Review Paper:

Recent Designed Simple Synthesis Approaches, Surface Modification Superparamagnetic Iron Oxide Nanoparticles and Biologically Inspired Biocompatible Nanoparticles for Biomedical Applications

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Abstract

In biomedical applications, iron oxide nanoparticles (IO NPs) offer several excellent advantages. In biological systems, iron oxide nanoparticles have a non-toxic nature. Iron oxide nanoparticles may be employed in a variety of biological applications since they have magnetic and semiconductor characteristics. In order to get over current limitations, recent research has focused on developing next-generation nanoparticle systems with enhanced surface modifications for internalization and targeting. Superparamagnetic iron oxide nanoparticles (MNPs) have a variety of biological applications, including cell separation, hyperthermia, tissue healing and magnetic resonance imaging contrast enhancement. This review clarifies how IO NPs are used in many biological applications.

According to this review, iron oxide plays a positive function in biological activity because of its simplicity of synthesis, various magnetic behaviors, biocompatibility and biodegradability. When iron oxide nanoparticles are used in a biological way, their size, shape, surface modification, aggregation and electrical properties all have a unique effect. Based on this review work, the IO NPs may be specified for biocompatibility, hyperthermia, drug delivery, magnetic resonance imaging, tissue repair and magnetofection.

Keywords: Iron Oxide, Surface Modification, Biocompatibility, Hyperthermia, Magnetic Resonance Imaging and Magnetofection.

Introduction

Iron oxide nanoparticles (IO NPs) of different sizes have been made and studied for a wide range of uses, especially in medicine, over the last few decades[22,30]. These include drug delivery[36], MR imaging[49] and theranostics. Inorganic nanoparticles which act as a bridge between the molecular and solid phases, connect chemical approachability in solution to bulk phase physical properties[50]. As a result, they are suitable buildng blocks for nanostructured materials and devices with variable chemical and physical properties[30,58]. Iron oxide magnetic nanoparticles (IONPs) in particular are physically and chemically robust, biocompatible and ecologically benign, making them ideal for therapeutic applications. Iron oxide cores are covered with polymers, known as dispersants, to allow superparamagnetic iron oxide NPs to be disseminated in aqueous environments and at physiological salt concentrations. Without a polymer shell, NPs will quickly agglomerate and precipitate out of solution due to interactions with other NPs or biological molecules[1]. Magnetic nanoparticles' targeting abilities can be bolstered by immobilizing certain ligands on a surface which is predicted to increase nanoparticle affinity for the regions (or site) of interest. Polysaccharides, antibodies, peptides, proteins and aptamers are the most common ligands[15].

Iron is abundant in the environment and its oxides are common natural compounds and are easily synthesized in the laboratory. Metal oxide nanoparticles are a valuable class of nanomaterials and they have several important applications in science and technology. Iron oxide nanomaterials have been expanding significantly because of their magnetic behavior. The important forms of IO NPs are maghemite (γ-Fe_2O_3), magnetite (Fe_3O_4) and hematite (α-Fe_2O_3) which are significant in the various aforementioned applications. These magnetic nanoparticles have unique advantages over other nanoparticles i.e. inexpensive to produce, biocompatible, chemically and physically stable and environmentally safe.

IO NPs have been commonly used in scientific and technological applications for decades including magnetic hyperthermia, thermo ablation, targeted drug delivery[13], bioseparation, biosensing, magnetic resonance imaging (MRI)[3], lithium batteries, supercapacitors, catalysis and so on. For example, hemoglobin is made of them. They also play an important role in the human body as well as in a number of biological and geological processes.

