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Nanotechnology advancements transforming molecular diagnostics: Applications in precision healthcare

Raffaele Conte[1,*], Roberta Foggia[2], Anna Valentino[1], Anna Di Salle[1], Fahd Kandsi[3], Anna Calarco[1]

[1]*National Research Council - Research Institute on Terrestrial Ecosystems, Via Pietro Castellino, 111 Naples, Italy.*
[2]*AMES Group, Via Padre Carmine Fico, 24 Casalnuovo di Napoli, Naples, Italy.*
[3]*Université Mohammed Premier, BV Mohammed VI B.P. 524 Oujda, Morocco.*

[*]**Corresponding author:** raffaele-conte@cnr.it

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Abstract:

Nanotechnology's impact on diagnostics is transformative, introducing cutting-edge tools that operate at the nanoscale, thereby revolutionizing clinical laboratory procedures. The development of nanoscale sensors, imaging agents, and other diagnostic tools has significantly elevated the sensitivity, precision, and efficiency of diagnostic processes. Indeed, engineered sensors that utilize nanomaterials (size < 100 nm) can detect minute biomarkers, allowing for early disease detection. Additionally, nanotechnology's integration with molecular diagnostics creates a synergistic effect, expediting diagnostic procedures and advancing personalized medicine. This convergence facilitates tailored treatments based on individual genetic and molecular profiles, optimizing therapeutic interventions. This review focuses on the application of these technologies in the clinical laboratory setting, analyzing the interrelationships of nanotechnology and molecular diagnostics. As these cutting-edge technologies continue to advance, they hold the potential to redefine the standard of care, ultimately contributing to a future world where healthcare is not only more sophisticated but also more patient-centric and optimized for individual needs.

Keywords: Molecular diagnostic; Molecular tracking; Nano-biochips; Nanobiosensors; Nanotechnology

1. Introduction

Nanotechnology (NT) represents a multidisciplinary frontier, intricately combining principles from physics, chemistry, biology, materials science, and engineering. It starts with Feynman's famous 1959 lecture, "There's Plenty of Room at the Bottom" with the discussion on the possibility of manipulating individual atoms and molecules and continues thank to Drexler's work (often referred to as the father of nanotechnology) with his book "Engines of Creation" where the concept of nanotechnology and its potential applications was popularized. NT delves into the exploration of phenomena at the nanoscale, where quantum effects and surface properties apply [1]. Operating within the size range of atoms and molecules, these materials exhibit distinct behaviors compared to their bulk counterparts, accentuating quantum effects and imparting unique optical, electrical, and mechanical properties [2]. Employing sophisticated techniques such as top-down and bottom-up approaches, researchers are able to create intricate structures and devices that harness the extraordinary properties observed at the nanoscale [3]. The impact of nanotechnology spans across diverse industries. For example, in medicine, nanotechnology facilitates targeted drug delivery, ushering in a new era in medicine where medications can be precisely delivered to specific areas within the body [4]. In electronics, nanotechnology fuels a relentless drive toward miniaturization, resulting in electronic devices that are not only smaller but also more efficient [5]. In materials science, NT plays a pivotal role in the development of materials that not only boast enhanced strength but are also lighter than their conventional counterparts [6].

In medical diagnostic, NT introduced a transformative era marked by heightened sensitivity, specificity, and precision. At the forefront of this revolutionary paradigm are metic-

ulously engineered nanoparticles (NPs) serving as potent imaging agents or sensors (figure 1). Magnetic resonance imaging (MRI) [7], computed tomography (CT) [8], and ultrasound [9] have been used for nanoscale tools capable of enhancing the visibility of tissues and structures with unprecedented clarity. In addition, nanobiosensors represent a significant leap forward in disease detection, enabling the early identification of specific biomolecules associated with illness [10]. Furthermore, the landscape of point-of-care diagnostics has undergone a profound redefinition with the incorporation of nanoscale components into miniaturized diagnostic devices [11] like DNA-based nanostructures allowing the detection of genetic mutations [12] or lab-on-chip biosensing platforms for diagnosis of viral infection [13, 14].

