6 Peroxidase-Like Activity of Metal Nanoparticles for Biomedical Applications

Swachhatoa Ghosh and Amit Jaiswal

Abstract

Nanoparticles are versatile proponents in modern-day research. Upon scaling down to the nano-range, materials exhibit a host of interesting properties, for use in imaging, sensing, and therapeutic approaches. Enzymes as biocatalysts require optimal thermodynamic conditions for maximal activity. Their purification protocols are both labor intensive and uneconomical, paving the need for development of simpler alternatives. Metal nanoparticles act as redox enzymes due to the electron exchange escalated by their superficial atoms. Also, monolayer-protected metal nanoparticles electrostatically interact with different substrates, promoting catalysis. This enables their use in industries, in detection of environmental pollution, and in biomedical applications and other clinical approaches. Peroxidases are an essential family of enzymes, capable of removing harmful metabolic by-products from the cellular environment and involved in the maintenance of cellular defense and integrity. This chapter lays its focus on the peroxidase-like activity of metal nanoparticles and their role in development of biosensors and immunoassays and detection of tumor cells while discussing its present catalytic limitations and future outlook.

Keywords

Nanoparticle · Peroxidase · Immunosensors · Biosensors · Catalysis

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Abbreviations

ABTS	2,2′-Azino-bis(3-ethylbenzothiazoline-6-sulphonic acid)
ADP	Adenosine diphosphate
AUR	Amplex ultra Red
BSA	Bovine serum albumin
CSF	Cerebrospinal fluid
DS	Dermatan sulfate
GAG	Glycosaminoglycan
HS	Heparin sulfate
SERS	Surface-enhanced Raman scattering
TMB	3,3′,5,5′-Tetramethylbenzidine
MS	Mass spectroscopy
GC-MS	Gas chromatography/mass spectroscopy
MALDI-MS	Matrix-assisted laser desorption/ionization
CGM	Continuous glucose monitoring
CTAB	Cetyltrimethylammonium bromide
TnI	Cardiac troponin I
GOx	Glucose oxidase
FAD	Flavin adenosine dinucleotide
$FADH_2$	Reduced flavin adenosine dinucleotide
hMSC	Mesenchymal stem cells
MNP	Magnetic nanoparticle
PBS	Phosphate buffer saline
MRI	Magnetic resonance imaging
ELISA	Enzyme-linked immunosorbent assay
ROS	Reactive oxygen species
HER2	Human epidermal growth factor receptor 2
OPD	o-Phenylenediamine dihydrochloride

6.1 Introduction

The fundamental role of enzymes is to act as biological catalysts. With the evolution of science over the years, enzymes are no longer restricted to proteins. Ribozymes, which are RNA molecules with enzymatic activities, can catalyze specific reactions, as can be done by certain DNA molecules. Enzymes may enhance the rate of a biochemical reaction by several folds. Cells contain many different enzymes, and their catalytic activity decides the regular events taking place within a living system (Cooper and Hausman 2004). Natural enzymes exhibit high sensitivity and selectivity towards the reactions they catalyze but involve intensive purification protocols, making the production costly. They also undergo rapid degradation when subjected to harsh temperature and pH changes in the reaction system. Therefore, the hunger for developing alternate enzyme-like systems with improved robustness

and easier production methods has been persistent in the scientific arena. Bionics is an emerging field dedicated to the study and design of engineering systems and modern technology, using naturally found biological systems (Bonser and Vincent 2007). Biomimicking is a concept that was popularized by Charles Darwin, as early as the nineteenth century. Artificial enzymes developed primarily to mimic the natural enzymes were found to be highly stable, robust, and easy to manufacture (Dramou and Tarannum 2016). Materials upon reduction to the nanoscale undergo drastic change in their physicochemical behavior, promoting their use in imaging (Han et al. 2019; Ahlawat et al. 2019), catalysis (Singh et al. 2017), sensing (Pallela et al. 2016; Skrabalak et al. 2007; Majarikar et al. 2016), diagnostics (Banerjee and Jaiswal 2018), and therapy (Yadav et al. 2019; Roy and Jaiswal 2017a). Catalytically active nanomaterials emerged as promising enzyme mimics, due to the attributes they shared with natural enzymes (Lin et al. 2014a). Metals like gold, silver, and platinum, considered to be inert under ordinary conditions, become efficient catalysts in nano-dimension (Lin et al. 2014b). This is primarily due to the increased surface area to volume ratio of nanoparticles, exposing a greater number of superficial metal atoms, leading to increased catalytic activity. Nanoparticles solely or with surface functionalization and their small size and varying morphology largely resemble natural enzymes and further boost their ability to replicate these catalysts (Wei and Wang 2013). Functionalization with polymers, ligands, drugs, proteins, surfactants, and other small molecules not only renders stability and regulates optical and physiochemical properties of nanoparticles but also aids in the interaction of the substrate molecules with its surface (Mahato et al. 2019). This enhances the catalytic output. However, unmodified nanoparticles are proven to be better catalysts. This is largely because the enzymatic activity stems from the nanoparticle itself and does not rely on the functional groups on its surface (Wang et al. 2012). The superficial atoms on the surface of metal nanoparticles contribute in electron exchange with the reaction system, spurring their redox-like properties (El-Sayed et al. 2017). Enhanced catalytic properties of porous nanoparticles are due to greater number of surfaces available for interaction. The inner and outer walls of the outer shell and the inner gold core of gold nanorattles exhibit varied catalytic profiles. The lack of stabilizers inside the gold nanorattle is responsible for increased catalytic activity of the inner core. Pasquato et al. termed their thiol-protected nanogold "nanozymes," in analogy with synzymes, which were enzyme-like polymers (Manea et al. 2004). Today, this term holds meaning in defining nanosystems which can show enzymatic activity in vitro and is already an intensely explored field (Lin et al. 2014b), primarily in sensing (Howes et al. 2014). The distinctive optical properties of metal nanoparticles, in combination with functionalized bioreceptors, impart sensing of miniscule analyte proportions in biological fluids (Chandra et al. 2011; Akhtar et al. 2018). Point-of-care devices and nanoparticles are together being used for rapid immunosensing applications. These multifaceted nanoparticles are increasingly being explored for simple healthcare (Roy and Jaiswal 2017b; Khandelia et al. 2013, 2014; Singh et al. 2019) and diagnosis (Chandra et al. 2017). In this chapter, the focus will primarily lie on the mechanism of peroxidase-like action, the properties of metal nanoparticles that