This review covers the fundamental methods of IO NPs synthesis including the key benefits and drawbacks, as well as pharmaceuticals bound to IO NPs in the manufacture of

drug-delivery nanosystems. Recent advances in biomedical applications, translational achievements and the use of IO NPs in antimicrobial treatment alternatives are also discussed providing new insights into IO NPs research. Finally, a number of concerns are raised about the toxicity of IO NPs, as well as new ways to coat them to make them more biocompatible.The emphasis of this study is on several aspects of the stability of IO NPs, ranging from their actual description to their application through the molecular strategy of the surrounding shell, as well as their impact on the magnetic characteristics of superparamagnetic IO NPs.

Iron Oxide Nanoparticles Synthesis: Several synthesis techniques have been devised to regulate size, shape, crystallinity, polydispersity and magnetic characteristics. Physical and chemical techniques for the production of iron oxide nanoparticles are both available[10]. Metal evaporation via sputtering, electro-deposition and ball milling are all part of the physical process. All of these techniques have the advantage of being able to be set up for large-scale production and synthesizing high-purity nanomaterials. However, the size and form of nanoparticles may be precisely controlled using the physical synthesis approach. To tackle these annoyances, a variety of chemical methods that rely on solution-phase colloidal chemistry have been employed.

A lot of chemical processes are used to make metal salts. These include metal salt reduction, reverse micelles, sol-gels, hydrothermal, sonochemical, hydrolysis and thermolysis of precursors, electrospray method and more. Because of its colloidal nature, the synthesis of superparamagnetic iron oxide nanoparticles (SPOIN) is a difficult procedure. The synthesis of iron oxide nanoparticles (IO NPs) presents a number of problems, including the determination of the monodisperse population of appropriately sized nanoparticles. Choose a consistent technique that can be industrialized without the need for a time-consuming purifying procedure (magnetic filtration, size-exclusion chromatography and ultracentrifugation).

Diverse techniques for the production of IO NPs have been developed in the last few decades for various medicinal and technological applications[10,78]. Sol-gel, co-precipitation, microemulsion and hydrothermal techniques are among the most popular collective procedures. The regulation of size and form, crystallinity, polydispersity, morphology and porosity are all important problems for synthesis techniques. These fundamental features are influenced by reaction parameters which have a significant impact on mechanical, magnetic, optical and electrical properties. This is necessary in order to regulate the performance of IO NPs in a variety of applications.

Co-precipitation Synthesis Method: Co-precipitation is an efficient chemical process for obtaining magnetic IO NPs. Since, it can be manufactured on a large scale and readily distributed in water, this technique is commonly employed in biomedical applications. At room temperature, IO NPs are synthesized by reacting ferrous [Fe(II)] salt and ferric [Fe(III)] salt in a basic media ($NH_3.H_2O$) with a 1:2 molar ratio of reacting species, resulting in a black-colored precipitate[12,63].

$$Fe^{2+} + Fe^{3+} + 8OH^- \longrightarrow Fe_3O_4 + 4H_2O\downarrow$$

Generally, an oxygen-free environment is required for the co-precipitation method, so we ideally use nitrogen gas (N_2) purging to protect the oxidation of nanoparticles and the reaction goes through N_2 gas protection.

Iron oxide nucleation is more efficient around pH 9-11, but iron oxide nucleus development is easier at higher pH (>11). However, because the particle is kinetically regulated, control over morphology, particle size and content is restricted in co-precipitation techniques. Thus, experimental variables such as medium ionic strength, pH value and the mole ratio of ferrous [Fe(II)] and ferric [Fe(III)] iron salts influence the shape, size and composition of iron oxide nanoparticles (sulfate, nitrates, chlorides and perchlorates).

To further enhance the stability of SPIONs, they can be synthesized by the Massart method and coated *in situ* to make use of capping ligands such as organic anions (citric acid, gluconic, carboxylate, or hydroxyl carboxylate ions) and polymer surface complexing agents (starch, polyvinyl alcohol, dextran). The fundamental purpose of a capping agent is to prevent nucleation by chelating iron ions and adsorption of a capping agent like citrate on nuclei causes hydrolysis, impeding nucleus growth or controlling nanoparticle size[27,43,44,61,69].

