitive diagnostic techniques remain crucial to effective control and treatment applications [4, 11].

AuNPs have been combined with established analytical techniques to detect Dengue and other viruses [4], as described here (Figure 2):

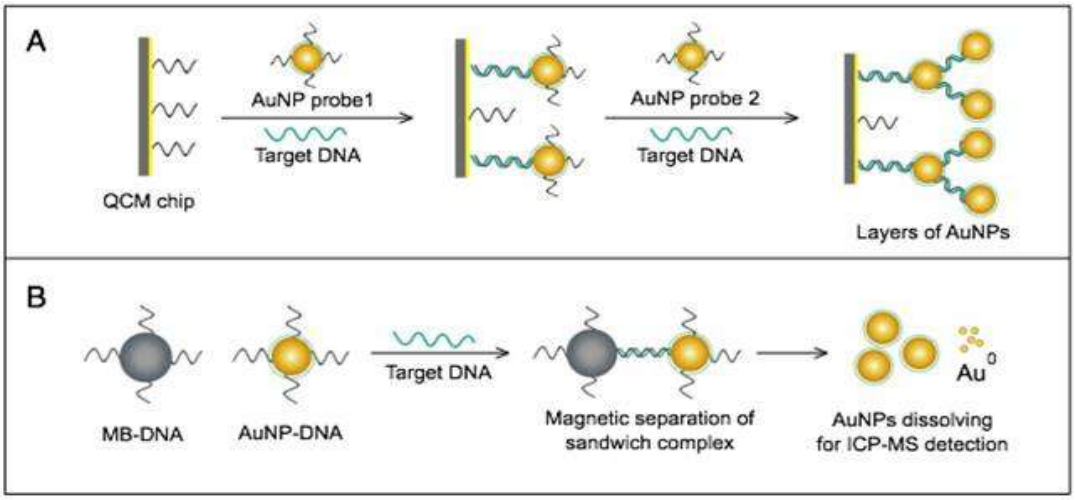

Figure 2. *Use of gold nanoparticles in the molecular diagnosis of viral infections such as Dengue [4].*

- Detection through target-induced assembly of multi layers of AuNPson a quartz crystal microbalance (QCM) chip. The surface of the chip is coated with gold film, on which the target DNA or RNA is captured and labelled by two types of virus-specific AuNP probe in order to amplify the detection signal by nanoparticle-based mass enhancement.
- Inductively coupled plasma mass spectrometry (ICP-MS)-based detection using magnetic separation and labelling with AuNP-DNA probes. Magnetic bead (MB)-DNA and AuNP-DNA sandwich the target DNA sequence, forming three-component hybridization complexes, which are separated magnetically and analyzed by ICP-MS.

