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Magnetite nanostructures: a novel delivery system for enhanced antimicrobial therapy

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ABSTRACT

Resistance of microorganisms for antibiotics is a serious and increasing public health problem in today's world. Therefore novel approaches for controlling microbial infection are urgently needed for which nanomaterials can be a very promising approach. Inefficient delivery of antimicrobials results in inadequate therapeutic index and side effects. Nanostructured biomaterials, nanoparticles in particular, have unique physicochemical properties such as ultra small and controllable size, large surface area to mass ratio, high reactivity and functionalizable structure. These properties can be applied to facilitate the administration of antimicrobial drugs, thereby overcoming some of the limitations in traditional antimicrobial therapeutics. Iron oxide magnetic NPs (MNPs) have many advantages and are considered very promising drug carriers. Iron oxide MNPs have been used with various synthetic and natural drugs to inhibit the development of microorganism causing serious infectious diseases. This review highlights potential of MNPs in developing novel routes for fighting antimicrobial resistance.

Keywords: *antibiotics, infection, nanomaterials, antimicrobial, magnetic nanoparticles, magnetic field, microbial resistance.*

1. CURRENT STATUS OF MICROBIAL INFECTIONS AND ANTIMICROBIAL AGENTS

Microbial infections are still a major cause of morbidity and mortality in the world. The effect on morbidity and mortality due to infectious diseases is very noteworthy among the population. Antimicrobial agents are substances that kill or inhibit the growth of microorganisms. Many infectious diseases have been overcome, since the discovery of antimicrobial drugs in the 1960s [1]. Typically, antimicrobials kill bacteria by binding to some vital compounds of bacterial metabolism, leading to inhibition of synthesis of functional biomolecules or impeding normal cellular activities, e.g. β-lactams (penicillins and cephalosporins) inhibit bacteria cell wall synthesis; tetracyclines, macrolides, and clindamycin inhibit protein synthesis; metronidazole and quinolones inhibit nucleic acid synthesis; and sulphonamides and trimethoprim have an inhibitory effect on enzyme synthesis. Antimicrobials such as penicillin are only effective against a narrow range of bacteria, whereas others, like ampicillin, eradicate a broad spectrum of both Gram-positive and Gram-negative bacteria [2]. Despite the development of many antimicrobial drugs, many infectious diseases remain difficult to treat; may be due to difficulty in transport of drug through cell membranes and they may have low activity inside the cells leading to weak inhibitory or bactericidal effects on the intracellular bacteria. In addition, antimicrobial toxicity to host tissues leads to a significant restriction to their use. Aminoglycosides, for instance, cause ototoxicity and nephrotoxicity and have to be given in controlled dosages. Another major issue with antimicrobials agents is the acquired resistance of infectious microbes.

In spite of availability of existing potent antimicrobial agents and other modern antibacterial means, microbial infections are still a challenge. Presently available antimicrobial agents clinically used today are having significant shortfalls, which include weak antimicrobial activity, risk of microbial resistance and difficulty in functioning in a dynamic environment.

Most of the currently used antimicrobial agents generally affect three microbial targets: cell wall synthesis, translational machinery, and DNA replication [3]. Unfortunately, bacterial resistance may develop against these targets.

The mechanisms by which resistance can be developed includes:

- Enzymes that modify or degrade the antibiotic such as β-lactamases and aminoglycosides;
- Modification of cellular components such as cell wall as seen in vancomycin resistance [3] and ribosomes in tetracyclines resistance;
- Efflux pumps that provide multidrug resistance against numerous antibiotics [3].

During the last few decades antimicrobial resistance has increased due to the continuous use of antibacterial agents. The resistance has resulted in genetic and biochemical modification of microorganisms in order to ensure their bacterial survival and multiplication. Antimicrobial resistance may be defined as a means of genetic events leading to biochemical alterations in bacterial genome. The biochemical changes resulting into resistance can be point mutations or gene amplifications. Also, usage of antibacterial drugs has increased the accumulation of genetic material which codes for bacterial resistance which can be transferred from one microbe to another resulting in multi-resistant clones.

In antimicrobial resistant bacteria the drug molecules are structurally distorted leading to prevention of binding of antimicrobial agent by the following mechanisms:

- Blocking of entry of antimicrobial agent into bacterial cell;
- inactivation of antimicrobial agent (e.g. enzymatic degradation);

• Rapid exit of antimicrobial agent from the bacterial cell through powerful efflux pumps before they bind to any site [4].

Thus, there is an increased concern regarding ever increasing microbial infections in addition to drug resistant bacterial strains. Moreover new antimicrobial agents are needed to treat bacterial infections and/or to develop new drug moieties which can be more effective than the currently used antibiotics. This had leaded the way for the development of newer and additional anti-microbial agents. In 2001 the WHO (World Health Organization) global strategy for the control of Antimicrobial Resistance [5] has provided a framework for reducing infectious diseases, reducing spread of infections, upgrading their use and access to antimicrobials. Since 1930s until now some new classes of antimicrobial drugs have been discovered, but the antimicrobial resistance still remains an issue to be solved yet. In many conditions antimicrobials has turn out to be less effective which has resulted in the usage of large concentration of antimicrobial agents to treat life-threatening infections. However, antimicrobial agents used in large concentration can be lethal for patients, leading to severe side effects. In order to resolve such issues, alternative antimicrobial drug delivery approaches have been proposed. Therefore, one of the most promising antimicrobial approach relies in the application of nanosized carriers which can transport as well as control the release of antimicrobial agents [6].