Sol-Gel Method: This is a traditional wet-chemical method that is widely used in ceramic engineering and material science. For most of the parts, the sol-gel process is used to create nanostructured metal oxide[14,16,32]. Starting with a colloidal solution, the sol-gel method relies on the hydroxylation and condensation of metal alkoxide or alkoxide precursors in solution, resulting in a "sol" from nanometric particles, which is then dried or "gelled" either by solvent removal/chemical reaction to find a three-dimensional (3D) web of metal oxide.

In general, water is used as a solvent and the precursors are hydrolyzed by either acid or base, resulting in the formation of colloidal gel in the basic medium and polymeric gel in the acidic medium[45]. The reaction takes place at the ambient temperature, but heat is required to achieve the crystalline state[40,54]. The reaction mechanism of magnetite particles can be understood by the sol-gel process.

$$Fe^{3+} + H_2O \xrightarrow{\text{Deprotonation}} Fe(OH)_x^{3-x}$$

$$Fe(OH)_x^{3-x} \xrightarrow[\text{Dehydration}]{\text{Oxidation, pH 9 -11, } \sim 60^oC} Fe_3O_4$$

Various parameters control the growth of reactions such as reaction kinetics, condensation reaction, hydrolysis, temperature,

pH and so on and the sol-gel method lies in particle volume fraction, particle size distribution and particle dispersion[65]. This process has sovereignty such as monodispersity, stability and controlled particle size, as well as the features of the sol-gel matrix, homogeneity of the product, control of the microstructure and the ability to design the structure based on the experimental conditions[81,83].

The iron-oxide silica composite is also made using the sol-gel technique and is more reactive than ordinary iron oxide because of its large surface area. Alcohol is used to dissolve Fe (III) precursors and tetraethyl orthosilicate (TEOS) in water. The TEOS and Fe precursors form a gel and heat is released to make a final iron-oxide-silica composite.[5,19,26,79]

Microemulsion Method: A microemulsion is a solution comprised of three parts; a polar phase (typically water), a nonpolar phase (commonly oil) and a surfactant. It is macroscopically homogenous, thermodynamically quite stable and isotropic dispersion of oil and water-immiscible liquids; it is made up of nano-sized water droplets distributed in oil. However, microemulsions are stabilized by an interfacial film of surfactant molecules (surface active agents)[63,64]. The surface-active agents alleviated nanocavities offer confinement effects; it limited the growth, particle nucleation and agglomeration. The monodispersity of nanoparticles can be achieved by changing the nature (non-ionic surfactants as polyoxyethylene, cationic surfactants as di-n-dodecyl dimethylammonium bromide) and quantity of surfactant, oil phase and physiological condition.

The production of magnetite nanoparticles by the expenditure of reverse emulsion and nano-emulsion system involved AOT {Aerosol Orange T (dioctyl sodium sulfosuccinate)}- BuOH /cHex /H_2O and the molar ratio of surfactant AOT/ H_2O is 2.85, while the molar ratio of AOT surfactant/BuOH co-surfactant is 1[67]. The nanoparticles are made using a sequential synthesis method. Magnetite nanoparticles are formed by adding ammonia ($NH_3.H_2O$) or tetrabutyl ammonium hydroxide as a precipitating agent into a microemulsion composed of iron source and sodium hydroxide.

Later, it was discovered that the way nanoparticles accumulate during synthesis is affected by experimental constraints such as reaction media, pH, temperature and washing cycles among others. Acetone lysed the nano-emulsion and eliminated the nanoparticles from the surfactant and ethanol was used 2-3 times for washing and drying the nanoparticle. Then NPs were collected and shown to have superparamagnetic performance. The main benefits of using this sort of microemulsion system are the construction of controlled sizes of nanoparticles and the modification of the size of the aqueous micellar core[20,41].