2. Nanotechnology-based biochips and microarrays

Nanotechnology-based biochips and microarrays represent a novel approach to comprehensive chemical analysis systems, promising to make molecular diagnostics more accessible and cost-effective. Several examples of such devices include nanofluidic arrays and protein nanobiochips [15]. One notable application of nanofluidic devices involves the isolation and analysis of individual biomolecules, such as DNA, with potential implications as innovative cancer detection methods [16]. A noteworthy device in this context implicates the creation of silicon [17] or carbon [18] nanotubes on a substrate through standard photolithographic and etching techniques, followed by a chemical oxidation step converting these nanowires into hollow nanotubes [17]. Nanotubes used for biomolecule isolation typically have diameters of 50 nm. In the process of trapping DNA molecules, a silicon nanotube connects two parallel microfluidic channels, with electrodes supplying current to drive DNA into the nanotubes. The sudden change in electrical current occurs each time a single DNA molecule enters or exits the nanotube. The application of nanofluidic technology holds significant promise across various domains, including systems biology, personalized medicine, pathogen detection, drug development, and clinical research [17].

Similarly, protein microarrays are generated by printing complementary DNAs onto glass slides and subsequently translating target proteins using mammalian reticulocyte lysate [19]. Epitope tags fused to the proteins facilitate immobilization in situ, eliminating the need for protein purification, addressing stability issues during storage, and capturing sufficient protein for functional studies. This technology has been utilized to map pairwise interactions among 29 human DNA replication initiation proteins and to

