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Perceptive Review on Properties of Iron Oxide Nanoparticles and Their Antimicrobial and Anticancer Activity

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ABSTRACT

During past years, researchers have focused on developing a technique that can be applied in biomedical field. Ongoing research efforts focused on iron oxide nanoparticles (IONPs) as one of those developed techniques. They have been widely used because of their unique properties. They are biocompatible, biodegradable with unique non-toxic magnetic properties. They can be synthesized with reliable surface modification. Thanks to these peculiar characteristics, IONPs appear as the starting point for the development of new therapeutic strategies in microbiology and oncology. Hence, the latest published works on newly developed surface modified IONPs are described, tackling the main benefits and drawbacks of the method of development, with particular emphasis on the possible applications in clinical practice as anticancer or antimicrobial agents. Looking forward, more progression in synthesis technologies of IONPs must continue to be optimized and developed to give rise to a new golden age in oncology and a breakthrough in the fight against cancer and infection.

Keywords: Iron oxide nanoparticles, anticancer, antimicrobial, surface modification

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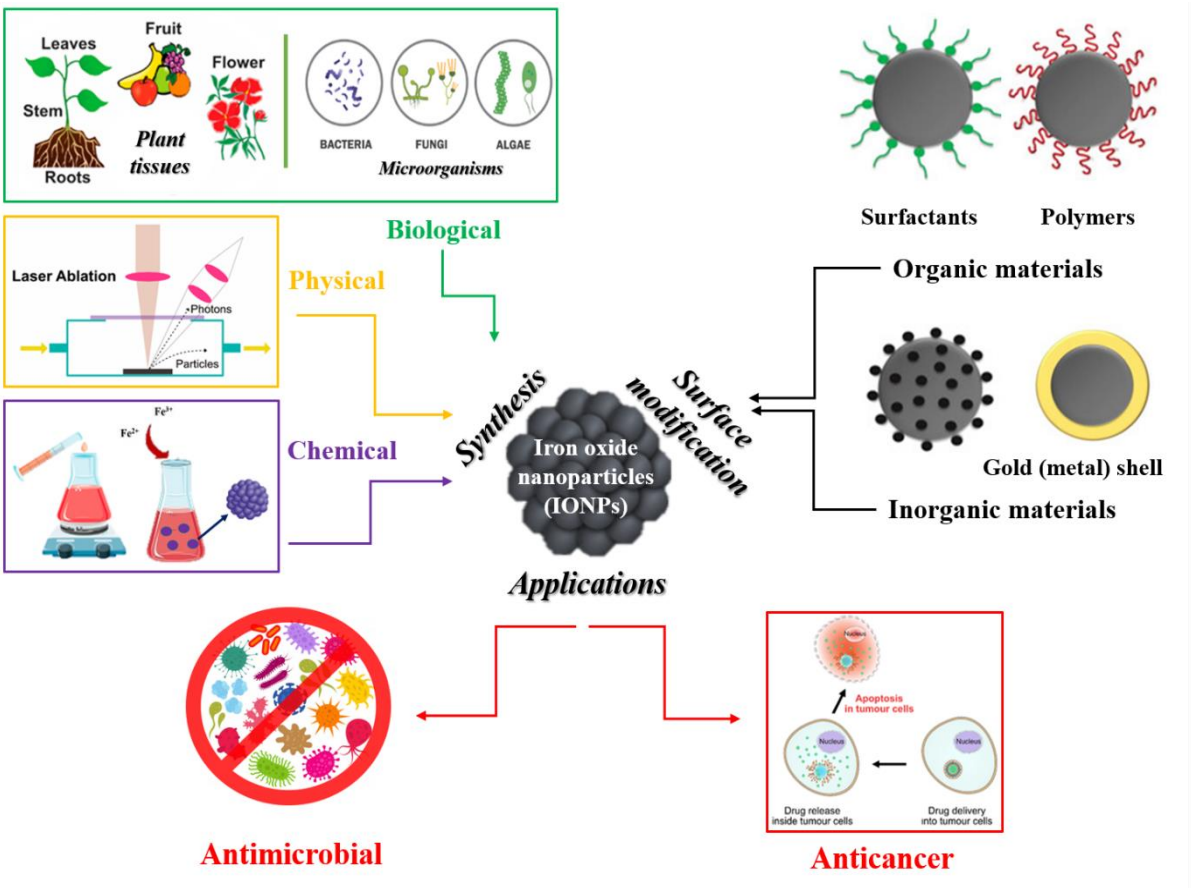
GRAPHICAL ABSTRACT



INTRODUCTION

In the past decade, nanoparticles (NPs) have gained an extra credit in drug delivery system and have been rapidly developed and applied in various sections of biomedicine. [1] The main purpose of developing a new technology for drug delivery was to overcome limitations of conventional drug delivery such as solubility problems, uncontrolled drug release and lack of drug targeting. [2] The NPs with a dimension range of 10-100 nm provide more efficient and convenient route of administration with low toxicity and longer circulating time. Also, it reduces the health care cost with an advantage to deliver two or more drugs as a combined therapy to give a synergistic effect. [3]

Iron oxide nanoparticles (IONPs) have been widely utilized as a drug carrier owing to their magnetic properties, in addition to their biocompatibility, biodegradability and non-toxic characteristics.[4] Surface modification of IONPs added extra rewards for many biomedical applications such as cancer drug targeting and magnetic resonance imaging (MRI). [5] The selectivity of iron oxide in delivering the drug to the designated illness spot with low side effects, and the ability of accumulation in a specific tissue when subjected to an outside magnetic field gave them an extra interest in cancer treatment and diagnosis. [6]

Moreover, bacterial resistance to different types of antibiotics directed researchers to look forward to a way that overcomes this resistance. The small size of nanocarrier as IONOs and their ability to change the metabolic activity of bacteria, revealed a broad-spectrum antibacterial activity against Gram-positive and Gram-negative bacteria. [7]

This review will provide theoretical evidence on approaches of IONPs synthesis, addressing the key benefits and drawbacks. In addition, the physicochemical properties and shape of IONPs have been summarized. Besides, selected examples from recent literature have been included to demonstrate the significant improvement in the IONPs antimicrobial and anticancer activity upon surface modification. Moreover, a set of concerns is delivered on IONPs toxicological traits, as well as innovations on coating approaches to describe more biocompatible nano-systems. Ultimately, forthcoming recommendations for expanding the use of IONPs in biomedical field were concluded.