contribute towards their activity, and their biomedical applications with focus on biosensing, immunoassay platforms, and cancer cell detection. Lastly, their pitfalls and scope for further research will be discussed.

6.2 Metal Nanoparticles as Catalysts

The ability of metal nanoparticles to act as catalysts was discovered by Haruta when he demonstrated room temperature oxidation of carbon monoxide by gold nanostructures (Haruta et al. 1987). This led to countless experiments on the catalytic property of metal nanoparticles. The properties of materials transform upon reduction from bulk to nanometer range. Noble metals, considered to be inert under ordinary conditions, show unparalleled catalytic properties on attaining nanometer dimensions. Bulk metals tend to reflect light falling on them. Electron clouds on the surface of metal nanoparticles, however, resonate with different wavelengths of light depending upon their frequency. The variation in size and shape is responsible for absorbing different wavelengths of light and hence most metal nanoparticles with different sizes and shapes differ in color. The melting temperature also drops with decrease in size (Noguez 2007a). The number of coordinatively unsaturated atoms on the surfaces and edges of the nanoparticles is far less than in the bulk form and thus becomes highly reactive. Atoms at the corner, steps, and edges of the nanoparticles have the lowest coordination number and tend to be a lot more interactive with substrates and reagents (Noguez 2007b). These, therefore, show the highest catalytic potential (Navalón and García 2016a). As nanoparticles increase in size, they may undergo aggregation, limiting their reactivity. Adsorbing nanoparticles on the surfaces of insoluble solids provides stability against sintering and growth (Navalón and García 2016b).

Considered to be hard, crystalline and diametrically opposite of proteins, nanoparticles in reality, resemble them quite remarkably. Their overall size, charge, and shape along with the organic functional groups on their surface make them protein-like catalysts. Interaction with the substrate is dependent on the media parameters for both nanoparticles and natural enzymes, as even the former may have bulky functionalities on its surface (Kotov 2010). Thus, the superficial atoms on the metal nanoparticles, the size of the nanoparticles, and their thermodynamic properties decide their catalytic potential.

6.3 Peroxidase-Like Activity of Metal Nanoparticles

Peroxidases are a group of enzymes, capable of catalyzing the oxidation of hydrogen peroxide into hydroxyl radicals. These radicals further participate in electron exchange with substrates producing color on oxidation. Found in a range of organisms, peroxidases remove the toxic hydrogen peroxide, released as a by-product of respiration. The standard reactions are demonstrated using colorimetric substrates like TMB, ABTS, and so on. The redox reactions catalyzed

by peroxidases can be replicated by the superficial metal atoms of metal nanoparticles, thereby promoting catalysis. Fe_3O_4 nanoparticles were first reported to be potent peroxidase mimics by Gao et al. (2007a). The reduced specificity of horseradish peroxidase makes it a model enzyme for studying peroxidase-catalyzed reactions. Initially, peroxidases were mostly involved in detoxification of polluted water. Nobel metal nanoparticles show peroxidase-like action. CTAB-reduced PtNCs exhibited peroxidase-mimicking activity, while galvanic replacement of tellurium produced highly porous Pt nanotubes (Ma et al. 2011; Cai et al. 2013). Unmodified AuNPs show the highest catalytic activity, when compared to charged nanoparticles (Wang et al. 2012). Bimetallic nanoparticles of iron and platinum, in a cage of apoferritin (Aft-FePt), were reported as peroxidase-mimicking platforms and were more efficient as compared to single metal counterparts Aft-Fe and Aft-Pt, as Pt localized over Fe (Zhang et al. 2012; Xie et al. 2012). FeCo nanoparticles also demonstrated increased peroxidase activity as compared to their singular forms (Xie et al. 2012). Heavy metals like Hg^{2+} and antioxidants result in catalytic inhibition. The loss in catalytic activity in presence of heavy metals was regained in their absence and promoted heavy metal detection. BSA-stabilized AuNPs were used as sensing platforms for dopamine, xanthine, and uric acid (Zhao et al. 2012; Wang et al. 2011; Tao et al. 2013a).