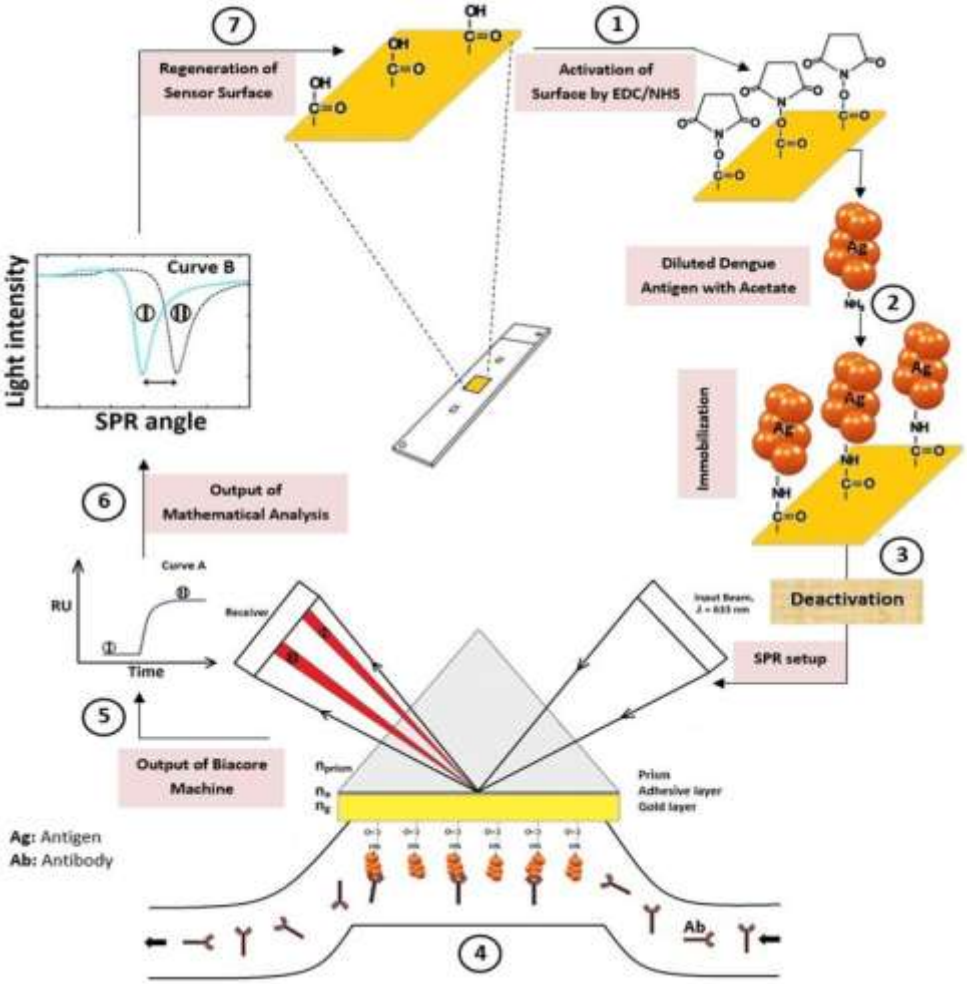

Figure 3. *Rapid human IgM-based Dengue diagnostic test using a surface plasmon resonance biosensor [12].*

Surface plasmon resonance (SPR) is another diagnostic tool with high sensitivity and specificity. This novel technique is applied to a rapid immunoglobulin (Ig)M-based Dengue diagnostic test for which all four known Dengue virus serotypes have been tested as a ligand on a biochip [12] (Figure 3). A serum volume of only 1 µl from a suspected Dengue patient is sufficient to indicate the SPR angle variation required to determine the ratio of each Dengue serotype in a sample, with 83-93% sensitivity and 100% specificity [12].

A nanodiagnostic method using nucleic acid sequence-based amplification (NASBA) and AuNP probes was developed for colorimetric detection of *Mycobacterium tuberculosis*, the bacterial causative agent of tuberculosis. The primers targeting 16S rRNA are used to amplify mycobacterial RNA by the isothermal NASBA process. The amplicons are hybridized with antigen-specific AuNP probes. The RNA–DNA hybrids are detected colorimetrically by the accumulation of AuNPs [13].

Diagnosis of RSV and Dengue Using Quantum Dots

QDs are inorganic fluorophores that offer significant advantages over conventional fluorescent markers when irradiated with low energy light the colour or frequency of which depends on the size of the dot. QDs of different sizes can be embedded into a given micro bead, producing distinct spectra of colour once excited. With just a simple excitation, both high sensitivity and abroad spectrum of excitation are achieved, which makes it a useful tool for genotype determination, image-guided surgery and molecular diagnostics [14].

QDs also enable multiplex diagnostic platforms and integration of diagnostics with therapeutics. Antibody-conjugated nanoparticles can rapidly and sensitively detect respiratory syncytial virus (RSV) and Dengue virus, by which relative levels of expression of the target surface antigen may be estimated [14, 15]. A major development is the use of dual colour QD fluorescence energy transfer nano beads that can be excited simultaneously with a single light source [15].

The QD system can detect the presence of viral particles within hours [15]. It is also more sensitive than comparable diagnostic tools, enabling the detection of virus earlier in the course of an infection. When RSV infects lung cellsit leaves part of its coat containing F and G proteins on the cell surface. QDs have been linked to antibodies that recognize coat proteins unique to the RSV capsid. As a result, when QDs come into contact with either viral particles or infected host cells they bind to each surface. In addition, colocalization of these proteins was demonstrated by confocal microscopy [15].

Diagnosis of Malaria and Dengue Using Magnetite Nanoparticles

Magnetite nanoparticles are colloidal iron oxide (Fe_3O_4) materials that exhibit super paramagnetic properties at ambient temperatures. The surface of the iron oxide nanoparticles are modified and conjugated with antibodies, proteins and nucleic acids in order to detect infecting pathogens, be they viruses, bacteria or protozoa. Magnetite nanoparticles possessing an iron oxide core and silver shell have been harnessed for the efficient and early detection of the major human malaria parasite *Plasmodium falciparum*. Such magnetite nanoparticles with haemozoin as a biomarker showed the capacity to detect β-haematin crystals through magnetic field-enriched surface-enhanced resonance Raman spectroscopy, thus allowing rapid diagnosis of malaria [6].