PROPERTIES OF IRON OXIDE

Iron (III) Oxide or ferric oxide is an odourless mineral substance which occurs abundantly in nature with a dark red colour. It shows different crystalline forms (polymorphs), with similar chemical composition, hence, different structural, physical, chemical and magnetic characteristics. [8] It occurs in nature in three phases as maghemite (g-Fe_2O_3), hematite (α-Fe_2O_3), and magnetite (Fe_3O_4) [9] These stages simply go through several level of alterations in response to pressure or heating exposure. Typically, under ambient conditions, the most thermodynamically stable polymorph is hexagonal hematite (α-Fe_2O_3). [8] Iron oxide is insoluble in both water and organic solvent, whereas it has high solubility in concentrated mineral acid. Therefore, its formulation is suffering from many limitations due to its low solubility in biological media. Many factors would influence the dissolution rate of iron oxide and its solubility, such as the temperature of the media and the characteristics of the solution phase (pH) and the general characteristics of the oxide (crystal structure, presence of impurities). [10] Its solubility increases with decreasing its particle size and increasing its bulk lattice energy. [11] Melting point and the optical properties vary from crystal form to another. Iron oxide starts to soften at 1,492 °C. The magnetite melts to a liquid form at 1,580 °C and exhibits an absorption in near-IR and visible region. While hematite and maghemite do not have any absorption signal near-IR region. [12]

PHYSICOCHEMICAL PROPERTIES OF IONPS

The reactivity of iron is very crucial in macroscopic applications (particularly rusting), however, at the nanoscale it is more prominent. It is pyrophoric at finely divided state. Its intense reactivity renders it challenging to discover and unwelcomed for applications. However, IONPs can be promptly stimulated by self-heating, external magnetic field application, and shifting alongside the attraction field into magnetic resonance. [13]

IONPs are usually the most desirable and used NPs since iron is abundant in our body and can be tolerated at doses higher than other metals. [14] IONPs have been widely researched and used, as they have enticed considerable attention due to their exceptional characteristics, such as superparamagnetic features, large surface area, reliable surface modification, easy-going synthesis, straightforward separation procedure, and low toxicity. [15, 16]. The charge of these NPs, the solution stability, zeta potential, crystallization, synthetic methods and the coating of the NPs are fundamental parameters that affect their application in the biomedical field, [17] including MRI as contrast agents, hyperthermia, transfections, drug delivery and anti-tumor applications, cell tracking, and tissue repair. [18]

The surface functionality and water-solubility of IONPs influence their interaction with the biological system. Hydrophobic coatings of IONP with poly (acrylic acid), polyethylenimine, or glutathione, yielded stable water-soluble charged NPs. The coating and the ratio of surfactant to NP can significantly alter the reflexivity of IONPs as MRI contrast agents. [19] The charge of IONPs can affect its distribution in the body. The modified NP surface with opposite (positive) charged polymers can encourage the delivery of antigen into cytoplasm. It promotes cell-mediated immunity by helping the cross-presentation of antigen into dendritic cells and T-cell activation. [20] In contrast, anionic IONPs are internalized by adsorptive endocytosis due to efficient interaction with the cells.

It is also noteworthy to highlight that the characteristics of IONPs rely on their shape and size. [21] However, the pH, the nature of the salts used, the ionic strength, the temperature, and the ratio of ferric to ferrous concentration affect the shape and size of the NPs. [22] In addition, ferric injection rate and cooling method affect the particle size. [23] IONPs of 10–20 nm ferromagnetic materials present unique traits of magnetism and are thought to be the best for intravenous administration, while greater sizes (>20 nm) have a restricted absorption rate. [13] Moreover, the average circulation time is affected by the size of the IONPs, particles sequestered by the spleen if they are greater than 200 nm, whereas particles smaller than 10 nm are cleared through the kidney, and move from the lungs to the lymph nodes when they are less than 34 nm.[17]

APPROACHES OF IONPs SYNTHESIS

A range of iron oxide nanostructures have been successfully synthesized. They can be synthesized by biological or chemical or physical techniques. IONPs with

appropriate surface chemistry (size, shape, solubility, and stability) are obtained. [24] These techniques can be classified into two major groups based on the solvent used. They could be aqueous or non-aqueous techniques, where better shape and size control was obtained by the non-aqueous-based methods, whereas they are somewhat extra costly contrasted to the aqueous-based techniques. [25] The most popular and efficient synthesis methods used in biomedical fields are presented in this review, highlighting the benefits and drawbacks of each method.[16]

Physical techniques

1. Pyrolysis method

It is a technique where a laser radiation hits gaseous organometallic precursors to generate IONPs. The energy is transmitted in a selective way to produces homogeneous and pure sample with great shape distribution and size control. It operates at atmospheric pressure; therefore, it is less expensive than other methods. The magnetization and the size of the resulting NPs are directly affected by the working pressure, precursors concentration, and laser intensity.

2. Laser ablation synthesis in solution

It is a technique where a pulsed laser beam is targeting specific substance engrossed in liquid solution. The beam initiates alterations in the structure of the ablation target as well as the liquid solution. This method depends on the solvent used, therefore, controlling the particle size and their clustering is very difficult. However, the size of iron oxide crystal can be decreased to few atom clusters by using phosphonates aqueous solution and bulk iron.