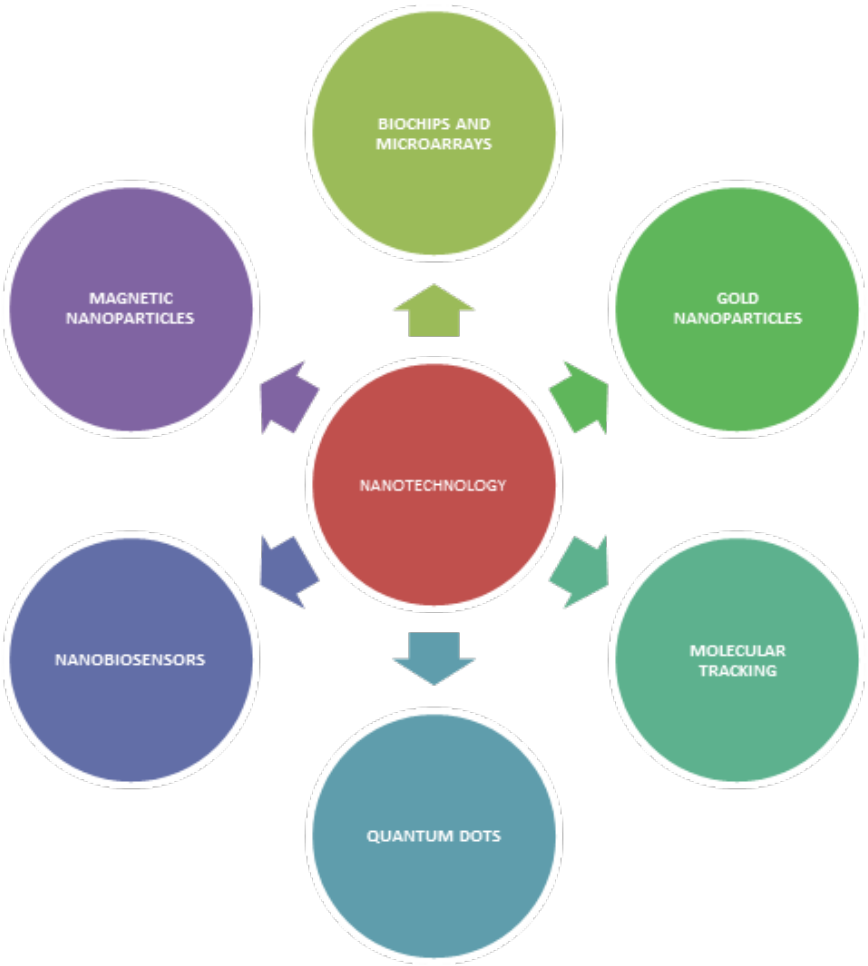

Figure 1. Nanotechnology applications in molecular diagnostic.

replicate the regulation of Cdt1 binding to select replication proteins, including mapping the geminin-binding domain [19].

3. Nanoparticles for molecular tracking

Examples of the use of nanoparticles used for tracking applications are the superparamagnetic iron oxide NPs (SPIO) that are gaining traction as an optimal tool for noninvasive cell tracking [20]. However, SPIO have limited efficiency in intracellular labeling, spurring the exploration of alternative labeling approaches.

A promising solution involves the utilization of perfluorocarbon nanoparticles specifically used to label endothelial progenitor cells extracted from human umbilical cord blood. This method enables the in vivo detection of progenitor cells through MRI [21]. In particular, only cells containing these nanoparticles become visible in the scan by adjusting the MRI scanner to the specific frequency of the fluorine compound contained in the nanoparticles. This approach effectively eliminates background signals that typically interfere with medical imaging. Moreover, the absence of interference allows for the precise measurement of low amounts of labeled cells, estimating their quantity based on the image brightness [21].

Given the range of available perfluorocarbon compounds, distinct cell types could be labeled with different compounds, administered, and subsequently individually identified by tuning the MRI scanner to each cell type's frequency. Partlow et al. (2007) have also demonstrated that the labeled cells retain their typical surface markers and remain functional post-labeling. Moreover, they are able to migrate and to be incorporated into blood vessels forming around tumors in mice [21].

4. Gold nanoparticles (AuNPs)

Gold nanoparticles have found widespread applications in the biomedical field, [22] particularly for the detection and identification of metabolites, proteins, and nucleic acids (DNA and RNA). Diagnostic techniques based on gold nanoparticles offer an unprecedented improvement in sensitivity enabling the use of smaller samples and reducing the reliance on complex apparatus for analytical procedures [23].

Various colorimetric approaches based on gold nanoparticles have been proposed for the highly sensitive and specific detection of biomolecules [23, 24]. These methods often rely on the colorimetric changes observed in an AuNPs solution upon aggregation, a process that can be induced by alterations in the medium's dielectric or interaction with the specified target. In the former, the binding and adsorption of the target alter the effect of changes in the medium's dielectric on the AuNPs. The latter method depends on the target's ability to mediate interparticle interactions, either by facilitating cross-linking of AuNPs or by keeping them apart through steric hindrance. The resultant aggregation causes a shift in the localized surface plasmon resonance (LSPR) band, which can be visually observed or measured using standard spectrophotometry. This is exemplified in several hybridization-based protocols, where single-stranded DNA (ssDNA) probes are functionalized onto the AuNPs, allowing for the sequence-dependent identification of DNA/RNA targets [25].

The specific electrical shifts in the plasmonic properties of AuNPs can also be leveraged for detecting biomarkers through light scattering. These methodologies rely on the pronounced light scattering exhibited by larger and anisotropic AuNPs, with changes in the spectral profile indicating their binding or association with molecules [26].

In electrochemical detection, AuNPs play a pivotal role by facilitating the binding of enzymes to electrodes and mediating electrochemical reactions as efficient redox catalysts [27]. Additionally, AuNPs are well-known for their ability to quench fluorescence dyes placed in proximity to their surfaces. This characteristic is extensively employed in molecular detection schemes, where binding to a specific target induces a conformational change in the recognition moiety. This alteration shifts the fluorophore's distance from the AuNP surface, resulting in an increase or decrease in fluorescence emission [28].