6.4 Biomedical Applications of Peroxidase-Like Metal Nanoparticles

6.4.1 Biosensing

6.4.1.1 Detection of Heparin in Blood

Heparin or unfractionated heparin (UFH) is an anticoagulant drug used for prevention and treatment of thrombosis. The activity of antithrombin, a natural anticoagulant, is instantaneously upregulated upon intravenous administration of heparin. Inside the body, heparin non-specifically binds to plasma proteins and interacts with the cell surfaces of leukocytes and endothelial cells resulting in rapid clearance and reduced therapeutic activity (McRae Simon and Jeffrey 2004). Patients are generally subjected to different doses of the drug to study its response (Baughman Robert et al. 1998). You et al. devised a highly sensitive and selective fluorescent probe for detection of heparin from human serum (You et al. 2018). The probe consisted of adenosine-analogue functionalized gold nanoparticles which aggregated in presence of surfen, a small molecule antagonist of heparin sulfate, and lost their peroxidase activity. On adding negatively charged heparin, the surfen molecule detached from the AuNPs dismantling the aggregates (Fig. 6.1). The increase in their peroxidase action on administration of heparin was monitored spectroscopically and used for estimating a dose as low as 30 nM in spiked blood serums.

Hu et al. developed a heparinase sensing system by conjugating heparin to boost the enzymatic activity of BSA-stabilized AuNCs. Heparinase is a heparin-degrading

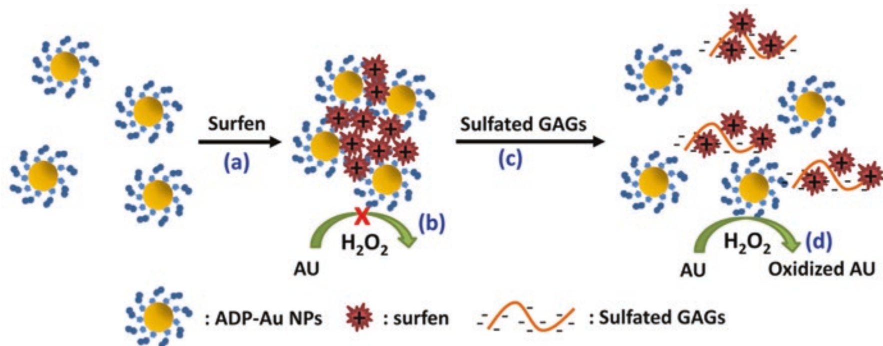


Fig. 6.1 Schematic demontration of sensing mechanism for detecting heparin- (**a–b**) surfen-induced aggregation of ADP-AuNPs, inhibiting the oxidation of AUR (**c–d**) in presence of sulfated GAGs like heparin, the surfen binding is disrupted allowing oxidation of AUR. (Reproduced with permission from You et al. (2018). Copyright 2018 American Chemical Society)

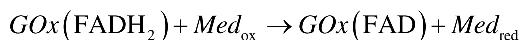
enzyme with pathological impact in tumor metastasis and membrane vascularization (Vlodavsky et al. 2011). It breaks heparin into small fragments inhibiting the peroxidase activity of the nanocomposites. The limit of detection achieved for heparinase was 0.06 μg/ml with a high signal to noise ratio, proving to be a sensitive and reliable system. Detection of both heparin and heparinase can be useful for detection of thrombosis and other cerebral conditions (Hu et al. 2018).

6.4.1.2 Detection of Glucose

Blood-Glucose Monitoring Sensor

Glucose is an essential fuel responsible for the major metabolic activities taking place in the human body. In India and globally, diabetes mellitus is considered to be a silent killer. This clinical condition is characterized by insulin insensitivity leading to hyperglycemia. Different types of glucose sensors have been flocking the market since the past 40 years (Chen et al. 2013). Glucose oxidase-based enzyme electrodes are applied for easy-to-use glucose monitoring, due to their cost-effectiveness and increased stability (Wang 2001; Wilson and Turner 1992).

$$GOx(\mathrm{FAD}) + \mathrm{glucose} \rightarrow GOx(\mathrm{FADH_2}) + \mathrm{gluconic\ acid}$$



$$GOx(\mathrm{FADH_2}) + Med_{\mathrm{ox}} \rightarrow GOx(\mathrm{FAD}) + Med_{\mathrm{red}}$$

The amperometric glucose biosensors involve the following three steps: (a) electron (and protons) transfer from glucose causing reduction of FAD to $FADH_2$ in the reaction centers of GOx; (b) electron transfer from $FADH_2$ centers to the mediator, transforming the mediators from Medox to their reduced state Medred; and (c) the final shuttle of electrons through the mediators to the electrode (Chen et al. 2013). Three generations of amperometric glucose sensors are known, based on the