The micro-emulsion technique (water-in-oil) has been used with great care to precisely regulate the size and size distribution of a diverse array of nanoparticles. It also demonstrates the wide range of topologies including spherical aggregates, bi-continuous and tubular bi-continuous. In recent years, iron oxides encapsulated with silica precursors have demonstrated that encapsulation improves nanoparticle stability and protects them against oxidation and reduction[46].

Hydrothermal Method: Exclusively for metals and metal oxides, the hydrothermal technique is the most suitable wet chemical methodology for the production of inorganic nanocrystals. In the hydrothermal method, reactions are completed in aqueous media in autoclaves (Teflon-lined stainless steel), where there is a relatively higher temperature (200^oC) and pressure (2000 psi) and it affects the formation of nanocrystals[28,29,52]. The hydrothermal technique offers a number of advantages including highly reactive reactants, easy morphological control and crystallization[11,21,77,88].

In addition, certain metastable and unusual condensed phases have developed at greater pressures. As for nanospheres, nanoplates, nanosheets, nanocubes, nanorods, nanorings, nanowires etc. have been fruitfully synthesized by this method[51]. With increasing precursor concentration, particle size and size dispersion improve. As a result, residence time has a greater impact on particle size than concentration. Exclusively for metals and metal oxides, the hydrothermal technique is the most suitable wet chemical methodology for the production of inorganic nanocrystals.

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The synthesis of quasi-sphere polyhedron nanocrystalline IONPs with 50 nm may be done via a hydrothermal method with $Na_2S_2O_3$ acting as a phase control agent. The mole ratio of $Na_2S_2O_3/FeSO_4$ decides whether the iron oxide nanoparticles phase can be created[20]. At temperatures 90-200 ^oC, the microwave hydrothermal method produced spherical IONPs with an average size range of 150-200 nm. Precursors included hydrated ferrous sulfate and ferric chloride with NaOH as the hydrolysis reactant[38].

The Fe/NaOH ratio was discovered to be the controlling factor in iron oxide production. Microwaves, in contrast to conventional hydrothermal techniques, make reaction kinetics easier. The morphology of IONPs looks like octahedral, prepared by using EDTA in mild conditions. $FeCl_3$ and $N_2H_4.H_2O$, NaOH and EDTA were used as starting reagents and EDTA works as surfactant and helped with the shape control[90].

Nanoparticles Surface Coating Methods: Because they have strong magnetic attractions among particles such as Van der Wall forces and high surface energy, the bare surface tends to agglomerate which is an unavoidable problem associated with iron oxide nanoparticles over longer periods[86]. Because biological media are mostly water, iron oxide nanoparticles should be spread in water for biomedical applications.

As a result, aggregated IONPs are quickly removed by the reticuloendothelial system in living organisms and an excess of iron is toxic to organisms[31,34,59]. These issues have been overcome by putting a shell on the surface of IONPs and making them biocompatible, hydrophilic and functionalized[31,56]. A few designed coatings and techniques were used to safeguard the iron oxide core from corrosion and it was made for specific applications.

Numerous surface modification techniques are available such as silica coating, polymer coating, noble metal coating, liposome coating, small molecular coating etc. Truly, fabricating these nanoparticles is applicable in a biological system and it is a very important step. It provides a biocompatible surface for bio-conjugation and additional physical properties like optical and CT resonance.

SiO_2 Coating Nanoparticles: The SiO_2 coating is frequently utilized in colloid surface modification. It provides a biocompatible surface with molecules for further bio-conjugation such as drugs, dyes and quantum dots combined into silica shells during the development of silica shells[31,56,82,84]. Silica-coated iron oxides have several advantages. It is also covalently attached to object organs via antibody-antigen gratitude with several biomolecules and ligands. SiO_2 coating is commonly prepared by hydrolysis of TEOS (tetraethyl orthosilicate) in the existence of iron oxides core NPs in alkaline medium[25,31,75,80].