Chemical techniques

They are the most simple, tractable, and efficient methods among the three different techniques. The composition, size and the shape of the NPs can be certainly controlled. Chemical-based synthesis is the most adopted method owing to the high yields and low production cost. [13]

1. Sol-gel method

It interchanges around hydroxylation and condensation of molecular precursors in the solution. [13] In such a system, the precursor for an integrated gel network of polymer is formed from a stable sol dispersion of colloidal particles in a solvent. The relatively high reaction temperature used prefers IONPs with saturation magnetization and greater crystalline. IONPs can be simply dispersed in aqueous and polar solvents because of the hydrophilic ligands attached to the surface of IONPs. Nevertheless, sol-gel method suffers from lack of safety during the process where large quantity of alcohol discharged during the calcination phase, and comparatively large cost of the metal alkoxides. [21]

2. Chemical co-precipitation method

It is one of the highly effective and traditional technique that is used to synthesize IONPs. [26, 27] This method involves mixing ferric and ferrous ions in a ratio of 1:2 molar's in very basic solution at 20 - 22 °C or at higher temperature.[21] The mechanism of the chemical reaction for the co-precipitation technique can be shortened in the equation:

$$2Fe^{3+} + Fe^{2+} + 8OH^- \rightleftharpoons 2Fe(OH)_3 + Fe(OH)_2 \rightarrow 4H_2O + Fe_3O_4\downarrow$$

This method has extraordinary advantages, such as being appropriate for mass production and inexpensive. [28] However, it is unsuitable for the preparation of untainted, specific stoichiometric phase. It suffers from wide particle size distribution of products due to aggregation of IONPs and lacks biocompatibility due to the use of strong base in the reaction procedure. [21]

3. Microemulsion method

It is developed when a colloidal material is dispersed in a solvent, that is not harmonizing with the material through a surfactant that forms a monolayer film at the oil/water interface. Sodium dodecyl sulfate, bis(2-ethylhexyl) sulfosuccinate, poly-vinylpyrrolidone (PVP), and cetyltrimethylammonium bromide (CTAB) are the most popular surfactants that are broadly applied. It is evidenced to be a easy and adaptable method for production of nanosized magnetic NPs. [31]

4. High-temperature thermal decomposition method

It is regularly applied to make iron oxide with various shapes, such as nano-spheres and nano-cubes. Many researchers preferred this method. [29, 30] This technique showed many advantages over other techniques, it is traditional and facile technique for getting hollow and shape controlled IONPs. It is especially appropriate for the development of good-quality iron oxide nanocrystals, specifically for the substances with elevated vapor pressure close to their melting points, they produce crystalline phases which are not stable at the melting point. The thermal decomposition approaches can be segmented into, traditional reaction approach where a reaction mixture is prepared at room temperature and then heated in an open or closed reaction vessel, and hot-injection approach where the precursors are inserted into a hot reaction mixture [21] It depends on the decomposition of organometallic composites at elevated temperature, where oxidation with surfactants and organic solvents is performed.

5. Hydrothermal and solvothermal synthesis

Iron salts are combined with sodium citrate, acetates, and urea, in organic or aqueous solvents under elevated vapor pressures (0.3 - 4 MPa) and elevated temperatures (130 - 250 °C). The dispersion is placed into the autoclave and heated for 8 - 24 h at 200 °C. Excellent quality for drug delivery can be obtained (achieving sizes of 10 - 200 nm) by changing the combination parameters such as pressure, temperature, and reaction time. However, the yields obtained are lower than the coprecipitation or thermal techniques. [31]

Biosynthesis techniques

It is an alternative approach for the chemical technique to obtain uniform NPs. They are manufactured employing biological resources like fungi, bacteria, plants or algae. [32] The development of nano-sized substances by microbial cells has arisen as a novel approach for the manufacture of metal NPs.[25] This technique is eco- friendly, reliable, and economic at neutral pH, and ambient temperatures and pressures that can be used in environmental remedy. [23] It is a green chemical route where the obtained product usually gives a good biocompatible IONPs. [21] The main reaction that occurs in this technique for the manufacture of IONPs is the oxidation/reduction reaction. The biosynthesis technique is based on the formation of elemental metal from the target ions that is grabbed by the

microorganisms from the environment using the enzymes generated by the cell activities. In this method, the traditional biosynthesis for magnetic IONPs is by using magneto tactic bacteria and iron reducing bacteria such as *Magnetospirillum gryphiswaldense or Geobacter metallireducens.* [21] In addition to bacterium *Actinobacter* species that produces maghemite NPs under aerobic conditions when reacted with a ferric chloride precursor. [21] Unfortunately, this method is very slow, laborious, [13] and still requires many studies to help in controlling the shape and size of magnetic IONPs. [21] However, the main advantages of using this bacterium in the manufacture of NPs are their simplicity of genetic manipulation, rapid growth rates [25], low cost and high yield. [13]

Plants can be utilized to produce crystalline IONPs with an average size of ~39 nm using a reducing agent of aqueous extract of *Psoralea corylifolia* seeds [33] or *Punica granatum* fruit peel extract. [34]

IRON OXIDE NANOPARTICLE MORPHOLOGY

The manufacturing conditions play a vital function in determining the phase composition, the properties, shape, and size of IONPs. Physical synthesis including deposition of gas phase and electron beam lithography yield spheres [35] and rods NPs. [36]

A diversity of iron oxide nanostructures like spindles, spheres, irregular elongated sheets, rhombic, hexagonal, facets , octahedral , hollow , cages, bipyramids, truncated, rods, elongated irregular nanotubes, flakes , cubes, and wires were manufactured by different chemical techniques such as electrochemical deposition, co-precipitation, thermal decomposition, sol-gel process, and hydrothermal and chemical vapour deposition. [13] Whereas biological techniques by microbial incubation results in small platelets, rod-like, and irregular spheres morphologies. [37]

Morphology of the NPs varies according to the preparation methods. The two main morphologies of hematite which are obtained from ferric chloride aqueous solution in acidic media by hydrothermal treatment and soda precipitation are rhombohedral shape. The presence of ligands like fluorine anion in ferric chloride solution resulted in hexagonal bipyramidal shape, while the spheroidal shape of magnetite resulted from hydrolysing ferric and ferrous in sodium hydroxide solution. [38]

The morphology of the particles can be determined by many techniques [39] ***I)*** The microscopic techniques including the transmission electron microscopy (TEM) and the scanning electron microscopy (SEM). TEM is used to characterize the crystal structures as cubes, plates, discs, or ellipsoids. Whereas SEM is used to investigate the surface structure (polished or rough). It offers 3-D image with high resolution and large depth of field to provide the composition, topography of the surface, and crystallography. ***II)*** The spectroscopic technique which delivers further information to pinpoint the chemical compositions. For example, X-ray diffraction can be used to characterize the structure of the crystals, their intensity, angle position, and width. ***III)*** The physical adsorption analysis to describe the textural properties, such as the pore size, and specific surface, which can be analysed using Brunauer-Emmett-Teller (BET) equation. While the desorption part of isotherm is used to describe the shape and distributions.