Over the last few decades, the applications of nanotechnology in medicine have been extensively explored for effective drug delivery. Nanotechnology deals with the understanding and control of matters in the 1- 100 nm range. These nanosized materials, at such scale have unique physicochemical properties including ultra small size, large surface to mass ratio, high reactivity and unique interactions with biological systems [7]. The pharmacokinetics and therapeutic index of the drugs can be significantly improved in contrast to the free drug counterparts by loading drugs into nanoparticles through physical encapsulation, adsorption, or chemical conjugation. nanoparticle-based drug delivery systems has advantages of improved serum solubility of the drugs, extended systemic circulation time, at a sustained and controlled release drugs, drug delivery specifically to the targeted tissues and cells, and delivery of multiple therapeutic agents to the same cells for combination therapy [7, 8, 9]. Moreover, drug-loaded nanoparticles can enter host cells through endocytosis and then release drug payloads to treat microbes-induced intracellular infections. Therefore, a number of nanoparticle-based drug delivery systems have been approved for clinical uses to treat a variety of diseases and many other therapeutic nanoparticle formulations are currently under various stages of clinical tests [7, 10]. Hence, in the era of current drug development, attention has been especially focused on new and emerging nanoparticle-based systems for antimicrobial chemotherapy.

Nanomaterials (NM) may be strategically advantageous as active antibacterial groups since their surface area is exceedingly large relative to their size. Nanosized particles may provide high activity although only a small dose of the particles is used. Consequently, NM could serve as an alternative to antibiotics to control bacterial infections. Most of the resistance mechanisms seen with antimicrobial agents fail by use of nanoparticles; since nanoparticles act mainly by direct contact with the bacterial cell wall, without the need to penetrate the cells. This raises the hope that nanoparticles would be less prone than existing antibiotics to promote resistant bacteria.

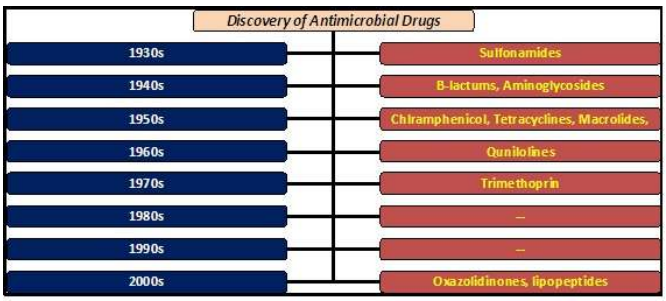

Figure 1. Discovery of Antimicrobial drugs from 1930s-2000s.

Generally, nanoparticles act by two major lethal pathways, which occur simultaneously in many cases and often related to each other:

(1) Disruption of membrane potential and integrity and

(2) Production of reactive oxygen species (ROS), also known as oxygen-free radicals, the NM acting as nanocatalysts [11, 12, 13].

2. NANOTECHNOLOGY AS A TOOL FOR DRUG DELIVERY

Nanobiotechnology is the science that investigates the interactions between nanoscale materials and biological systems. Nanotechnology is referred as the manipulation of matter with at least one dimension sized from 1 to 100 nanometers.

Nanoparticles are typically defined as solids with less than 100 nm in all three dimensions. The nanoparticles exhibit new thermal, mechanical, magnetic and optical properties such as small size, large surface area to mass ratio, and high reactivity delivery, that allow for their widespread application in biomedicine [14]. Most often, they are particles having diameters about 10 nm or less similar to most biological molecules and structures [15]. Nanoparticles are composed of mostly inorganic materials or organic (e.g. polymeric) materials. These materials may or may not be biodegradable. The significance of such Nanomaterials is ascribed to its characteristics such as size effects, magnetic and electronic properties and the role played by surface phenomena as their size is reduced [16]. Some nanoparticles commonly consist of magnetic elements such as iron, nickel and cobalt and their chemical compounds. Such nanoparticles can be synthesized and modified with various chemical functional groups and conjugated with biological molecules or structures, such as drugs of interest, opening a wide range of potential applications in biomedicine [14]. Metal and magnetic nanoparticles have been continuously used and modified to enable their use as a drug delivery system.

3. THE EVOLUTION OF MAGNETIC DRUG DELIVERY

As described by Paul Ehrlich (1854-1915) if an agent selectively targets a disease-causing organism, then a toxin for that organism could be delivered with the agent of selectivity. Hence, a 'magic bullet' might be created which can be able to kill the targeted organism exclusively [17]. Since then, various strategies have been proposed to deliver a drug to the vicinity of an invading organism.

In 1960, Freeman *et al*. proposed that magnetic nanoparticles could be transported all the way through the vascular system and can be concentrated in a specific body part with the help of a magnetic field [18]. The use of magnetic microparticles and nanoparticles has evolved since 1970s for the delivery of antimicrobial agents. In 1976, Zimmermann and Pilwat used magnetic erythrocytes for the cytotoxic drug delivery [20]. Widder *et al*. studied the targeting of magnetic albumin microspheres of doxorubicin in animal models [20]. In the 1980s, several researchers utilized this concept for delivering drugs with magnetic microcapsules and microspheres [21, 22, 23]. Hafeli *et al*. prepared biodegradable poly (lactic acid) magnetite and the β-emitter 90Y microspheres for targeted radiotherapy for subcutaneous tumors [24, 25].

However, all these early approaches utilizing magnetic drug delivery were of microsized systems. Magnetic nanoparticles were first used in animal models by Lubbe *et al* [26]. In 1996, the first Phase I clinical trial of epirubicin magnetic NPs was conducted in advanced and unsuccessfully pretreated cancers patients [27]. Although more than 50% of nanoparticles were destroyed in liver in these trails. Afterwards, various scientists have developed magnetic vectors and proved their potential applications. Several other start-ups currently manufacture magnetic microparticles and nanoparticles employed in magnetic resonance imaging, magnetic fluid physiological condition, cell sorting and targeting, bioseparation, sensing, enzyme immobilization, immunoassays, and factor transfection and detection systems.