Also, Raman spectroscopy has seen advancements through the integration of AuNPs. In fact, in Surface Enhanced Raman Spectroscopy (SERS), weak Raman signal intensity of biomarkers is significantly amplified through the metal surface association of the AUNPs. For example, non-spherical AuNPs provide up to 10^12 to 10^14 enhancement of the SERS signal, introducing a new dimension to the multiplexing detection of biomarkers [29].

5. Quantum dots

Quantum Dots (QDs) present distinct advantages over traditional fluorescent markers, including high sensitivity, broad excitation spectra, stable fluorescence with simple excitation, and independence from lasers. Their red/infrared colors make them well-suited for whole blood assays, contributing to their diverse applications in molecular diagnostics and genotyping [30].

In cancer diagnosis, luminescent and stable QD bioconjugates allow for the visualization of cancer cells in living animals, facilitating high-resolution cell tracking through fluorescence microscopy. For instance, QDs coated with a polyacrylate cap and covalently linked to antibodies have been employed for immunofluorescent labeling of the breast cancer marker Her2 [31]. Additionally, carbohydrate-encapsulated QDs with luminescent properties prove valuable for cancer imaging [32].

QDs also find application in viral diagnosis, notably for respiratory syncytial virus (RSV) [33]. The rapid and sensitive detection of RSV, crucial for infection control and antiviral drug development, is facilitated by Dual-color QDs excited simultaneously with a single light source. This QD system enables the swift detection of RSV particles, offering heightened sensitivity for early virus detection during an infection. In the case of RSV infection in lung cells, QDs linked to antibodies specific to unique structures in the RSV coat adhere to viral particles or infected cells, enabling their visualization [33].

6. Magnetic nanoparticles

Different types of magnetic nanoparticles are used for molecular diagnostic. For example, iron nanoparticles ranging in size from 15 to 20 nm with saturation magnetization, have been successfully synthesized and incorporated into copolymer beads composed of styrene and glycidyl methacrylate (GMA). These beads were further coated with polyGMA through seed polymerization [34]. The resulting Fe/St-GMA/GMA beads effectively suppress nonspecific protein adsorption and found application for bioscreening [34].

Similarly, ferrofluids, such as Immunicon's Cell Tracks™ Technology, comprising a magnetic core surrounded by a polymeric layer coated with antibodies, are usedfor capturing cells with diagnostic applications [35]. Moreover, a family of calcium indicators for MRI is created by combining superparamagnetic iron oxide (SPIO) nanoparticle with the calcium-sensing protein calmodulin to reveal small lymph-node metastases [36].

7. Nanobiosensors

Nanobiosensors play a crucial role in detecting molecules with extraordinary sensitivity thanks to their ability to be electronically gated to respond to the binding of a single molecule [37]. Prototype sensors have successfully identified nucleic acids, proteins, and ions, operating in both liquid and gas phases [38].

The detection schemes employed by these sensors use cost-effective, low-voltage measurement approaches, directly detecting binding events. This eliminates the need for expensive, complex, and time-consuming labeling chemistries such as fluorescent dyes or the use of bulky and costly optical detection systems. As a result, these sensors are not only inexpensive to manufacture but also portable.

Examples of nanobiosensors are cantilevers biosensors, devices that convert a reaction into a nanoscale mechanical motion that can be directly measured by deflecting a light beam from the cantilever surface; Cantilevers offer an alternative to PCR and protein microarray methods. A notable advantage is that there is no need for labeling or replicating the target molecules. They provide rapid and label-free recognition of specific DNA sequences, including single-nucleotide polymorphisms, oncogenes, and genotyping. The potential significance of nanocantilevers emerges in their role as ultra small sensors for detecting viruses, bacteria, and other pathogens [39].