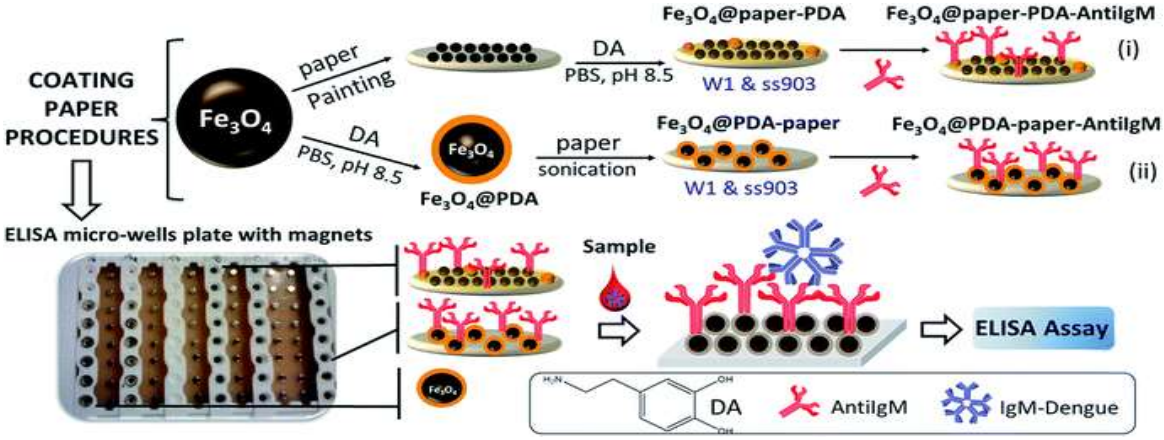

Figure 4. *Use of magnetite nanoparticle-coated paper conjugated with human anti-Dengue IgM for ELISA-based detection [17].*

Magnetite nanoparticles can be combined with other analytical techniques in order to improve their detection performance. A magnetic enzyme-linked immunosorbent assay (ELISA) based on core shell magnetite with polydopamine nanoparticles supported on cellulose filter paper was developed to detect anti-Dengue human IgM [16] (Figure 4). Magnetite nanoparticles deposited on filter paper conjugate with IgM using polydopamine (PDA) as a linker. Structural features, magnetic behaviour, coating homogeneity and the ratio of nanoparticles linked to antibodies were each determined. This method showed 100x more sensitivity and had a 700x lower limit of detection than more traditional methods [17]. This diagnostic technique is noted for its low cost, ease of manufacturing, speed of handling and producing few artifacts that may result in false positive results.

Diagnosis of *Mycobacterium Tuberculosis* and HIV/AIDS based on Bio-MEMS

The diagnostic principle of nano mechanical deflection due to adsorption of specific antigens is employed where by a cantilever surface is rendered bio sensitive by the deposition of a sensing layer that either contains bio receptors or to which these are covalently bonded [18]. This process is known as functionalization.