By changing the experimental factors like reaction time, iron oxide (seed) concentration and TEOS/ Fe_3O_4 ratio, the thickness of the SiO_2 shell was regulated to range from 12 to 45 nm[31].

Gold Coating Nanoparticles: The coating of noble metals is a well-known method to defend Fe_3O_4 nanoparticles that form corrosion at low pH. The prospect of coating Au or Ag with a surface plasmonic feature is quite exciting since it offers new optical properties. In the presence of Fe_3O_4 nanoparticles, the gold coating is attained by reducing the gold precursor[37]. The experimental conditions alter the properties of Fe_3O_4 nanoparticles like surface chemistry, solubility, size and so on.

Thermal decomposition to produce Fe_3O_4 nanoparticles, as well as magnetic core/shell (Fe_3O_4/Au or Fe_3O_4/Au/Ag) nanoparticles synthesized at room temperature was by reducing $HAuCl_4$. It is tough to coat gold on top of an IO surface. When gold precursors are reduced quickly, gold nanoparticles develop instead of covering the shell. To slow down the reduction of $HAuCl_4$ in the chloroform solution of IO NPs, oleylamine was employed as a weak reducing agent. Because of oleylamine desorption on the surface of IO nanoparticles and the IO surface capped by oleylamine, gold-coated iron oxide nanoparticles were soluble in a nonpolar solvent. Dried IO NPs are mixed with sodium citrate and CTAB (Cetyltrimethylammonium bromide) and CTAB forms a stronger capping and replaces oleylamine, resulting in water-soluble core/shell nanoparticles[73].

The co-precipitation method is also well known for coating gold atop iron oxide nanoparticles. This method is water-soluble and iron oxide surfaces are abundant in OH groups, so the coating of gold and metal is quite difficult. Surface modifications are now required and organic linkers such as APTES (3-aminopropyl triethoxysilane) are used to functionalize the surface with an amino group and it has a high affinity for gold. Nitric acid is also used to functionalize the surface with a positive charge.

Polymer Coating of Nanoparticles: The polymer coating is similar to the silica coating method and it provides shielding and bio-compatible organic surfaces for functionalization. Its production is similar to that of silica and frequently it is produced by polymerization of precursors in the existence of Fe_3O_4 nanoparticles[73]. For nanomedical purposes, multifunctional polymers have various functions like optical imaging, magnetically targeted drug delivery and cancer-targeted MRI at the same time. In the MRI, nanoparticles combined into the biodegradable PLGA poly (D, L-lactic-co-glycolic acid) matrix and iron oxide nanoparticles work as magnetically targeted delivery and contrast agents. PLGA-coated NPs have a matrix for loading drugs such as doxorubicin and organizing the release of hydrophobic drugs into the cells. As a result, cancer-targeting folate is PEG-conjugated with PLGA NPs to kill malignant cells with therapeutic drugs (doxorubicin)[39].

Biomolecules: Biomolecule functionalized magnetic IONPs are biocompatible and used in biological segregation, sensors, detection and other biomedical applications. Several biomolecules, as well as biotin, proteins, antibodies, enzymes and avidin, have been tied up to the surface of IO nanoparticles[8,33,62,68].

Functionalized IO nanoparticles with biological molecules can be used to separate proteins, cells, biochemical products, DNA and other materials that move when you move them.