Magnetic Propreties of Nanoparticles [28]

Magnetic nanoparticles for bioseparation are made of one or more magnetic cores coated with matrix of polymers such as silica or hydroxylapatite with terminal functionalized groups.

The magnetic core is usually made of either of magnetite (Fe_3O_4) or maghemite (gamma Fe_2O_3) with superparamagnetic or ferromagnetic properties.

Magnetic cores can be produced using magnetic ferrites, such as cobalt ferrite or manganese ferrite.

Theory of Magnetic Drug Targeting [29]

For magnetically controlled drug targeting it is assumed that magnetic nanocomposite particles having high saturation magnetization could be used for potential application. These particles have relatively magnetic properties along with discrete randomly oriented magnetic moments. When the magnetic particles are placed in the external magnetic field, their moments rapidly rotate into the direction of the field and thus improve the magnetic flux density. In order to manage the motion of such particles within a circulating system, a magnetic force and a hemodynamic drag force are combined to generate a total vectoral force on the particles. The magnetic force of the external field must be greater than the drag force or hydrodynamic force so as to effectively overcome the influence of a fluid flow in order to achieve the desired external magnetic field. Therefore, the magnetic force on the magnetic particles will be governed by:

$$\vec{F} = \vec{\nabla}(\vec{m} \bullet \vec{B}_0) \quad \text{(Newtons)}$$

Where F is the magnetic force, m is the total magnetic moment of the material in the microsphere, Δ is the gradient that is assumed to be derived from characteristics of the field alone, and the magnetic flux density- also known as the Bfield. Hence these quantities influence to some degree to which an external magnetic field may be used to internally guide particles in the body. The del operator is defined for magnetic field distribution at xyz directions.

$$\nabla \equiv \frac{\partial}{\partial x} a_x + \frac{\partial}{\partial y} a_y + \frac{\partial}{\partial z} a_z.$$

It is eminent that the gradient of a scalar function at any point is the maximum spatial change of the magnetic field. The Bfield tends to align with the net magnetic moment of a particle in a fixed direction while the gradient leads to a force so as to moves the particles. The second factor characterizes the magnetic properties of the particles. The magnetic moment of a material m, is proportional to the applied magnetic field H, and the intrinsic magnetic susceptibility of the material, χm.

$$m = \chi_m H$$

The magnetic volume susceptibility for various materials ranges from aluminium at 2.07 x 10-5 to magnetite 1.0 x 106 5.7 x 106 and as high as 106 for various ferromagnetic rare-earth materials. The force which counteracts the magnetic force on the particle in the fluid stream is due to the liquid flow (blood flow). Stokes law governs the hemodynamic forces on a particle in the liquid. The equation is given by:

$$F = 6 \Pi \eta \upsilon r$$

Where, F is the drag force, is the viscosity of fluid, and is the relative velocity of a spherical particle and r is the radius of the particle.

Also, there are other variables for drug delivery together with tissue c porosity, particle distribution, and allowable cell damage caused by incompatible sphere size and variable blood flow and body. An extremely porous tissue permits small particles to be simply manipulated out of the blood stream and into tissue. However, a comparatively tight tissue structure would need a lot of magnetic field elicited force to drag the nanoparticles out of the bloodstream, and such interfacial transport may additionally cause damage to the tissue. As a result, the magnetic nanocomposite particle size and external forces required for effective particle manipulation are extremely dependent on the area in which drug delivery is performed. Factors Affecting Magnetic Targeting of Drug [30]:

1. Size of the particles in ferrofluid.
2. Surface characteristics of particles.
3. Concentration of the ferrofluid.

4. Volume of the ferrofluid.
5. Reversibility and strength of drug/ferrofluid binding (desorption characteristics).
6. Access to the organism (infusion route).
7. Duration or rate of injection/infusion.
8. Geometry, strength and duration of the magnetic field application.

Limitations of Magnetic Drug Delivery [31, 32, 33]

The magnetic gradient decreases with the distance to the target.

The geometry of the magnetic field is extremely necessary and should be taken under consideration when coming up with a magnetic targeting method because the magnetic carriers accumulate at the target site similarly as throughout the cross section from the surface supply to the depth marking the effective field limit.

Once the magnetic flux is removed magnetic agglomeration are often developed owing to the small size of NPs, a requisite for superparamagnetism.

Limitations additionally arise in extrapolating applications of MNPs from animal models to humans

The fates of magnetic carriers are insufficient and, in several instances, there's inadequate characterization.

Magnetic targeting is an expensive, technical approach and needs specialized manufacture and quality control system.

It desires specialized magnet for targeting, advanced techniques for observation, and trained personnel to perform procedures. Magnets should have relatively constant gradients, so as to avoid focal over dosing with poisonous drug. A massive fraction of magnetite, which is entrapped in carriers, is deposited permanently in targeted tissue.

4. MAGNETIC NANOPARTICLES (MNPs)

Nanoparticles consisting of ferromagnetic elements such as iron, cobalt, nickel, or their oxides and alloys exhibiting magnetic properties are called magnetic nanoparticles [34]. Elemental manganese upon physicochemical treatment can display magnetic behavior [35]. In brief, the magnetic properties of a material replicate their magnetization arising from magnetic moments of unpaired electrons. Since thermal fluctuations of magnetic moments reverse their direction, some magnetic nanoparticles display superparamagnetic properties. Such properties are termed as the non-appearance of magnetic behavior when the magnetic field is not present [36, 37].