SURFACE MODIFICATION OF IONPS

Ferrimagnetic IONPs have drawn interest because of their special features and wide-ranging applications like medical diagnosis, [5] biosensors, [40] and catalysis. [41] The successful use of IONPs in biomedical applications depends mainly on their stability under biological environments. IONPs must bring together definite features in biocompatibility, high degree of magnetization, and active surface functionality for their best use in the field. [42]

IONPs hold high surface energies due to their great surface-to volume ratio. Therefore, particles with bare surface have high tendency to form aggregation to reduce the energy of the surface. The causes of agglomeration can be related to large surface area and Van der Waals interactions among particles. Having no surface coating substance causes hydrophobic interactions between particles which will lead to the aggregation and formation of agglomerates, causing an increase in the size of particles and strong dipole-dipole attraction between them. Consequently, rapid clearance of the agglomerated particles by the reticuloendothelial system [13, 43]. Moreover, IONPs with bare surface possess high chemical activity that render them airborne oxidized, causing loss of magnetism and dispersibility. [21, 43]

Thus, suitable modification of IONPs surface is essential for making them stable and biocompatible and that is crucial for their function as vehicles for drug delivery. The main purposes for surface modification are reducing the likelihood of accumulation of the naked particles hence increasing the blood circulation time by avoiding elimination, protecting their surface from oxidation, thereby enhancing their dispersibility and stabilization of the colloidal system. Also, improving the surface activity, the biocompatibility, and the physicochemical and mechanical properties of IONPs. Furthermore, providing a surface for targeting ligands and drug molecules to be conjugated, and minimizing nonspecific interactions thus reducing toxicity. [15, 44]

Several methods can be done to achieve the surface engineering. This can be accomplished by forming a core-shell structure in which the core of iron oxide is layered with a coating substance forming an outer shell, or beads can be formed by dispersing the particles through a polymeric matrix. [42] Furthermore, with one-half of IONPs and the other half with efficient substances Janus particles can be created. Moreover, a shell-core-shell structure can be created by integration of IONPs between two efficient substances. [21] The coating technique is the commonest surface modification line for conjugating both the organic and inorganic substances on the surface of IONPs. In addition to preventing the aggregation and oxidation of IONPs by this technique, it also has a role of offering an opportunity for additional functionalization that can enhance their physicochemical features and make them suitable options for the biomedical sector. [15]

The substances used when modifying the surface are classified as organic and inorganic substances. The silica, the carbon, the metals and their oxides or sulphides are considered as inorganic materials while small molecules or surfactants, polymers, and biomolecules are considered organic materials. [15] The typical substances to stabilize IONPs must be biodegradable, biocompatible, and can be coated either during or after synthesis. [45]

Polymeric coating materials are the most used in surface coating. They can be categorized as synthetic and natural (**Table 1**). polyvinyl alcohol (PVA), poly (lactic-co-glycolic

acid) (PLGA), Poly (ethylene-co-vinyl acetate) (PVP), polyethylene glycol (PEG), and poly(acrylic acid) (PAA) are synthetic polymeric systems. Gelatine, dextran, chitosan, alginate, starch, pullulan are natural polymer systems. To enhance dispersibility of the polymers in an aqueous medium, several surfactants are commonly utilized, like carboxymethyl cellulose sodium, sodium oleate, and dodecyl amine. [42] Dextran is a polysaccharide polymer of variable chain length and branching degrees. Due to its exceptional biocompatibility as well as good water solubility, it was utilized as a coating polymer. Surface modification of NPs by dextran impacts their physicochemical features. [15] It reduces the saturation magnetization of the IONPs, decreases the toxicity of bare IONPs to the cells, enhances their biocompatibility and consequently improves their potential application. [46] Chitosan is a natural hydrophilic, alkaline, harmless, biodegradable, and biocompatible polymer. IONPs coated with chitosan are commonly further functionalized with other polymers including PAA and PEG. [15] Chitosan is a linear polysaccharide composed of three kinds of functionalities (amino, primary, and secondary hydroxyl groups) that function as a framework for the combination of therapeutics, imaging and ligand targeting. Chitosan has antimicrobial activity against several microbes, and chitosan coated IONPs show better antimicrobial activity and can therefore be improved as a coating resistant to microbials for biomedical instruments. [48]

Table 1. List of polymeric coating materials that were used to stabilize the IONPs along with their advantages.

Material used	Advantages	Ref.
Dextran	Biocompatible, biodegradable and water-soluble material, improves the stability and biocompatibility of the colloidal system and enhances the time of blood circulation	[46, 47]
Chitosan	A natural alkaline cationic linear polymer, biocompatible and biodegradable, nontoxic and shows excellent antibacterial and antifungal properties	[48]
Silane coupling agent	Modify IONPs surface directly, well biocompatible, great density of surface functional end groups, enabling attachment to other metals, polymers or biological molecules	[49
Polyethylene glycol	Enhance the hydrophilicity and improve the biocompatibility, increase the time of blood circulation, easily functionalized, and internalization efficiency of the NPs.	[50]
Poly(vinylpyrolidine)	Stabilizes the colloidal solution, minimizes the agglomeration and control the particle size, enhances the blood circulation time	[51, 52]
Poly(vinylalcohol)	Prevents the agglomeration giving rise to monodispersibility, biocompatible polymer with low toxicity	[50]
PAA	Enhances the biocompatibility and stability of the particles in addition to bioadhesion	[13]
Poly (D, L- lactide)	Improves the biocompatibility, biodegradability, and low cytotoxicity	[13]
Poly(N-isopropylacrylamide)	Thermoresponsive copolymer and used in drug delivery and cell separation.	[53]
Polypeptides	Increases the colloidal stability and biocompatibility, functionalize the NPs with targeting moieties, specific cell targets.	[54]
Silica	Great surface area, no requirement for organic solvent usage, porosity, chemical inertness, non-toxicity and biocompatibility	[55]
Gold, Silver	Noble metals, stable chemical features, biocompatibility, oxidation and corrosion resistance.	[49, 56]
Gelatin	Hydrophilic biopolymer, emulsifier, biocompatible, suitable coating since its composition includes many functional groups like amino and carboxylic groups for binding a drug, can deliver anticancer and therapeutic agents.	[57]
Starch	A drug carrier with respect to its biocompatibility, biodegradability and safety, drug target delivery, good for MRI.	[58]
Alginate	Anionic polysaccharide water-soluble biopolymer, improves the colloidal stability and biocompatibility, capacity to serve as a muco-adhesive, cell tracking by MRI and drug delivery, alginate gels are broadly utilized in the encapsulation and to control the release of drugs	[50, 58]
Ethyl cellulose	Preservation of drug concentration at wanted location for extended duration, controlling the colloidal properties, reduce the oxidation behaviour of particles, used for targeted drug delivery applications	[59]
Albumin	Biodegradable, reproducible, increases particles biocompatibility, lack of immunogenicity and toxicity, good magnetic targeting agent and separation without affecting cell viability and proliferation	[60]
Liposomes	Simple and easy surface modification, biocompatible and biodegradable, low toxicity, improves solubility, increases load, no minimization of superparamagnetic activity on coating of naked IONPs, increases permeability, enables selective targeting and localization	[44]