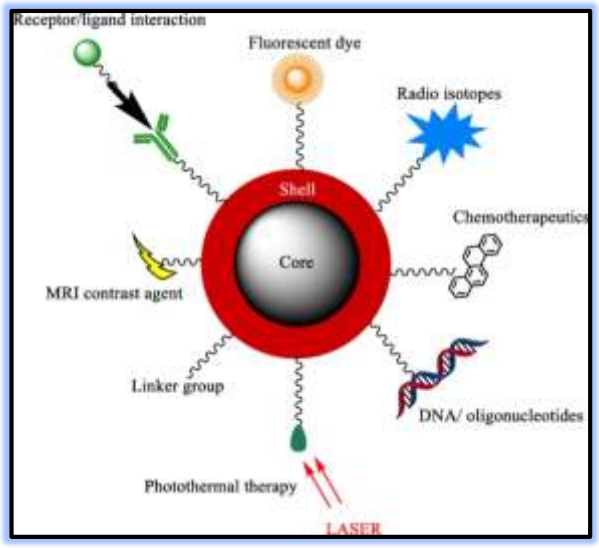

Figure 1: The uses of core/shell nanoparticles are depicted in this diagram[2].

Applications of Iron Oxide Nanoparticles

Drug Delivery: At the nanoscale (<100 nm), magnetically targeted IO nanoparticles serve as drug delivery vehicles. The nanoparticles are conjugated with chemotherapeutic drugs such as doxorubicin and paclitaxel. The lack of poor targeting of chemotherapeutic agents (doxorubicin and paclitaxel) to target specific tissues/organs can be conquered when the chemotherapeutic drugs are guided to their target using an externally applied magnetic field. Even conjugated genetic materials like DNA and RNA can be delivered to target tissues/organs in the same way[17,59,66]. Iron oxide as a drug delivery vehicle has received a lot of consideration over the last few years because it has a huge potential for well-planned drug delivery and minimally invasive side effects.

Iron oxide is loaded with anticancer drugs e.g. doxorubicin (Dox.) or paclitaxel and we can observe the effectiveness of Dox through *in vitro* cytotoxicity, apoptosis studies and fluorescence microscopy. IO nanoparticles also represent a loftier MRI contrast agent and have a high magnetization value along with super para magnetism at room temperature. Folic acid-coated nanoparticles (or folate-conjugate nanoparticles) show strong MRI contrast with regards to HeLa cells and without folate-conjugate nanoparticles. After comparison, their potential key role is visible in the MRI-based identification of cancer. Suppose silica-coated nanoparticles have a magnetic core, which is necessary for MRI imaging and silica is a porous material, so its shell will be porous for carrying the anticancer drug doxorubicin (Dox.). Doxoxamide works as a fluorophore molecule for imaging. All of the unique features are combined into a single nanoparticle that has a lot of potential for use in the body.

Hyperthermia: The use of iron-oxide nanoparticles in the hyperthermia (heat) treatment of cancer (malignant tumors) is as old as medicine itself. According to Hippocrates, the founder of medicine, surface malignancies might be scorched by the use of hot iron. Current methods for heating and destroying tumors include perfusion heating, magnetic fluid hyperthermia, high-frequency radiation and others[26,60]. One of the most popular methods used in cancer treatment is magnetic induced hyperthermia, which involves exposing cancer tissues (malignancy tumors) to a changing magnetic field. Live tissues and the deep regions of the living body do not absorb magnetic fields; magnetic particles such as iron oxides are subjected to magnetic field variations, causing heat to be produced as a result of magnetic hysteresis loss. Whatever heat is released, it leans on the nature of the materials and magnetic field parameters.

Immersion of iron oxide nanoparticles around the tumor site in an oscillating magnetic field generates heat up to a specific degree (meaning the temperature depends on the strength of the magnetic field, material property, oscillating frequency and blood flow in the tumor site to decrease the temperature). Cancer cells (malignant tumors) die at temperatures above 43.1^0C, but normal cells can survive at this temperature[24].

As can be seen in cancerous cells and animals, magnetic nanoparticles (like iron oxide) used for hyperthermia have an evident therapeutic influence on different kinds of tumors[9,57]. A very small number of magnetic particles (tenth of a milligram) combined with a suitable external magnetic field increased the temperature of cancer cells nearby which causes cell necrosis. The cancerous cell re-expressing the luteinizing hormone-releasing hormone (LHRH) receptor, can be highly specific for killing the following treatment with LHRH-coated iron-oxide 'nano clinics and DC magnetic field[48,72].