Iron oxide nanoparticles of size having approximately 10 nm in diameter like maghemite (γ-Fe_2O_3) display a superparamagnetic behavior (superparamagnetic iron oxide nanoparticles called as SPIONs) leading to improved dispersive properties in the nonappearance of a magnetic field [38]. Later on these nanopartcles are guided to in order to accumulate at the site of interest in presence of magnetic field. Such approach has enormous importance in targeted drug delivery applications [36].

Hence, magnetic nanoparticles having smaller particle size, consisting of iron oxide and display magnetic behavior only in the existence of a magnetic field are termed as superparamagnetic iron oxide nanoparticles (SPIONs) [36]. When the magnetic field is removed permanent magnetic behavior of magnetic particles result in destruction within the organism. Hence it is necessary to use SPIONs in biomedical applications [39].

Iron oxide nanoparticles such as magnetite Fe_3O_4 or magnemite Fe_2O_3 and gadolinium nanopartcles such as chelated organic gadolinium complexes have capacity to separate into iron and oxygen within the body. Due to this they are generally used as contrast agents in MRI for biological applications, since they can be safely eliminated and utilized in metabolic and oxygen transport (academia) systems [36]. Magnetic nanoparticles also exhibit low cytotoxicity and has been approved by the United States FDA for clinical MRI applications (academia) [40-46]. Most of the magnetic nanoparticles used as targeted delivery systems are chemically iron oxides (Table 2). Iron is essential to every organism. In addition endogenous iron oxide nanoparticles were also found in the human hippocampus [40, 41]. On the other hand, iron oxide cause direct cellular toxicity due to the production of reactive oxygen and nitrogen species (ROS and RNS) [42]. Therefore, magnetic nanoparticles are mostly prepared by core-shell methodology.

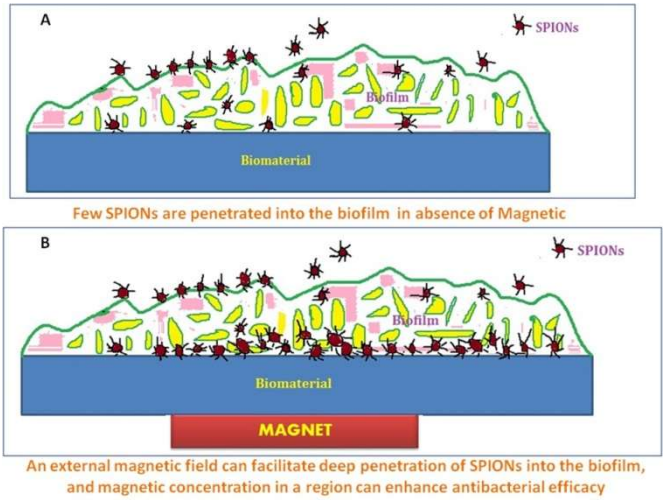

Figure 2. Application of SPIONs in Antibacterial Therapy.

The magnetic core of iron oxide nanoparticles consists of magnetite (Fe_3O_4) and/or maghemite (γ-Fe_2O_3) and their shell surface coating consists of organic compounds such as surfactants, synthetic or natural polymers, or inorganic material, such as silica, carbon, precious metals or oxides [36, 43]. Significance of core-shell type magnetic nanoparticles is due to following reasons [36, 44]:

- protection of magnetic core from oxidation;
- protection of shell surface from chemical reactions;
- prevention of aggregate and agglomerate formation due to Van der Waals forces, hydrophobic effects and magnetic attractions [36];
- various therapeutics attachment is possible;
- Cellular uptake rate can be amplified.

Biocompatibility of MNPs depends on the type of shell surface coating in addition to their size. Surface coating may be biodegradable (e.g. certain polymers) or non-biodegradable (e.g. silica). Uniqueness of the particle surface (e.g. hydrophilicity and

surface charge) is determined by the type of the coating whereas total size of magnetic nanoparticles is determined by the thickness of the coating [36]. Magnetic nanoparticles with smaller particles lesser than 100 nm can be prepared by coating with an inorganic material; whereas larger magnetic nanoparticles with particles above 100 nm are prepared by polymer coating [45, 46].

Magnetic nanoparticles used for biomedical applications are primarily prepared as ferrofluids, i.e. magnetic liquids. Therefore their surface charge is established by ionization of surface groups or by adsorption of charged species around the liquid on the particle surface which leads to the formation of layer around the particle. The variation in potential between the surrounding liquid medium and the layer around the particle is called the zeta potential. Particles with zeta potential larger than 30 mV, either positive or negative, will repel each other, reside as under and result in a stable ferrofluid [36, 47].

Fundamental prerequisites for Magnetic NPs

Magnetic NPs used for biomedical applications should posses certain specific characteristics as follows

Superparamagnetism

Superparamagnetism occurs in magnetic materials consisting of minute crystallites (Fe-based NPs turn into superparamagnetic at sizes <25 nm) [17, 48]. In superparamagnetic materials, the fluctuations affect the direction of magnetization of entire crystallites. The magnetic moments of individual crystallites compensate for each other and the overall magnetic moment becomes null.

Particle Size

In NPs with large particle size, energetic considerations favor the development of domain walls. But, when the particle size decreases below particular limit, the development of domain walls becomes unfavorable and each particle consists of a single domain. This happens with superparamagnetic NPs. Superparamagnetism used in drug delivery is essential because as the external magnetic field is removed, magnetization disappears and thus agglomeration is prevented [17].