Oleic acid (OA) is a highly utilized surfactant for IONPs stabilization with tight bond between the OA carboxyl group and the IONP surface. It is nontoxic and highly soluble in organic solvents. OA used for coating the surface of IONPs for managing their particle sizes, preventing accumulation of NPs, achieving biocompatibility, increasing dispersibility as well as stability. Bilayer OA coated IONPs could be dissolved in inorganic solvents at the optimal pH, that can be suitable for their efficient applications particularly in biomedical sector. [49]

PEG is a synthetic hydrophilic, non-toxic, and biocompatible polymer that is frequently used in the functionalization of NPs. PEG coating prevents opsonization by proteins, reduces the uptake by the reticuloendothelial system, which enhances the blood circulating time. [50] Because of the improved permeability, it decreases the non-specific body consumption that aids in tumour growth. The PEG serves as a spacer for the addition and extension of various biological molecules. The IONPs will be accumulated and driven to a specific area of concern if a targeting ligand, like an antibody or protein, are linked to them. [60] The high mobility of the surface provides great steric exclusion that aids in the stabilization of the surface in aqueous systems. It is also efficient in avoiding the adsorption and adherence of proteins. As a result, the covalent immobilization of PEG on the surface of magnetic NPs increases the biocompatibility and the stability of NPs. Additionally, it is possible to combine PEG chains having some functional groups with other biopolymers in order to produce biodegradable copolymers like poly (ethylene glycol)-co-poly(d,l-lactide) PELA. [62]

PVA is a synthetic, hydrophilic, and biocompatible polymer of little toxicity. Its coating inhibits the aggregation of the NPs, giving rise to monodisperse particles. It can transform into a polymer gel with unique properties. [63] PVA has the benefits of preventing protein adsorption and cell adhesion along with the great biocompatibility and thus it can act as an outstanding biocompatible and water-soluble coating material for IONPs. [61] PVA's multiple hydroxyl group results in an improved crystallinity, resulting in great tensile strength and modulus of elasticity for biomedical uses. [50]

Silica is the widespread and commonly utilized inert inorganic material for modifying the surface of IONPs. There are many advantages of silica coating including chemical stability, agglomeration prevention, and cytotoxic effects reduction. Thus, it has revealed good biocompatibility, hydrophilicity, and stability. In addition, the silica layer has many reactive silanol groups (Si-OH) that can provide a binding site used for further surface functionalization. [15] Because of these silica properties, iron oxide core-silica shell hybrid NPs have benefits in several utilizations. IONPs were modified with silica to create kind of multifunctional NPs which both IONP and fluorescent dyes are incorporated to produce highly fluorescent multiple IONP core-silica shell NPs. These unique NPs effectively showed fluorescence and magnetism and could be widely used in nanomedicine. [55] Carbon-based compounds are also utilized as an inorganic substance for coating the surface of IONPs to increase the dispersity, biocompatibility, and stability. These NPs can be used in many fields including electrode ultracapacitors, catalysts, and in lithium-ion batteries as anode materials. [15] Metallic core shell of IONPs have an internal iron oxide core and an external metal surface coated. Gold is the most common noble metal used for surface coating, which is very suitable for adding functionality to magnetic NPs and improving their stability in aqueous dispersions. Another noble metal that can be utilized for covering the surface is silver. [15] Modification with gold, not just enhances the stability of IONPs and the surface binding capacity to ligand, but similarly prevents the development of damaging free radicals. Due to the special catalytic, optical, mechanical, and structural features of gold NPs, they are used in many fields. Owing to the high biocompatibility, there has been substantial development in their biomedical application in therapeutics, biosensors, diagnostics and medical chemistry like genetic science, photo thermolysis of tumor cells, immunoassays, optical imagery and targeted drug delivery, and microorganisms identification and control. Furthermore, wide variety of functional materials can be combined with gold NPs, such as dendrimers, peptides, proteins, polymers, surfactants, ligands, drugs, nucleic acids, and oligonucleotides. [56]

BIOMEDICAL APPLICATIONS OF IONPS

During recent years, IONPs represent the most outstanding class of magnetic NPs especially in the biomedical field (**Figure 1**). What makes IONPs suitable for drug loading, or protein absorption and in vivo applications is their large surface-to-volume ratio. IONPs are used to increase the vaccine efficiency, [64] for targeted drug/gene delivery, [65] and in bacterial separation for the diagnostic needs. [66] In addition to magnetic resourance imaging, cell tracking can be a major step in cell-based therapy. The application of IONPs in cell labelling and quantification by MRI improves the regenrative studies and therapy. [67] They impact stem cell proliferation and differentiation in a dose dependent manner by enhancing the osteogenic differentiation. [69]

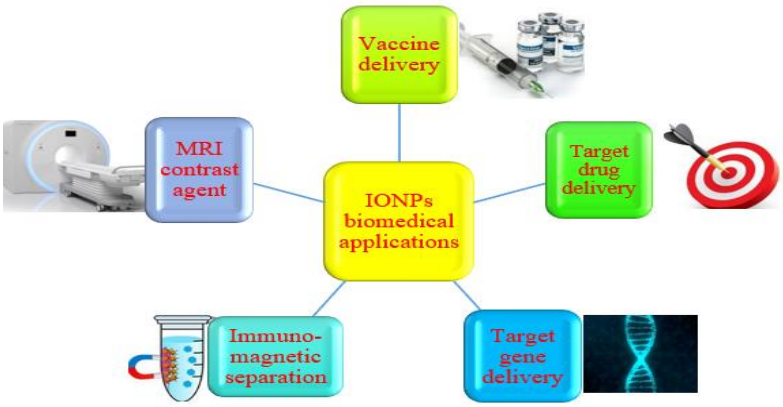


Figure 1. Biomedical applications of IONPs

The utilization of IONPs as anticancer and anti-microbial has received significant consideration in therapeutic nanomedicine as the treatment of cancer. They can increase the drug activity in combination therapy (IONPs and chemotherapeutic drugs) or as hyperthermia agents. [68] In addition, the affinity of bone marrow, spleen, liver to IONPs after their engulfment by mononuclear phagocytic system signifies the cause for the use of these NPs as contrast agents and delivery tools for chemotherapeutic agents.