Also Surface plasmon resonance (SPR) technology stands out as a prominent example of optical nanobiosensors. Optical-detectable tags are created through surface-enhanced Raman scattering (SERS) of active molecules at the glass-metal interface. Each type of tag leverages the Raman spectrum of a distinct small molecule. SERS bands, with widths 1/50th that of fluorescent bands, allow for a higher degree of multiplexing compared to fluorescence-based quantification tags. The spectral intensity of SERS-based tags linearly correlates with the number of particles, enabling their use for multiplexed analyte quantification. SERS-based tags can be detected using cost-effective instrumentation. These particles can be examined in the near-infrared range, facilitating detection in blood and other tissues. Moreover, they exhibit stability and resistance to photodegradation [40, 41]. SERS technology also allows the direct detection of biological species, including spores and biomarkers of pathogenic agents, through a DNA-based method known as surface-enhanced Raman gene probes (SERGen) that works through hybridization to DNA sequences complementary to these probes [42].

Another type of biosensors is the viral nanobiosensors. Herpes simplex virus and adenovirus can be assembled with magnetic nanobeads to design nanosensors able to link with clinically relevant viruses [43]. This innovative approach allows the detection of as few as 5 viral particles in a 10-mL serum sample. Notably, this system surpasses the sensitivity of ELISA-based methods and represents an improvement over PCR-based detection methods due to its cost-effectiveness, rapidity, and reduced susceptibility to artifacts [43]. Table 1 recaps the main applications of nanodevices.

Furthermore, graphene based nanobiosensors and platinum nanoparticles are extensively used in tumor diagnosis. For example, Sadeghi et al. coupled reduced graphene oxide nanosheets (rGONs) and rhodium nanoparticles (Rh-NPs) on the surface of graphite electrode. The graphite electrode-based aptasensor (g-aptasensor) demonstrated exceptional performance against HER2-overexpressed SKBR3 cancer cells, with a linear dynamic range of 5.0 to 10.0×10^4 cells/mL, an analytical limit of detection (LOD) as low as 1.0 cell/mL, and a limit of quantification (LOQ) of 3.0 cells/mL [44]. Javad Mohammadnejad et al. fabricated a novel electrochemical nanobiosensor based on reduced graphene oxide (RGO) and gold nanoparticles (AuNPs) to detect miRNA-128. It was found that the modified electrode has excellent selectivity and sensitivity to miR-128, with a limit of detection of 0.08761 fM in label-free and 0.00956 fM in labeling assay [45]. Similarly, Lad et al. developed a platinum based nanobiosensor for monitoring toxicological behavior of carboplatin by measuring Carboplatin-DNA interaction changes in vitro. The surface of electrode was modified with platinum nanoparticles to develop an electroanalytical method, which can monitor Carboplatin-DNA interaction. As the time elapse, the change in electrode potential gets elevated, while at one time the change in electrode potential get stopped due to formation of Carboplatin-DNA adduct. This adduct is responsible for producing toxic effects. The developed nanobiosensor showed detection limit up to 10 ng/mL, which can be a crucial for DNA damage studies [46].

8. Conclusion

The ongoing advancements in nanotechnology for advanced diagnostics represent a significant leap forward in the trajectory of medical innovation. The integration of nanoscale tools and sensors into diagnostic methodologies has demonstrated unprecedented potential to elevate the standards of medical diagnostics. Beyond the immediate benefits of enhanced accuracy and speed, this integration has the transformative power to democratize access to diagnostic procedures, transcending geographical and socioeconomic

Table 1. Applications of nanodevices.