Biodegradability of Magnetic Core

SPION are considered as biodegradable because iron is recycled by cells using usual biochemical pathways for Fe metabolism [49, 50]. But for non-biodegradable cores, a specific coating is required in order to prevent exposure of the magnetic core and to assist intact excretion through the kidneys (e.g. contrast agents based on gadolinium) [17, 50].

5. DRUG DELIVERY USING MAGNETIC NANOPARTICLES

Different organic materials such as polymeric NPs, liposomes, micelles have been reported as drug delivery nanovectors. Such materials acts by passive targeting, active targeting with a recognition moiety (e.g. antibody), or active targeting by physical stimulus (e.g. magnetism in magnetoliposomes). Nevertheless, these organic systems has limitations such as inadequate chemical and mechanic stability, swelling, susceptibility to microbiological attack, insufficient control over rate of drug release [51] and high cost. A major drawback of dendrimers and dendritic polymers is their high cost.

Due to the limitations of organic NPs used for drug delivery, inorganic vectors (inorganic magnetic NPs) are gaining popularity of intense research.

The main advantages of magnetic nanoparticles (organic or inorganic) are as follows:

1) visualization (superparamagnetic NPs are used in MRI);

2) magnetic nanoparticles can be guided or detained at the site with the help of magnetic field;

3) magnetic nanoparticles can be heated in a magnetic field to enhance drug release or to generate hyperthermia/ablation of tissue.

Behavior of magnetic NPs [51] is attributed to the important parameters such as:

- shell surface chemistry;
- particle size (which determines magnetic core, hydrodynamic volume and size distribution);

-Magnetic properties (which determines magnetic moment, remanence, coercivity).

The surface chemistry is important in order to avoid the action of the reticuloendothelial system (RES) and to increase the half-life in the blood stream. Coating of nanoparticles by neutral and hydrophilic compound (i.e. polyethylene glycol (PEG), polysaccharides, etc.) increases the circulatory half-life ranging from min to hours or days [51].

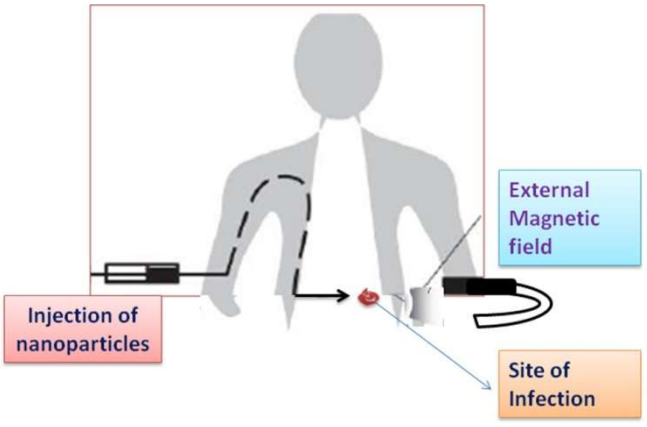

Figure 3. Drug Delivery by Magnetic field guided mechanism.

Fate of MNPs

The fate of MNPs with respect to their distribution all over the body depends on a range of physicochemical parameters such as: particle size, toxicity, surface charge, protein adsorption capacity, surface hydrophobicity, drug loading and release kinetics, stability, carrier systems degeneration, hydration behavior, electrophoretic mobility, porosity, specific surface characteristics, density, crystallinity, contact angle, and molecular weight [51, 52]. In addition, the fate and probable toxicity of magnetic NPs also depends strongly on the dose and route of administration like oral or parenteral (intravenous, pulmonary, transdermal and ocular) [51].

Oral administration

Several researchers have reported the application of magnetic NPs coated with an organic shell used as oral drug delivery vectors [53]. Also magnetic NPs are used as MRI contrast agents in gastrointestinal tract. The most important restriction for the oral

delivery of peptides and proteins is that they are degraded in gastric acid, have low absorption, undergo first-pass metabolism and shows considerable increase in initial drug concentration. Feng *et al*. has studied fate of chemotherapeutic nanoparticles in oral delivery wherein it reports that particles below 5 μm can be removed via lymphatic drainage, particles upto 500 nm can cross epithelial cell membrane by endocytosis, and particles less than 50 nm can reach paracellular passage between intestinal epithelial cells [51, 54].

Intravenous administration

The carrier for magnetic NPs used in parenteral applications should be nontoxic, nonimmunogenic, and has size such that it avoids embolization of capillary ducts. On reaching bloodstream NPs are cleared by moocytes into the liver, spleen, and bone marrow where NPs are captured by resident cells (e.g. Kupffer cells in the liver) prior to degradation. Some NPs which are present in the Kupffer cells may be incorporated into the bile and removed in the feces depending on biodegradability and their size. Remaining NPs are filtered by kidneys and secreted in urine. Generally, smaller NPs are rapidly eliminated by kidney, whereas larger NPs are uptaken by liver, spleen, and bone marrow [55-61]. Large NPs are removed by cells which are able to undergo endocytosis (i.e. by B and T lymphocytes). Biodegradable magnetic NPs are taken up by any cell by means of pinocytosis [51].

Influence of the Magnetic Field on Human Body

The magnetic fields required to generate magnetic effect in the body must be very large. All body components are either dia-, para-, superpara-, ferri-, or ferromagnetic in nature. Even RBC which contains hemoglobin (Fe containing pigment), shows comparatively low response to large or steep field gradients. Although this low value is sufficient to be used in functional MRI (fMRI). The other natural Fe-containing compounds in the body are hemosiderin, ferritin, transferrin, and the cytochromes [51].