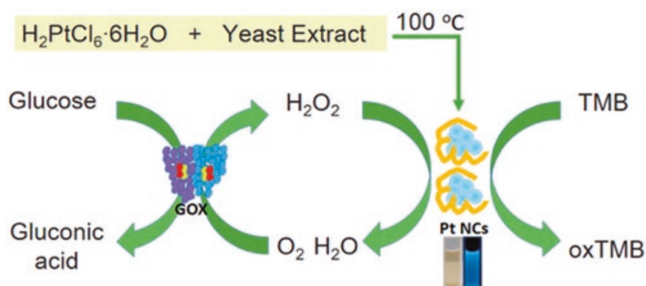


Fig. 6.2 Synthesis procedure of yeast-stabilized PtNCs and its mechanism of catalysis for glucose detection. (Reproduced with permission from Jin et al. (2017) Copyright 2017 American Chemical Society)

mediator (Med). The first generation of amperometric glucose biosensors uses O_2 for regeneration of GOx (FAD) and detects glucose based on either the amount of O_2 consumed or the amount of H_2O_2 generated in the process. These generations of sensors were simple and easy to use but were sensitive to oxygen for detection. The reliability on oxygen was addressed in the second-generation glucose sensors by using artificial electron shuttlers like ferrocene derivatives and conducing organic salts, transferring electrons into and out of the enzyme active site. The need to stabilize these artificial electron shuttlers resulted in development of third-generation glucose sensors. Electrochemical potential of GOx was used for detection of glucose in this generation of sensors.

Metal nanoparticles can be conjugated to glucose oxidase for triggering their peroxidase-like activity and promoting glucose detection. A unique yeast-stabilized PtNC system with peroxidase-mimicking ability was developed by Jin et al. for simultaneous oxidation of glucose and reduction of H_2O_2 (Jin et al. 2017) (Fig. 6.2). This system was then extended to human serum for colorimetric estimation of glucose. The limit of detection of this colorimetric system was 0.28 μM, which is significantly less when compared to other colorimetric sensors.

AuNPs, with intrinsic peroxidase-like activity, were used for colorimetric detection of H_2O_2 and glucose (Jv et al. 2010). A colorimetric glucose detection platform was developed by Wang et al. by combining the catalytic activities of GOx and MNPs. This sensor was fabricated based on the electrochemical depletion of electroactive species in the diffusion layer. Electroactive species like ascorbic acid and their role in glucose detection was tested by the interference-free micro-circumstance of the substrate electrode (GOD). The limit of detection for glucose was 0.005 mM with an overall range of 0.01–1 mM (Wang et al. 2005).

6.4.1.3 Detection of Cholesterol and Galactose Using Peroxidase-Like MNPs

Colorimetric detection of cholesterol was achieved by developing a nanostructured multicatalyst system consisting of MNPs and cholesterol oxidase, immobilized in large pore-sized mesoporous silica (Kim et al. 2011). This multicatalyst system

consisted of MNPs embedded in the wall of mesocellular silica pores, resulting in magnetic mesoporous silica (MMS) and cholesterol oxidases. Cholesterol oxidase immobilized in the MMS reacted with cholesterol to generate H_2O_2, subsequently activating MNPs in the mesocellular silica pores for colorimetric conversion of its substrate. The limit of detection for cholesterol was as low as 5 μM. Detection of galactose using a nanostructured multicatalyst system consisting of MNPs and galactose oxidase was reported by Kim et al. This multicatalytic system was tested as a promising analytical tool for galactosemia diagnosis, by determining the galactose concentration from the dried blood specimens obtained from clinical hospitals (Kim et al. 2012).

6.4.1.4 Plasmonic and Catalytic Activity of Gold Nanoparticles for Sensing Glucose and Lactate in Living Tissues

Lin et al. investigated the cross-talk between glucose, lactate, and ascorbate in the ascorbate modulating neuronal metabolism for better understanding of brain ischemia (Lin et al. 2014c). A microfluidic chip-based sensor was fabricated using three surface-modified indium-tin oxide electrodes as working electrodes. A stainless steel tube was used as a counter electrode and an Ag/AgCl wire as reference electrode. Electrochemical oxidation of ascorbic acid was achieved by using single-walled carbon nanotubes, while for glucose and lactate, a dehydrogenase-based mechanism was implemented. Fluctuation in the level of these metabolites may help in early diagnosis and treatment of neuronal conditions.

A peroxidase-like gold nanoparticle-impregnated metal-organic framework AuNP@MIL-101 was used for SERS activation of leucomalachite green (LMG) and subsequent detection of glucose and lactate in living tissues (Hu et al. 2017) (Fig. 6.3). This oxidase-integrated nanoparticle system for detecting glucose resulted in reduction of oxygen to hydrogen peroxide. This H_2O_2 facilitated the conversion of LMG, and the SERS signal intensity was used for quantification.