Anticancer activity

Until now cancer is the dominant cause of death worldwide. Due to the serious side effects of different chemotherapeutic agents and occurrence of drug resistance beside the high socio-economical cost, the continuous development of anticancer agents and improvement of diagnostic process is still required.

Different metal oxide NPs have shown cytotoxicity actions in cancer cells, but not in normal cells. [68] Cancer which is one of the most complex pathologies in the world contains multiple mechanisms and signalling pathways. Throughout the previous two years, doxorubicin was the major anticancer drug linked to IONPs. [4] In a recent study, the in vivo administration indicated their effectiveness as anticancer agents. The magnetic IONPs appears to mediate DNA lesions in normal cells, as well as in case of tumour cells.

One of the mechanisms of magnetic IONPs is to stimulate magnetic hyperthermia in the shape of heat generation. Employing a high frequency alternating magnetic field causes the release of energy. This performance's principle of action consists of boosting the cell temperature atypically to 41 - 45°C, which causes considerable damaging effects (**Figure 2**) that can be irreversible for cancer cells and reversible within the normal cells. The induced hyperthermia increased the metabolic rate, which increased the production of reactive oxygen species. This can cause protein injury that can lead to tumour cell death. [70]

Another mechanism by which the magnetic IONPs are able to injury the cancer cells was a novel proposed mechanism called enucleation, which is the departure of tumour content with intraperitoneal rupture into the perihepatic space, and this can be seen on arterial phase imaging as low attenuating lesion from peripheral enhancing rim. [71]

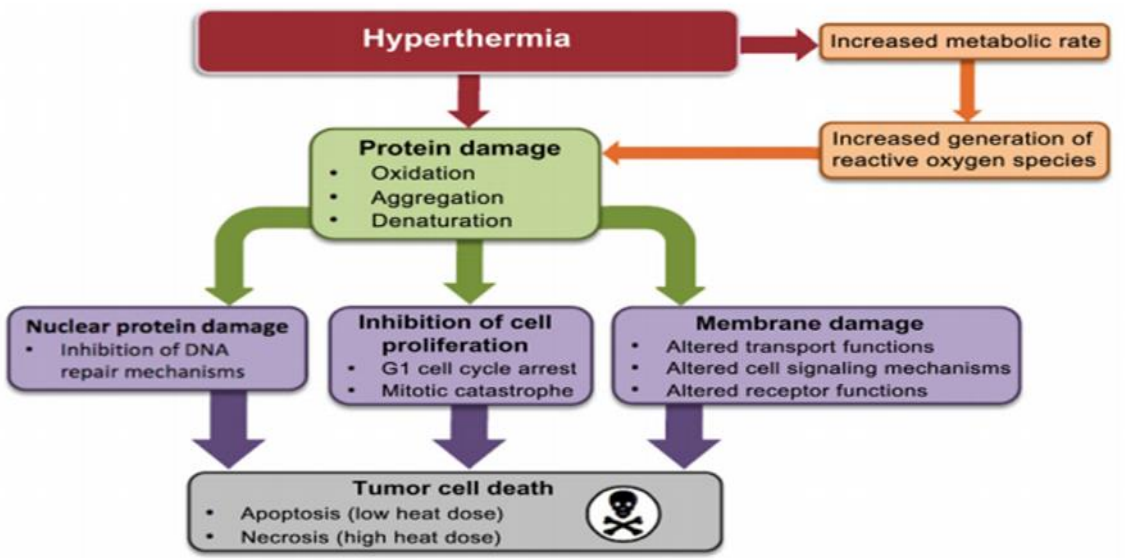


Figure 2. Effect of magnetic hyperthermia in cancer cells

Recently MRI is one of the highest effective tools for non-invasive clinical diagnosis in oncology. The polymer coatings of super magnetic IONPs facilitate MRI-guided drug delivery. [72] The polymer coatings of super magnetic IONPS increase their performance and application in magnetic hyperthermal therapy. [73]

Feraheme® (ferumoxytol injection), Feridex I.V.® (ferumoxides) and Gastromark™ (ferumoxsil) are different super magnetic IONPs formulations approved by the United States Food and Drug Administration (FDA) as MRI contrast agent. [74] Many studies showed promising therapeutic value in oncology using modified surface IONPs (**Table 2**).

Table 2. Summary of recent updates of IONPs with/without (coating materials/anticancer drug) and their promising therapeutic value in oncology.

Cancer type	IONPs with/without coating material	Clinical application	Ref.
Breast cancer	Hyaluronic acid coated IONPs	Induce magnetic hyperthermia and target CD44 receptor	[75]
	Superparamagnetic IONPs	Contrast agent for microwave images, which maps the cancerous tumours	[76]
	Superparamagnetic IONPs labelled with ^{225}Ac conjugated with Trastuzumab	Perspective tool for combined α-radioimmunotherapy and magnetic hyperthermia of HER2-Positive breast cancer	[77]