Device	Application	Reference
Nanofluidic array	Isolation and analysis of individual biomolecules	[16]
Silicon nanotubes	Trapping DNA molecules	[17]
Carbon nanotubes	Trapping DNA molecules	[18]
Protein microarrays	Mapping the geminin-binding domain	[19]
Superparamagnetic iron oxide nanoparticles	Non-invasive cell tracking	[20]
Perfluorocarbon nanoparticles	*in vivo* detection of progenitor cells through MRI	[21]
Gold nanoparticles	Detection and identification of metabolites, proteins, and nucleic acids	[22]
Gold nanoparticles	Detection of biomolecules	[23, 24]
Gold nanoparticles	Single-stranded DNA (ssDNA) probes allowing for the sequence-dependent identification of DNA/RNA	[25]
Gold nanoparticles	Leveraged for detecting biomarkers through light scattering	[26]
Gold nanoparticles	Redox catalysts	[27]
Gold nanoparticles	Molecular detection schemes	[28]
Gold nanoparticles	Enhancement of the SERS signal	[29]
Quantum dots	Molecular diagnostics and genotyping	[30]
Quantum dots	Immunofluorescent labeling of the breast cancer marker Her2	[31]
	marker Her2	
Carbohydrate-encapsulated quantum dots	Cancer imaging	[32]
Quantum dots	Diagnosis for respiratory syncytial virus (RSV)	[33]
Magnetic nanoparticles composed of styrene and glycidyl methacrylate	Bioscreening	[34]
Immunicon's CellTracks™ Technology	Bioscreening	[35]
Superparamagnetic iron oxide (SPIO) nanoparticle linked with calmodulin	Reveal of small lymph-node metastases	[36]
Nanobiosensors	Binding of molecules for diagnosis	[37]
Nanobiosensors	Identification of nucleic acids, proteins, and ions	[38]
Nano cantilevers	Detection of viruses, bacteria, and other pathogens	[39]
Optical nanobiosensors	Detection with Surface-enhanced Raman scattering	[40, 41]
Optical nanobiosensors	Direct detection of biological species and biomarkers of pathogenic agents	[42]
Optical nanobiosensors	Recognition of clinically relevant viruses	[43]
Reduced graphene oxide nanosheets (rGONs) and rhodium nanoparticles (Rh-NPs) on the surface of graphite electrode	Recognition of overexpressed SKBR3 cancer cells	[44]
Electrochemical nanobiosensor based on reduced graphene oxide (RGO) and gold nanoparticles (AuNPs)	Detection of miRNA-128	[45]
Platinum based nanobiosensor	Monitoring of the toxicological behavior of carboplatin	[46]

barriers. As we look toward the future, the trajectory of advanced diagnostics appears to be on the cusp of a groundbreaking era characterized by precision, accessibility, and patient-centricity. The marriage of nanotechnology with diagnostics not only promises to refine the detection and monitoring of various medical conditions but also to foster a paradigm shift in healthcare delivery. The concept of personalized medicine, where diagnostic and treatment strategies are tailored to individual genetic and molecular profiles, stands as a beacon on the horizon. The democratization of diagnostic procedures, made possible by nanotechnology, holds the potential to empower individuals in actively managing their health. The advent of cost-effective and portable diagnostic devices, powered by nanosensors and microfluidic technologies, could revolutionize point-of-care testing, bringing advanced diagnostics closer to patients. This decentralization of diagnostic capabilities aligns with the broader trend in healthcare towards proactive and patient-centered approaches.In conclusion, the integration of nanotechnology into advanced diagnostics signifies not just a technological milestone but a potential catalyst for positive shifts in healthcare paradigms. The journey ahead involves addressing challenges, such as standardization, ethical considerations, and regulatory frameworks, to ensure the responsible and equitable deployment of these technologies. As we navigate this path, the collective vision is an era where diagnostics become not only more precise and accessible but also deeply personalized, contributing to improved health outcomes and laying the foundation for a holistic and patient-centric healthcare future. The convergence of nanotechnology with advanced diagnostics holds immense promise, and the continued exploration of these frontiers is likely to unveil unprecedented opportunities for improving global health.

Authors Contributions
All authors have contributed equally to prepare the paper.

Availability of Data and Materials
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Conflict of Interests
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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