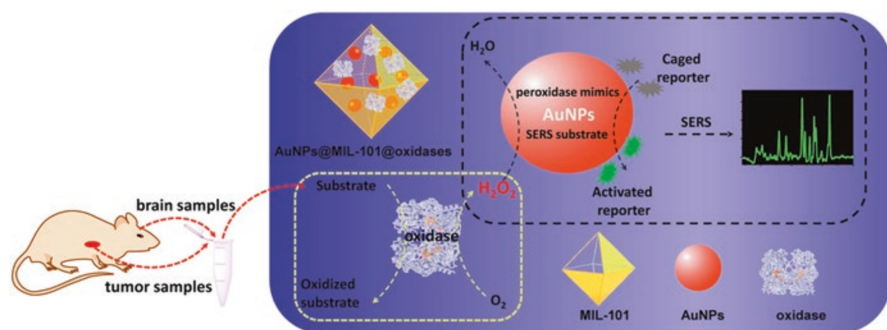


Fig. 6.3 Schematic showing AuNP@MIL-101@oxidase nanozyme. The mechanism of oxidation of substrate for H_2O_2 production and simultaneous peroxidase activity for obtaining activated SERS reporter (malachite green). The peroxidase activity of the integrated system enables enhancement in SERS signal. (Reproduced with permission from Hu et al. (2017) Copyright 2017 American Chemical Society)

A similar protocol was followed for detection of lactate from living tissues. The combination of plasmonic properties and SERS activity of gold nanoparticles show promise in successful real-time probing of analytes and can be used for designing novel immunoassays. In tumor tissues, the glucose metabolism is enhanced leading to low glucose levels. Hypoxia or low oxygen levels may enhance anaerobic glycolysis leading to lactate production, making these integrated nanosystems highly significant in clinical diagnosis.

6.4.1.5 Using Bare Nanoparticles for Detection of Food Contaminants

To fulfill the increasing demands for different food products, artificial taste enhancers are often added. The melamine debacle of China in 2008 is one such example (Pei et al. 2011). Melamine is a nitrogenous compound added to increase the protein amount in food artificially. This organic compound is reportedly toxic beyond a daily intake of 0.5 mg/kg of body mass and leads to reproductive damage and bladder or kidney stones (Ingelfinger 2008). Melamine detection is critical to evaluate the quality of food, and the intensive experimental techniques were replaced by nanozymes for ease of access. Nanoparticles have also been used for detection of harmful reactive oxygen species in food samples (Bajpai et al. 2018).

A simple colorimetric detection strategy consisting of bare AuNPs was developed by Ni et al. for sensitive detection of melamine in food (Ni et al. 2014). On exposure to melamine, the AuNPs form aggregates with melamine, resulting in better oxidation of the substrate and increased colorimetric detection. Melamine and H_2O_2 combine to form an addition compound which was used for detection of melamine according to Ding et al. The consumption of H_2O_2 was used as a measure of melamine levels in spiked raw milk and milk powder samples (Ding et al. 2010).

6.4.1.6 Detection of Nucleic Acids

A label-free, colorimetric sensor consisting of MNPs was developed for detection of known DNA samples. The DNA was first amplified using polymerase chain reaction and then conjugated with MNPs. The peroxidase-like activity shown by unconjugated MNPs was inhibited by the electrostatically adsorbed DNA. The electrostatic interaction between positively charged substrate OPD and negatively charged DNA and the adsorption of DNA on the surface of the enzyme inhibited the peroxidase activity of MNPs, reducing the signal output. This decrease in the colorimetric product led to the detection of *Chlamydia trachomatis*, a common bacterium found in sexually transmitted diseases, in human urine. Other nanoparticles like CeO_2 were also used for detection of nucleic acids (Park et al. 2011).

6.4.1.7 Detection of Thrombin

Zhang et al. developed an aptamer-based biosensor for detection of thrombin in blood samples. Aptamers are ssDNA or ssRNA and can be designed to specifically bind to a target molecule, replacing antibodies for detection. A sandwich-type assay developed using chitosan-conjugated MNPs and two thrombin aptamers could detect a 1 nM level of thrombin (Zhang et al. 2010a). Following the principle of

ELISA, streptavidin was subjected onto a 96 well plate, and the non-specific binding sites were blocked using BSA. A biotinylated 29mer aptamer I adsorbed onto the 96 well plate due to biotin-streptavidin interaction. On adding the sample and a 15mer aptamer 2 modified MNPs to the wells, a colorimetric change was observed, in presence of thrombin. This was due to the distinct aptamer binding sites present on opposite faces of thrombin. A 1 nM concentration of thrombin was successfully detected using this biosensor.

6.4.2 Detection of Tumor Cells

The hallmark of a typical cancer cell is the overexpression of certain proteins when compared to a healthy cell. Folate receptors, for example, are most commonly expressed on tumor cells. Antibodies against these receptors or folic acid functionalized onto nanohybrids help in easy detection of certain tumor types. Successful ultrasensitive detection of HER2-positive SKBR-3 cells was done by anti-HER2-fabricated AuNPs (Zhu et al. 2013). Amperometric detection of metastatic tumor cells was also achieved using gold nanoparticles (Pallela et al. 2016). Colorimetric detection, by peroxidase-mimicking AuNPs, has also been reported for easy and sensitive detection of tumors.