	Graphene oxide nanosheets by covalent linking with amine functionalized IONPs	Theranostic agent for targeting cancer in the metastatic phases	[78]
	Polyethyleneimine-coated superparamagnetic IONPs	Improve magnetofection for gene transfer	[79]
	Glucose oxidase and polydopamine functionalized IONPs	Selective anticancer effect and improve the photothermal therapy with near-infrared radiation	[80]
	Polyethyleneimine nanogels Incorporated with ultrasmall IONPs and Doxorubicin	Effective inhibition of tumour growth under the guidance of MRI (Good water dispersibility, excellent loading, colloidal stability, and efficiency)	[81]
	Polyvinylpyrrolidone with IONPs	Induce apoptosis in cancer cell line	[82]
	Siliceous and carrageenan hybrid shells that coat superparamagnetic magnetite NPs	Promising nanocarrier for doxorubicin	[83]
Glioblastoma	Superparamagnetic IONPs	Inhibits Tyrosine kinase with immunoglobulin-like and EGF-like domains expression by drive miR-4855p overexpression in glioma stem cells *in vivo* and *in vitro*	[84]
	Ultrasmall superparamagnetic IONPs	MRI contrast agent	[85]
	Magnetic IONPs with Polyethyleneimine (PEI)-polyethylene glycol	Potential site-specific magnetic targeting for chemotherapy (Salinomycin)	[81]
	IONPs coated with dimer captosuccinate	Less toxic, more rapid dissolving alternative for copper oxide NPs as anticancer	[86]
Non-Hodgkin lymphoma	Rituximab conjugated IONPs	Increases the anticancer nanomedicine and theranostic efficiency	[87]
Colon cancer	Niobium substituted cobalt-nickel nano-ferrite	Targeting colon cancer cells and cause their death	[88]
Colorectal cancer	IONPs	MRI contrast agent and enhances the antitumor efficacy of tumour necrosis factor-related apoptosis-inducing ligand	[89]
Gastric cancer	1-((3-(4-chlorophenyl)-1-phenyl-1H-pyrazol-4-yl) methylene)-2-(4-phenylthiazol-2-yl) hydrazine (TP) conjugating with (3-Chloropropyl) trimethoxy silane (CPTMOS)-coated IONPs	New anticancer candidate against gastric cancer cells to be studied in invitro mouse model	[90]
Pancreatic cancer	Magnetic IONPs	MRI contrast agents and as anti-pancreatic cancer	[91]
Ovarian cancer	Lanthanide-Doped Superparamagnetic IONPs with holmium (III) bio conjugated with Trastuzumab	Targeting action by simultaneous internal and localized irradiation and magnetic hyperthermia of specific cancers	[92]
Lung cancer	Glycine coated super-paramagnetic NPs	A potential non-invasive MRI contrast agent for lung cancer and other pulmonary disease	[93]
Osteosarcoma	Zinc with IONPs and silica coating	Effective magnetic hyperthermia agents	[94]
Hepatoma	Copper-iron oxide nano powders	Limited cytotoxic and mutagenic effect	[95]
Prostate cancer	Magnetic IONPs and cysteamine functionalized gold NPs	Diagnosis of prostate cancer with antibody-antigen immuno- complex	[96]

Antimicrobial activity

The appearance of highly resistant bacterial strains has aroused the interest in designing new antibiotic-carrier nano-systems to find alternatives to conventional antibiotics. They were designed to enhance the sensitivity and detection limit, and to increase the performance of microbial application. IONPs revealed superb safety to mammalian cells and great antimicrobial activity. This was obvious with hematite compared to conventional magnetite NPs. Many studies nowadays indicate that the capability of magnetic NPs to produce microbial toxicity is basically due to sequence of interactions, that lead to impairment of cell integrity because of membrane depolarization, oxidative stress development, and reactive oxygen species (ROS) generation, that trigger the inflammatory response. High ROS levels can damage cells by producing lipid peroxidation, mitochondrial damage, protein oxidation, DNA disruption, and gene transcription modulation, which leads to cell apoptosis [97] (**Figure 3**).

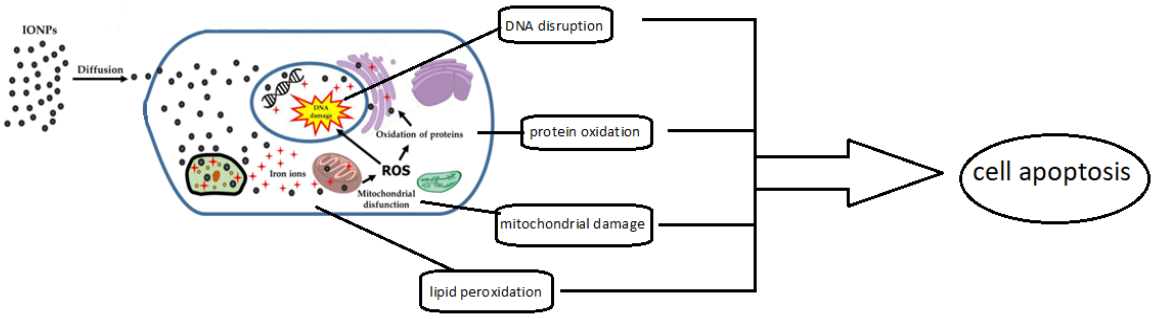


Figure 3. Microbial cell toxicity of IONPs; ROS: reactive oxygen species

IONPs conjugated with different antibiotics showed higher antimicrobial activity than NP alone, where the minimum inhibitory concentration (MIC) of IONPs conjugated with amoxycillin showed 3 - 4 times lower than the antibiotic alone on *Staphylococcus aureus* and *Escherichia coli* planktonic cells. [4] Furthermore, surface modified IONPs showed higher activity than undecorated surface.

Several studies were done and summerized in **Table 3** that invistigate the role of IONPs whether conjugated with antibiotic or not against different strains of drug resistance bacteria.

Table 3. List of IONPs (with/without antimicrobial) and their clinical application on different microorganisms.