Magnetic Resonance Imaging: In comparison to other imaging modalities, MRI provides a number of benefits. There are no radioisotopes or X-rays used and it offers excellent structural resolution and soft-tissue contrast[26,47]. However, because MRI provides inadequate anatomic elucidation, it has limited sensitivity and makes it difficult to

see ultrafine tissue changes. Gadolinium complexes are commonly utilized as MRI contrast agents in clinical applications to increase sensitivity. Gadolinium-based treatments are ineffective at low concentrations, but they are effective at larger doses. Gadolinium ions also have hazardous side effects, such as nephrogenic systemic fibrosis (NSF)[74]. This complex's design is only used for a brief time. Iron, unlike gadolinium, is plentiful in living creatures and is necessary for a variety of biological activities including the transfer of oxygen via hemoglobin. Iron has no harmful effects in the rat model up to 100mg/Kg while 600mg iron per Kg in the form of injection is not lethal.

IO NPs have been extensively studied as MRI contrast agents because of their unique properties, surface modification, biocompatibility and functionalization, as well as their beneficial contrast effects. Iron oxide has better sensitivity in the nanomolar range than the gadolinium complex (used as a T_2 MRI contrast agent)[6] or nanoparticles with high relaxivity. The current advancements in nanotechnology permit the controlled size of nanoparticles, surface modifications and the crystal structure of iron oxide nanoparticles. It is an important MRI contrast agent because it can easily distinguish between healthy and pathological tissues. Thus, iron oxide is approved for clinical use.

Tissue Repair: Iron oxide nanoparticles are used for tissue repairs by affixing two tissue surfaces after sufficiently heating the tissues to fuse them, or by welding or soldering proteins or man-made polymer-coated nanoparticles between the two tissue surfaces to increase tissue affixing. More than 50^{o}C is favorable for induced tissue union which is thought to be caused by protein denaturation followed by the complexity of the adjacent protein chain[55]. Iron oxide nanoparticles have strong absorbing light commensurable to the laser, also suitable for tissue-repairing procedures. Few nanoparticles such as silica or gold-coated IO nanoparticles have been produced to strongly absorb light. Nanoparticles were coated on the surface of two-part tissues at the chosen connecting point, with the chosen method reducing tissue damage by employing a less damaging wavelength of light[76,87].

Only stem cells have the ability to renew and give birth to other types of specialized cells. Thus, stem cells are used for transplantation purposes such as repairing injured tissues and giving signals so that they form the specific types of cells for the development of tissues. The absence of targeted techniques for the signals that determine the mode and destiny of tissue growth and neural stem cells is a significant barrier to developing such a treatment. Superparamagnetic iron oxide nanoparticles attach to cells and guide them to a specific place inside the body. Several growth hormones and proteins, for example, might be linked to iron oxide nanoparticles and transported to the damaged tissue location where they would play a critical role in tissue formation. Cell-based therapy has enormous potential for diseases like Parkinson's and Alzheimer's. Cell-based therapy has enormous potential for disease treatment and repair. Magnetic particles have the skill to target and trigger stem cells in specific areas of tissue injury and healing[7].

Magnetofection: Gene therapy is a well-known approach in treating a variety of ailments. Its primary goal is to transfer a foreign gene into a cell to compensate for an abnormal gene and produce therapeutic proteins. A foreign gene requires transport to the cell with the help of a vector, of which there are two types (viral and nonviral). Generally, viral vectors are used such as adenovirus, adeno-associated virus and lentivirus etc. and they are very efficient carriers. The efficiency of gene therapy depends on the ability to deliver the necessary genes to cells. Viral vectors have several potential risks. As a result, the nonviral method emerges as a safe option.