6.4.2.1 Hybrid AuNCs for Rapid Colorimetric Detection of Cancer Cells

AuNCs show peroxidase-like action under acidic pH. Graphene oxide can undergo easy surface functionalization due to the presence of a large number of surface functionalities. A GO-AuNC hybrid was prepared which was capable of showing peroxidase-like activity at physiological pH. Tao et al. proposed a simple, cost-effective, folate conjugated GO-AuNC nanohybrid which electrostatically interacted with positively charged TMB to produce a colorimetric product (Tao et al. 2013b) (Fig. 6.4). The folate receptor-overexpressing MCF-7 cells were targeted using this nanohybrid system, and the catalytic activity increased with increase in the number of cancer cells. Also, the developed composite was found to be highly selective for MCF-7 cells when compared with both healthy and cancerous cell lines. This strategy could be employed for further detection of other folate overexpressing cell lines.

6.4.2.2 Stem Cell Proliferation and Imaging

Biosafety concerns limit the use of nanoparticles in stem cell imaging. Huang et al. reported ferucarbotran, an ionic superparamagnetic iron oxide nanoparticle, to be safe for mesenchymal stem cell imaging and growth. Upon internalization into hMSCs, the intrinsic peroxidase-like activity of ferucarbotran resulted in the quenching of intracellular H_2O_2, thereby helping in the growth of stem cells. The SPIO nanoparticles underwent lysosomal degradation resulting in leaching of Fe from their surface. However, this could not block the stem cell progression, and an exact reason for the above observation was not known. However, the

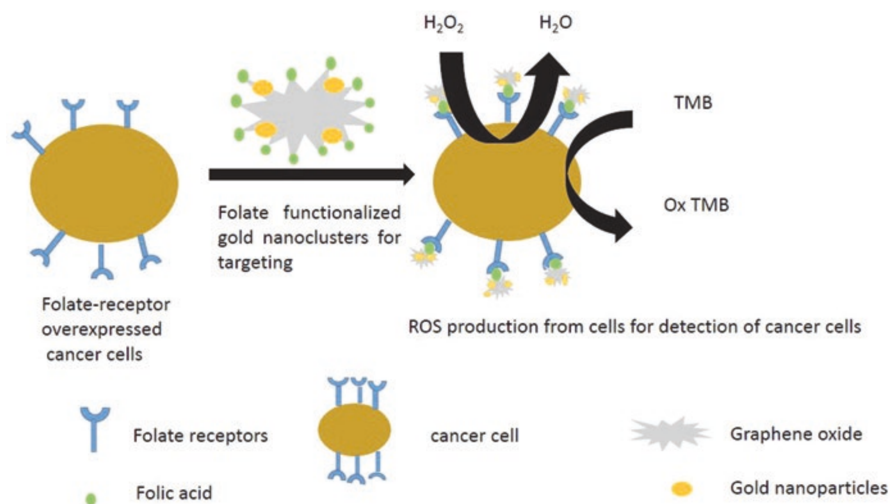


Fig. 6.4 Schematic showing folate-functionalized hybrid nanoclusters for detection of cancer cells. The ROS produced by the cells facilitates colorimetric detection of folate-overexpressed cancer cells

ferucarbotran-labelled hMSCs were very easily visualized using MRI. Thus, a simple, biocompatible cell-imaging platform was reported by Huang et al. and could be effectively used in regenerative medicine(Huang et al. 2009).

6.4.3 Immunoassays

In biological systems, immunoassays are used for tracking different hormones, proteins, and antibodies. Enzymes with colorimetric substrates like horseradish peroxidase and alkaline phosphatase are extensively used for conjugation with secondary antibodies and amplify the detection signal manyfold (Micheli et al. 2002). However, these enzymes have a short shelf life and undergo easy denaturation on long-term storage (Gao et al. 2008). Thus, nanozyme systems with peroxidase-mimicking properties have been used for developing immunoassays. A typical immunoassay platform developed using the enzymatic metal nanoparticles is shown in Fig. 6.5.

6.4.3.1 Immunoassay for Detection Cardiac Troponin I

Gao et al. developed a sandwich-type immunoassay, similar to ELISA, using dextran-functionalized MNPs for suitable detection of cardiac troponin I, a well-known biomarker of cardiac myopathy (Gao et al. 2007b). An antibody of TnI antigen was conjugated with MNPs and then mixed with serum to capture the cardiac troponin antigen. On binding with the antigen, the bound MNPs were separated using a magnetic field and then loaded along with the reaction buffer, into a microtiter plate, for measuring the absorbance at 652 nm. Both the catalytic and magnetic properties of MNPs were demonstrated using this immunoassay platform.