Nanoparticle	Microorganism	Clinical application	Reference
Alginate-coated magnetite NPs conjugated with tobramycin	*Pseudomonas Aeruginosa*	Damage bacterial proteins and DNA Provide a lower cost anti-bacterial coating material for drugs than silver NPs	[98]
Silica-coated IONPs conjugated with polyvinyl alcohol entrapped with Vancomycin	*Staphylococcus aureus* *Escherichia coli*	Provide lower dosages of antibiotic to attain the same therapeutic effect of antibiotic alone Reduce the induction of antibiotic resistance Fast drug delivery	[99]
Citrate coated IONPs	*Bacillus subtilis* *Escherichia coli*	Generate hydroxyl radical that damages bacterial cell wall by improving the peroxidase activity of IONPs nanozyme against bacteria	[100]
Magnetic IONPs	*Salmonella Enteritidis*	Have bacteriostatic activity that has potential to be used in poultry industry	[101]
IONPs	*Staphylococcus aureus*	Improve the antibacterial effect of macrophages	[102]
Vancomycin-loaded spore-targeting iron oxide NPs	*Clostridium difficile*	Enhance the action and potency of vancomycin	[38]
IONPs	*Escherichia coli* *Enterococcus hirae*	Have bactericidal effect	[103]
IONPs	*Escherichia coli (Ampicillin-resistant E. coli and kanamycin-resistant E. coli)*	Provide synergistic effect with antibacterial drugs	[103]

IONPS CYTOTOXICITY

Previous findings reveal that a dose level of iron up to 100 µg/ml is nontoxic.[104] Many factors affect IONPs toxicity. The rod shape of IONPs is more toxic than sphere shape due to the alterations in the surface area. [105] In addition, IONPs with positive charge exert more toxicity due to non-specific interactions and endocytosis adsorption with the negative charged cell membrane. This interaction leads to the increase of their intracellular accumulation which affects the cell membrane integrity.

Moreover, surface coating of IONPs plays a role in toxicity. Coating IONPs with polyethylene glycols (PEG350 and PEG2000) showed no *in-vitro* toxicity with *in-vivo* liver and kidney injury. Oleate-coated IONPs were found to be both cytotoxic and genotoxic, [106] while the cytotoxicity of silica surface modified IONPs was dose dependent. [4]

DRAWBACKS AND FUTURE RECOMMENDATIONS

The size, shape, and its distribution are crucial factors that influence the pharmacokinetics and biodistribution of IONPs. However, most methods of NPs preparations are

challenged with various drawbacks. It is a great challenge to produce stable, size controlled and monodispersed particles with tunable shape. In addition, a controlled manner, reproducible, scalable, high-quality functionalized and long-term stable IONPs are still a big challenge to achieve. Different formation mechanisms under different conditions of iron oxides still need to be investigated. [15] For all manufacturing techniques, the major challenge is designing non-agglomerated IONPs with efficient coatings that deliver the optimum performance *in-vitro* and *in-vivo* biological applications. [107] The iron oxide encapsulation via mini emulsion polymerization suffers from presence of iron oxide-free particles without magnetic material, non-uniform distribution of the magnetic material inside and among the particles, aggregates in the aqueous phase, and limited loading of the magnetic material in the particles. [108]

Co-precipitation method suffers from extreme agglomeration, irregular shape and wide variety of particle size distribution of IONPs owing to the rapid reaction in this method. This creates difficult separation procedure of explosive nucleation from the successive stage of slow crystal growth. More focus should be given to overcome these limitations of wide particle size distribution and utilization of strong base in the reaction procedure. Sol–gel method overcomes the co-precipitation drawbacks by providing yield with high quality IONPs. Nevertheless, the relatively high metal alkoxides cost, the uncontrolled morphology and the particle size of the yields, use of suitable solvent concentration, and the release of large quantities of alcohol during the calcination step and reactive time are compelled to be further upgraded. [21, 49]. The hydrothermal method involves special equipment compared to co-precipitation method, the cost of the production is comparatively greater, and there is a significant oxidation problem in the reactive process, all challenge their utilization to industrial manufacture. [49]

Delivery of conjugated drugs onto the surface of IONPs is facing some drawbacks. The major one is the low entrapment efficiency, owing to the limited number of drugs that can be conjugated in this way. In addition, the presence of stable covalent bonding between the surface of the particles and the drug results in drug release failure at the site of action. [44] Beside the burst release effect is another limitation for drug delivery purposes. Moreover, significant premature release of drug away from the site of action, due to absence of appropriate surface coating may lead to toxicity. Cross-linkable polymers are very promising candidates that can be used for reducing this burst effect and controlling the drug release rate[44] Moreover, the lack of knowledge about the nature of surface interaction of the IONPs to the target tissue, the affinity for the target, and the number of vectors are major drawbacks to clinical application.

Looking forward, more progression in synthesis technologies of IONPs must continue to be optimized and developed. Size tuning of the nanoparticle must be optimized, and IONPs must continue to advance the frontiers of biomedical applications, thus the clinical appraisal of applications other than MRI is anticipated. More of such IONPs-based materials will be available to the consumer market. In addition, with the enhancement of surface alteration technology and the manufacturing of efficient, stable, and ecological friendly surface alteration materials, multifunctional IONPs would clutch the attention for the progress of nanomedicine in the coming years with large and industrial-scale production of IONPs. The future holds potential promise for the use of IONPs in cellular and deep tissue imaging, early detection, gene delivery, disease diagnosis, as well as multifunctional therapeutics. Such efforts would offer faster, simpler, and less invasive diagnosis processes, and encourage insertion of IONPs in the future medical practice. [109] These data taken together, support the hypothesis that IONPS might represent a new era for anticancer and antimicrobial therapy.

CONCLUSION

IONPs with various sizes, shapes and properties have been developed and widely investigated as anticancer and antimicrobial. IONPs can be prepared by microbial, chemical, and physical techniques. IONPs morphology is affected by the technique used. Proper surface modification of IONPs is necessary to create stable and biocompatible NPs, to be used as drug delivery vehicles. Their activity as antimicrobial is introduced by the generation of reactive oxygen species (ROS) with DNA damage and lipid peroxidation due to bio-interaction with the nanometal. As anticancer, IONPs perform vital role in optimization of the diagnostic process and tumour targeting as well as ameliorate the therapeutic efficiency of the treatment by loading the chemotherapeutic agent in IONPs. The cytotoxic effect of IONPs rely on the charge, shape, and the nature of NP coatings. While the exploitation of IONPs for therapeutic drug delivery is still in its infancy, a further progressive thoughts and systemic appraisal of the IONPs will enhance their synthesis as a superior and effective drug delivery system that can bring innovations to the field of antimicrobial and cancer nanomedicine.

AUTHOR CONTRIBUTIONS

Conceptualization & design, R.A.H; Supervision, R.A.H; Literature search, S.A, F.M, M.K., A.B., R.A.H.; Writing original draft, S.A, F.M, M.K., A.B.; Review & editing, R.A.H.

CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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