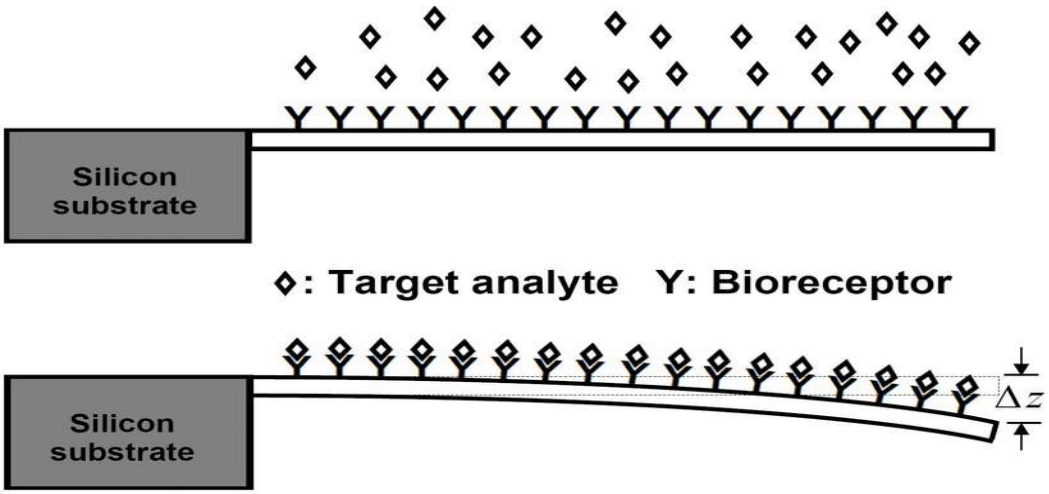

Figure 5. *Working principle of a cantilever array biosensor. Cantilever is functionalized by depositing a bio receptor layer (top). Surface stress-induced deflection upon binding between bio receptor and target analyte (bottom) [18].*

The most common bio receptors used in bio sensing are proteins, antibody/antigen complexes and nucleic acids. When analyte molecules are adsorbed to the functionalized cantilever surface the stress created bends the cantilever either up or down [18]. As the degree of bending depends linearly on the molecular species and its molar concentration, by measuring this deflection both variables be determined (Figure 5).

Detection founded on such a biomedical microelectromechanical system (bio-MEMS) is highly specific since a complementary biochemical interaction occurs between *M. tuberculosis* antigens and antibodies raised to them immobilized on the cantilever's upper surface. A diagnostic kit measures antigen concentrations up to pictogram levels [18].

Nanobiotechnology-based tuberculosis diagnostic kits are user-friendly and therefore do not require training in order to use and interpret. Moreover, manufactured in India they offer efficiency, portability and availability for the low cost of 30 rupees per test, which is around 50 Australian cents [7].

Bio-MEMS-based methodologies provide early detection of pathogenic microorganisms in sputum and stool samples, there by facilitating the possibility of intervention before infection spreads to other parts of the body. This is especially suitable for HIV/AIDS patients for whom life expectancy is increasingly long due to improved treatment regimens [18].

Perspectives on Current Drawbacks and Future Improvements

There are many positive aspects of harnessing nanobiotechnology-based diagnostics to further the early and rapid diagnosis and treatment of infectious diseases, as described above. Nevertheless, at the present time certain drawbacks of utilizing nanoparticles are recognized [19]. The use of nanoparticles across a broad spectrum of clinical medicine disciplines must comply with public health policies and adhere to environmental safety legislation.

- Due to their extremely small size, and thus a large surface area to volume ratio, nanoparticles can be highly reactive in bulk by comparison to larger particles. Hence, they may directly cause cytotoxic effects when adsorbed to human cells.
- Nanoparticles may trigger the release of free radicals that would harm human cells.
- There is insufficient research on the environmental impact of nanobiotechnology. Key issues such as the biodegradability of nanoparticles and the extent to which they may become incorporated in the food chain, thereby causing a potentially serious environmental threat, require ongoing investigation.

Further research is needed in order to determine the extent and impact of any negative impacts. If required, nanoparticles should be derived from natural substances that are biodegradable. Detailed guidelines surrounding legal implications and ethical considerations should be established for exploiting nano biotechnology to progress modern medicine, including in regard to improved detection of pathogenic microorganisms.

Conclusion

Nano diagnostics is the evolving application of nanoscale technology to meet the demands of clinical diagnostics, including characterizing the pathology of a condition, determining the stage of disease and identifying the causative organism [14]. Future development may lead to improved medical laboratory procedures, thereby providing novel and enhanced means of assessment of patient samples and early detection of disease biomarkers with high specificity and sensitivity. This nanoparticle platform offers the opportunity for on-the-spot diagnosis as the complex detection procedures are integrated into a simple, small and affordable device [20]. Once appropriate ethical considerations are met and procedural guidelines are framed at institutional and local government levels, nanobiotechnology-based molecular diagnostic procedures are set to revolutionize the way in which the diagnosis of infectious disease-causing pathogenic microorganisms is conducted. This will be of massive positive impact to the early diagnosis and treatment of established major global public health threats such as malaria, tuberculosis and HIV/AIDS as well as on the rapid and accurate detection of newly emerging pathogens in future decades.

Author Contributions

DR and AWT-R made substantial contributions to the conception of the work and to literature search, contributed significantly to writing the manuscript, revised it critically for important intellectual content, approved its final version, and agreed to its submission.

Conflict of Interest

The authors declare no actual or potential conflicts of interest in relation to this article.

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