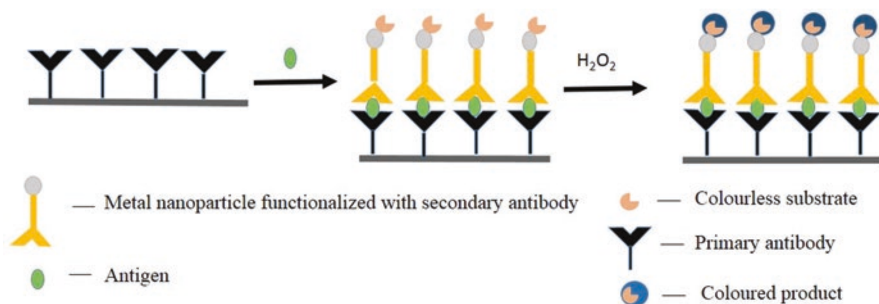


Fig. 6.5 Schematic showing a colorimetric immunoassay platform developed using gold nanoparticles

6.4.3.2 Immunoassay for Detection of Cancer

An ultrafast, specific immunoassay platform was designed for the colorimetric detection of HER2, a common biomarker for breast cancer. Kim et al. developed a nanocomposite of MNPs and PtNPs and immobilized it on mesoporous carbon for utilizing its synergistic catalytic potential in detection of HER2 and rotavirus like model pathogenic antigens (Kim et al. 2014). An antibody against HER2 was immobilized on the nanocomposite MMC-10/Pt-10, and cell lysates from human breast cancer cell lines SKBR-3 and MCF-7 along with human cell melanoma WM-266-4 were employed in different wells along with the nanocomposite. A typical ELISA type reaction demonstrated a dense blue color in the well containing SKBR-3 cell lysate, indicating the selectivity of the immunoassay developed. Among SKBR-3 and MCF-7, the level of HER2 expression is much higher in SKBR-3, justifying the observation reported by Kim et al. Rotavirus detection reported using the same nanocomposite was equally selective, and the magnetic properties of the MNPs were also demonstrated for the separation of the antigens. This nanocomposite with distinct catalytic and magnetic properties could be extended for point-of-care detection in clinical applications.

6.4.3.3 Chitosan-Modified MNPs for Detection of Mouse IgG and CEA

The use of MNPs for immunoassay designing is reliant on four major properties: easy dispersion in aqueous solution at physiological pH, proper surface functionalization for linking with antibodies, large enough saturation magnetization for separation using a moderate magnetic field, and easy separation of aggregated nanoparticles upon removal of applied magnetic field.

Chitosan-modified MNPs, used by Gao et al. for the development of a sandwich antigen-down type immunoassay, possessed all the above features and were used for detection of mouse IgG and carcinoembryonic antigen (CEA) detection (Gao et al. 2008) (Fig. 6.6). The wells of a 96 well microtiter plate were loaded with the antigen (mouse IgG) in bicarbonate buffer at 4 °C. The unbound antigen was washed thrice using PBS followed by addition of non-specific protein BSA to the wells. After 2 hours of incubation, the wells were again washed with PBS. Antimouse IgG

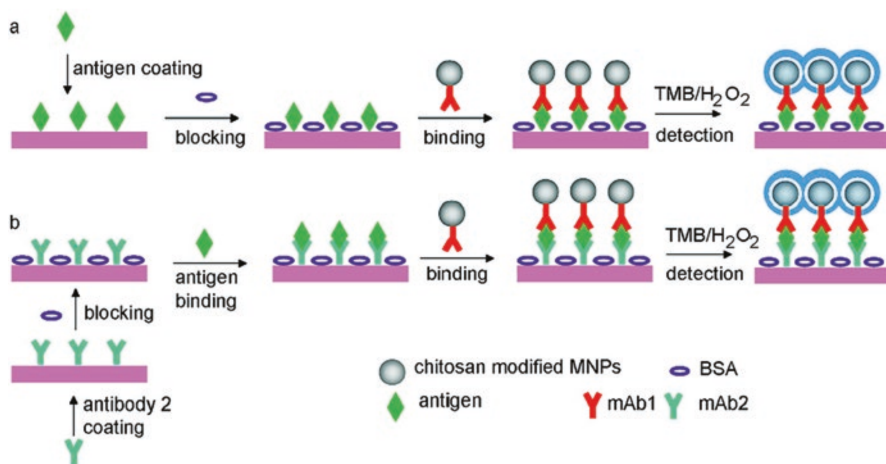


Fig. 6.6 Schematic showing the sandwich-type immunoassay developed by Gao et al. The binding of the antigen to the well was followed by addition of the antibody functionalized MNPs for detection of the chromogenic product. (Reproduced with permission from Gao et al. (2008) Copyright 2008 American Chemical Society)

antibody functionalized MNPs were then loaded into the wells and incubated for 1 h at room temperature. The unbound nanoparticles were washed out using PBS, and the reaction buffer was added into the wells. The MNPs, acting as peroxidases, oxidize the substrate producing a color change that can be detected at 652 nm for estimation of the amount of antigen.