In 1987 Schenck reported that, the US FDA classified magnets having field strength lesser than 2 T as nonsignificant risk devices [62]. Further positive results made FDA to extend this threshold to 4 T in 1996 and again up to 8 T in 2003 for adults. However the rate of human blood flow has been reported to be reduced by 30% in *in vitro* tests in experiments conducted with strong static magnetic fields (8 T) [51, 63]. Also it has been reported that magnetic fields more than 3 T may affect conventional behavior of erythrocytes. Clinically relevant adverse effects in physiological or neuro-cognitive functions occurring due to exposure to static magnetic fields effects (up to 8 T) has not been observed in human subjects studied [51, 64]. Kangarlu and Robitaille has reported that human imaging obtained at fields more than 10 T can most likely be achieved and such comes are currently being planned [51, 65].

Toxicity of MNPs

The toxicity of MNPs, depends on numerous factors includes but not limited to the dose, chemical composition, method of administration, size, biodegradability, solubility, pharmacokinetics, biodistribution, surface chemistry, shape, and structure. MNPs must be also be assessed with respect to the risk-benefit ratio in order to justify their risk. The most important characteristics of MNPs regarding cytotoxicity include their size, surface area, shape, composition, and coating (Macaroff, 2006; Arruebo, 2006). The toxicity of NP can be reduced by the modifying their surface characteristics (Park, 2006). It has been reported that the big surface-to-volume ratio of all nanosized particles will probably cause unfavorable biological responses, once such NP are inhaled and afterward absorbed via the respiratory organ or engulfed followed by absorption across the gastrointestinal tract [51, 67]. Apparently, it's conjointly been reported that, in 20-100 mg/ml concentrations, large magnetic particles show higher toxicity than smaller ones even when normalizing for surface area [68]. In any case, toxicity studies ought to take into account not solely acute toxicity however additionally that of degradation products, the attainable stimulation of cells with future release of inflammatory mediators [69] and long term toxicity .

The magnetic carriers used as potential drug delivery vectors [51] should be specifically analyzed for:

- Toxicity in cellular and animal models (acute, subacute, and chronic toxicity, teratogenicity and mutagenicity);
- Hematocompatibility;
- Biodegradation;
- Immunogenicity;
- Pharmacokinetics.

6. DESIGN OF MNPs FOR BIOMEDICAL APPLICATIONS

MNPs used for biomedical applications are commonly prepared as a core/shell structure. Core/shell structure are designed such that the inorganic magnetic core is enclosed by an outer layer of shell (coating). Proper selection of magnetic core and surface coating material is a key for successful design of MNPs [70]. Magnetic core chiefly determines the abilities (heating, sensing etc) of MNPs linked with application efficiency. Surface coating determines the interaction of MNPs with physiological environment.

Magnetic Core

The magnetic core must be crystalline and smaller than a critical size so that it shall contain only one magnetic domain which enables single-domain nanoparticles to display superparamagnetic behaviour in the absence and a very high magnetization in the presence of an external field. Due to this particles are dispersed and concentrated in solution in-vitro or in blood circulation in-vivo, without forming magnetized clusters. They may also respond to an instantly applied field with some kind of magnetic on/off switching behavior. The size distribution of magnetic cores must be small. The magnetic cores in a particular sample must have a unique and uniform shape (monodispersed) since; the magnetic and physico-chemical properties strongly depend on size and shape of the magnetic cores [70]. The elements used for the magnetic core are transition metals such as Fe, Ni, Co and Mn since they present high magnetization values. But if they are not specially treated they undergo oxidation very quickly in the synthesis step. Instead transition metal oxide compounds called ferrites are used since they are stable, have acceptable

magnetizations and are generally used for biomedical applications [70]. Superparamagnetic iron oxide (SPION), belonging to ferrite family, is the most commonly employed one in biomedical applications. İron oxide generally exists as two stable forms called magnetite (Fe_3O_4) and its γ phase maghemite (γ-Fe_2O_3).

Magnetic alloy nanoparticles consisting of 2 or 3 different kind of metals like FeCo, FePt and NiCu. FePt are also used as magnetic cores and becoming more popular due to its chemical stability and high magnetic anisotropy [70].

MNPs can be prepared with several techniques including inert gas condensation [71] mechanical milling [72], spray pyrolysis [73], sol-gel [74], vapor deposition [75] and wet chemical processes [76]. However for biomedical applications the most common method used for synthesis of MNPs is by hydrothermal chemical decomposition method. Basically, in such type of liquid phase synthesis method, some organometalic precursors are placed in reaction in the presence of some organic surfactants or polymers. An inert gas is continuously fluxed through the mixture during the decomposition process at high temperature, and desired nanomaterial is obtained as a precipitate at the end of the reaction [70].

Another common method used in MNP synthesis is the co-precipitation method. It has some limitations as compared with thermal decomposition method like lower crystallinity and lower monodispersity. However the method is easier and larger quantity of product can be obtained. The method is based on the simultaneous nucleation and growth of magnetic cores by dissolving metal salt precursors in aqueous environment with changing pH and temperature [77]. Instead this type of reaction can be carried out in a confined environment by microemulsion method in which the co-precipitation reaction takes place nano-reactors called micelles [70].

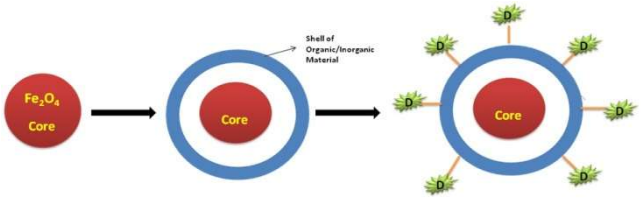

Figure 4. Schematic presentation of formation of the core-shell structure of MNPs.