Among the numerous gene delivery techniques, nonviral methods are mutually called transfections. A variety of non-viral gene carriers of inorganic nanoparticles are utilized. After being imposed on cells, gene carriers have two primary functions: to connect with DNA and to protect it from enzymatic assault. Sonoporation (using ultrasound waves), lipofection (using liposomes), electroporation (using an electric field), biolistic delivery by gene gun, cell-squeezing method and magnetofection (magnet-assisted transfection) are a few transfection methods.[42,70,89,92]

Magnetofection is the process of transferring magnetic iron oxide nanoparticles containing genes into specific cells under the impact of an external magnetic field. To achieve this tenacity, magnetic nanoparticles are often covered in a cationic polymer (polycation polyethylenimine). The cationic polymers connect to the negatively charged DNA, which is subsequently linked to the cell membrane and diffused into the cytoplasm through endosomal escape. Particles are dispersed into cellular vesicles where they are picked up by endocytosis. After that information reaches the cells, DNA is disclosed by the proton sponge effect (an osmotic pressure that ruptures the endosomal membrane and instigates enough pressure to disrupt the endosome and release DNA).

Magnetofection has demonstrated that the efficiency of this vector can be thousands of times higher. Magnetofection improves DNA delivery and is widely applicable to both nonviral and viral vectors, making it extremely fast. Using a magnetic field to bind, polyethyleneimine (PEI) to iron oxide nanoparticles improves transfection efficiency and reduces toxicity[91].

Toxicity and Biocompatibility of Iron-oxide Nanoparticles: The term "Biocompatibility" is explained as the aptness of materials to accomplish a suitable host response in a particular application and it has compatibility with a living system and it does not produce any toxic substances, no physiological reactiveness and no immunological rejection by a living system. Biomaterials

are substances that are metal oxide, iron oxide nanoparticles/iron coated nanoparticles etc. meant to interact with biological systems for therapeutic, diagnostic, or theranostic purposes. Biomaterials have a wider range of applications in medicine and are available to patients with increased longevity and higher quality of life. Cell separation, illness therapy, biological imaging, labeling and sensing are just a few of the biomedical uses for metal and metal oxide nanoparticles. Because of their toxicity, only a few kinds of nanoparticles have made it through pre-clinical studies.

The majority of biological processes in living systems need iron, including cellular respiration through redox enzymes and oxygen transport via hemoglobin. Iron is the most prevalent element in living systems. IO NPs are able to be injected into the body and absorbed into the human metabolic system since they are benign, safe and biocompatible.. When compared to IO NPs, biocompatible shell-coated IO nanoparticles are more efficient and less harmful. The inorganic coating layer on iron oxide nanoparticles anticipates shielding against oxidation and reactive species and allows superficial modification for multi-disciplinary biomedical uses[23,35,53].

Conclusion

This study covered the synthesis approaches, surface design and uses of iron oxide nanoparticles in biotechnology and medicine. This study seeks to examine the most widely utilized and inexpensive synthesis methods for magnetic IO NPs, which may be employed in biomedical applications such as biocompatibility, hyperthermia, drug delivery, magnetic resonance imaging, tissue repair and magnetofection. Future research could be a lot better if it used better and faster methods to learn more about how nanoparticle toxicity works. Despite the fact that iron oxides are less hazardous than other transition metals or semiconductor nanomaterials, toxicity concerns about iron oxide-based composite NPs persist. It is commonly established that naked iron oxide NPs cause extensive cellular internalization and considerable cell death.

However, iron oxide nanoparticles with surfaces made of materials that are hydrophilic and biocompatible have shown lower cytotoxicity in a variety of cell types. The review study indicates that iron oxide materials are less damaging to the environment and suitable for biomedical research. Recently, research on cancer detection and therapy moved on to the next phase. We believe that continued progress in design, synthesis and surface modification will open up new avenues for improving the efficiency and efficacy of IO NPs in biological applications as well as in broadening their field of use. Multimodal IO NPs could also be used to better diagnose and treat illnesses in the future.

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