For CEA detection, the wells were first coated with anti-CEA antibody. The unbound antibodies were washed with PBS and a blocking agent BSA was added. This was followed by further washing of the wells and addition of the antigen CEA into it. After incubation, the unbound CEA was washed using PBS, and the anti-CEA antibody-functionalized MNPs were added into the well. Upon washing the wells with PBS, the reaction buffer containing the peroxidase substrate TMB and H_2O_2 was added, and absorbance of the oxidized substrate at 652 nm was measured spectroscopically. Gao et al. also demonstrated the magnetic concentration and separation of the antigen from the wells. This immunoassay platform reported the sensing of 1 ng/mL CEA.

6.4.4 Detection of IgG Using Prussian Blue-Modified Iron Oxide Nanoparticles

The peroxidase-mimicking activities of Prussian blue-modified iron oxide nanoparticles were used in the development of an immunoassay platform for detection of IgG. Zhang et al. used a staphylococcal protein A(SPA) as the antibody for conjugating with the modified nanoparticles (Zhang et al. 2010b). This protein-nanoparticle composite was used for the detection of IgG immobilized

on 96 well plates. The catalytic and magnetic properties of the nanoparticles were intact and even increased on functionalizing with Prussian blue, confirming its usage for further designing of improved biosensing platforms.

6.5 Limitations

Nanozymes as artificial enzyme systems demonstrate high operational stability, low cost, and easy bioconjugation. Their catalytic potential is supported by the unsatiated atoms on their surface and further boosted by functionalization. Their applications in biosensing, immunoassay development, and tumor detection platforms is therefore a highly investigated field. In-depth studies of their catalytic behavior have revealed some limitations. Of the different catalytic behaviors manifested by metal nanoparticles, redox enzyme mimics seem to dominate. Docking and other forms of simulations can help discover other enzymatic properties of nanometals (Wang et al. 2016). Despite being functionalized by different organic groups, nanozymes cannot compete with the selectivity and catalytic properties of natural enzymes. New nanozymes with improved catalytic features need to be designed in the future. Better cascade mimics can be developed by focusing on the natural action of enzymes. Studying the stability of metal nanoparticles in response to functionalization may lead to detailed investigation of their theranostic properties. Their enzymatic properties have been scarcely explored for therapeutic purposes. A major reason for this is the cytotoxicity associated with the unshielded metal surface (Vlamidis and Voliani 2018). In-depth evaluation of their biocompatibility may promote their use in clinical medicine and therapeutics.

6.6 Summary and Outlook

- Metal nanoparticles are suitable alternatives to natural enzymes. A summary of the biomedical application of different metal nanoparticles arising from its peroxidase-like activity is represented in Table 6.1.
- Their small size, easy surface functionalization and reduced manufacturing cost enhance their applicability in scientific research.
- This chapter is a brief account of their peroxidase-like action in several biological applications.
- Biosensing of small molecules, clinically significant biomarkers and food adulterants can be successfully achieved through these enzyme mimics. Their sensitivity is enhanced by colorimetric oxidation of different peroxidase substrates.
- Nanozymes play a significant role in tumor cell detection and imaging, making their clinical applicability remarkable.
- Further efforts made into designing biocompatible nanocomposites with improved catalytic potential will lead to distinct therapeutic applications and approval for clinical use.
- Bionics and other fields which are exploring these mimics ambitiously can achieve a lot in the future.

Table 6.1 Biomedical application of different metal nanoparticles arising from its peroxidase-like activity

Application	Nanozyme	Detection method	Details	Reference
Biosensing	MNPs	Colorimetric	Glucose biosensor	Wang et al. (2005)
	AuNPs	Colorimetric	H_2O_2 and glucose biosensor	Jv et al. (2010)
	AuNP (impregnated on metal-organic framework with oxidase)	Colorimetric, SERS based	Glucose, lactate, and ascorbate detection	Hu et al. (2017)
	MNPs (with oxidase in mesoporous silica)	Colorimetric	Glucose and cholesterol biosensor	Kim et al. (2011)
	MNPs (with oxidase in mesoporous silica)	Colorimetric	Galactose biosensor	Kim et al. (2012)
	MNPs	Colorimetric	Nucleic acid detection	Park et al. (2011)
	Chitosan-modified MNPs with thrombin aptamers	Colorimetric	Thrombin detection	Zhang et al. (2010a)
	MNPs	Colorimetric	Melamine detection	Ding et al. (2010)
	AuNPs	Colorimetric	Melamine detection	Ni et al. (2014)
Immunoassay	MNPs	Colorimetric	Cardiac Troponin I (TnI)	Gao et al. (2007b)
	MNPs-PtNPs in mesoporous carbon	Colorimetric	Detection of cancer biomarkers	Kim et al. (2014)
	Chitosan-modified MNPs	Colorimetric	Mouse IgG and carcinoembryonic antigen (CEA)	Gao et al. (2008)
	Prussian blue-modified γ-Fe_2O_3 NPs	Colorimetric	IgG detection	Zhang et al. (2010b)
Cancer cell detection	Superparamagnetic iron oxide NPs	Colorimetric	Promotion of stem cell growth	Huang et al. (2009)
	GO-AuNCs	Colorimetric	MCF-7 cell detection in mice	Tao et al. (2013b)

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