Surface Coating [78, 79, 80]

The coating will contains long-chain organic ligans or inorganic/organic polymers, wherever these ligands or polymers are often introduced throughout (in-situ coating) or when (post-synthetic coating) synthesis. Unaltered coating is employed for co-precipitation synthesis technique whereas post-synthetic coating is used with thermal decomposition technique. Throughout unaltered coating, precursors of magnetic cores and coating materials are dissolved within the same reaction answer, and therefore the nucleation of core and also the coating occurs at the same time. In post-synthetic coating the surface coating materials are introduced when the magnetic cores are formed. In every procedures either end-grafting or surface-encapsulation are followed in order to link the surface molecules to magnetic cores.

The coatings on MNPs often serve manifold purposes such as it reduces leaching of the cores and it often facilitates the stabilization of NPs in slightly alkaline pH or significant salt concentration [51]. The external surface of silica coatings can be functionalized to allow the binding of biomolecules.

Additionally, coatings play an important role in retarding clearance by the RES. Uncoated NPs after systemic administration are rapidly up taken by the mononuclear phagocyte system and are cleared to the liver, spleen, and bone marrow. Antibodies bind to the surface of foreign bodies and accelerate phagocytosis of the particles. Biodegradable (e.g. dextran) and nonbiodegradable organic and inorganic coatings are used to obstruct detection and uptake by the macrophages. The most widely used coating for this reason is PEG which is a linear neutral polyether. Attachment of NP to PEG surfaces provides a 'stealth' shielding effect, delaying the action of the RES [81]. PEG shows very low toxicity and immunogenicity, and intact excretion is possible, either via the kidneys or in the feces [51, 82]. The organic/inorganic surface coating of MNPs plays a significant role in medicine applications for [70].

Preventing agglomeration of MNPs due to the higher interparticle interactions and ultimately providing the mixture stability of ferrofluids ready with MNPs;

Providing biocompatibility of MNPs by preventing escape of nephrotoxic particle from magnetic core into biological surroundings;

Serving as a base for additional anchoring of functional groups like biomarkers, antibodies, peptides etc. [70].

Table 1. Polymers / organic used as Nanomaterials [70].

Polymers / organic molecule	Properties	Advantages / Disadvantages
Chitosan	-natural, cationic, hydrophilic, linear, biodegredable	-They are used in non-viral gene delivery
Dextran	-natural, branched, hydrophilic, biocompatible	-They permits the anchoring of biovectors and drugs when functionalized with amino groups
Polyethyleneglycol (PEG)	-synthetic, neutral, hydrophilic, linear, biocompatible	-They are stable even at high ionic strengths of solutions with varying pH, enhances blood circulation time (a few hours) and spermits functionalization
Polyethyleneimine (PEI)	-synthetic, cationic, linear or branched, non-biodegredable, toxic	-They form strong covalent bonds with MNP's surface. They are used for DNA and RNA delivery, but exhibit cytotoxity

Polymers / organic molecule	Properties	Advantages / Disadvantages
Polyvinylealcohol (PVA)	-synthetic, hydrophilic, biocompatible	They irreversibly binds on MNP's surface but can be used in temperature sensitive heating or drug release applications due to its decomposition temperatures (40-50 ^{o}C),
Polyvinylpyrolidone (PVP)	-synthetic, branched, hydrophilic	They forms covalent bonds with drugs containing nucleophilic functional groups

Functionalization of MNPs [83]

In order to modify the application of MNPs in biomedical applications, the MNPs are functionalized by conjugating with functional teams. The surface coating permits appropriate base to attach useful groups on MNPs. These groups (antibodies, peptides, polysaccharides etc.) allow specific recognition of cell varieties and target the NPs to a particular tissue or cell by binding to cell surface receptor. Several linker molecules like 1-ethyl-3-(3-dimethylaminopropyl) carbodimide coordination compound (EDCI), N-succinimidyl 3-(2-pyridyldithio) propionate (SPDP), N-hydroxysuccinimide or N, N'-methylene bis amide (MBA) are also used to attach the initial hydrophilic coated molecules to those targets [83]. However in practice the fraction of targeted cells which interact with the protein attached MNP is relatively low even though the targeted cell population is recognized with high specificity. In fact the effectiveness of biomedical applications depend more on cell-nanoparticle interactions rather than particle targeting. The cell membranes play a vital role in cell-nanoparticle interactions. In order to explain the character of cell-nanoparticle interactions, sometimes lipid bilayers mimicking cell membrane are employed in studies (Umut, 2013).

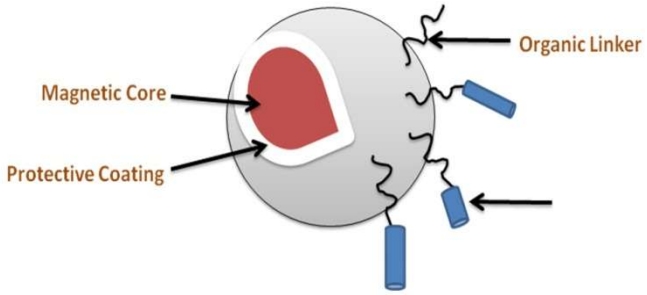

Figure 5. Representation of magnetic core-shell structure and multi-functional surface decoration; MNPs consist of a magnetic iron oxide core coated with a biocompatible material (e.g. polysaccharide, lipid, protein, small silane linkers, etc.) and Functional groups on the surface of coatings to link ligand molecules.

7. METHODS FOR PREPARATION OF MAGNETIC PARTICLES

Ramazan Asmatulu et al. method [83]

Magnetic nanoparticles were prepared by the chemical co-precipitation of Fe^{2+} and Fe^{3+} salts in the presence of a strong base. 2 g of $FeCl_3$ and 0.736 g $FeCl_2$ dissolved in 2ml of deoxygenated water. Dry nitrogen was purged into a 25ml 3-necked round bottom flask so as to ensure an inert atmosphere. The prepared salt solutions were added in the flask. This was followed by the addition of deoxygenated ammonium hydroxide (50/5 aqueous) until the pH of the solution was 9.5. The solution turns black immediately upon addition of the base, suggesting that magnetite (Fe_3O_4) was formed. The reaction was allowed to continue under a nitrogen purge for 3min. Then a stabilizer solution (oleic acid and dichloromethane) was added by a syringe. The reaction was stirred for an additional 0.5-2 hrs. It was then neutralized with 1M HCl to a pH 7 and allowed to stir for an additional 3min. The produced material was decanted into a beaker where it separated into aqueous and organic layers. The magnetite nanoparticles were positioned in the organic phase as they were stabilized by oleic acid. After removing the aqueous layer, the organic layer was rinsed with fresh deionized water to remove any remaining salt.

Kathryn M. Spiers et al. method [84]

Magnetite particles were synthesized by oxidative hydrolysis of a ferrous sulphate in an alkaline medium. A solution of 6.46 g potassium nitrate (KNO_3) and 44.9 g potassium hydroxide (KOH) in 240 ml oxygen-free double distilled water was added dropwise over 5 min to a solution of 80 g ferrous sulphate ($FeSO_4 \cdot 7H_2O$) in 560 ml water. The solution was heated to 900C and flushed with nitrogen. It was stirred continuously using a magnetic stirrer. About 23 g of magnetite is synthesized by his reaction. In order to limit the growth of the magnetite particles, 14.4 ml oleic acid was added 15 min after the addition of a KNO_3/KOH solution. The reaction vessel was immediately removed from the heat, and allowed to cool. Afterwards the solution was acidified to pH 3 by the addition of dilute nitric acid. Addition of hexane to this solution leads to the separation of magnetite into the organic phase, indicative of functionalisation of the magnetite by the oleic acid. The magnetite powder was collected and dried [17]

Huiling Bao et al.method [84]

Briefly the hydroxylated copolymer was dissolved in 1ml tetrahydroflourane (THF), and 10ml Fe^{2+} solution was added. The reaction was continued for 4 h with magnetic stirring at 700C. Thereafter, the reaction mixture was cooled to room temperature and the latex was separated from the medium by centrifugation. Then obtained latex was filtered with methanol, and the precipitation was washed with water and methanol three times in turn. After vacuum dried at 600^{o}C, the final product was obtained.

A. Pich et al. method [85]

Synthesis of iron oxide particles

Solutions of $FeCl_2$ and $FeCl_3$ were prepared in separate flasks by keeping molar ratio of $FeCl_3$:$FeCl_2$ constant at2:1. These solutions are added to stirred dispersion under nitrogen. NH_4OH solution was added drop-wise to start iron oxide formation. Immediately the solution became dark-brown demonstrating that

iron oxide has been produced in the system. After 30 min formed composite particles were removed from reaction vessel and cleaned by precipitation to remove all by-products [17]. Magnetic nanoparticles were washed with 0.01-M HCl solution. Magnetic dispersion with HCl solution was centrifuged (Universal 16 A) at a speed of 3000-U/min for 25-min to precipitate the particles. This procedure was repeated for four times. After that precipitated magnetic particles were cleaned with distilled water. Then calculated amount of sodium oleate was added to the required amount of magnetic dispersion (15-mg Na-oleate in 50-ml of 1.1-mg/ml magnetic dispersion, keeping the ratio constant at 1:1). Then the dispersion was heated to 80C for 5-min and finally sonicated for another 5-min.

A. Ibrahim et al. method [86]

Magnetically responsive polyalkylcyanoacrylate nanoparticles were prepared by anionic polymerization of the monomer in the presence of ultrafine magnetite particles of between 0.01 to 0.05 m. After 1g of glucose and 1g of citric acid had been dissolved in 100 ml of distilled water, 0.7 g of magnetite particles were dispersed by ultrasonic treatment for 15 min. The suspension was passed through a fritted glass filter (pore size 9-15 m) to avoid magnetite agglomerates. Dactinomycin (2 ml) and isobutylcyanoacrylate monomer (1.5 ml) were added and stirred ultrasonically (40W). After 3 hrs, nanoparticles were formed and filtered through a fritted glass filter (suspension A). To separate magnetized nanoparticles, the suspension was allowed to flow through a magnetic field at a rate of 1 ml per 3 min, using a pumping circulation tub system. Four permanent magnets were attached to the external surface of the circulation tubes. After removal of the magnets, the nanoparticles attached to the internal surface are washed with 10ml of an aqueous solution containing sodium chloride (0.7 %) and calcium chloride (0.2 %). This magnetically responsive particle suspension was finely resuspended by ultrasonic treatment for 15 min, at 40W and filtered through fritted glass.

4. CHARACTERIZAZTION OF MAGNETIC PARTICLES

Particle Size and Shape

Magnetic particles are of variable sizes. Conventional light microscopy (LM) and scanning electron microscopy (SEM) are most commonly used techniques to visualize microparticles. Both techniques can be used to determine the shape and outer structure of the microparticles. Particle size and its distribution are determined by light microscopy, scanning electron microscopy, transmission electron microscopy, etc. Confocal laser scanning microscopy (CLSM) is applied as a nondestructive visualization technique for microparticles. CLSM allows visualization and characterization of structures not only on the surface, but also inside the particles, provided the material is sufficiently transparent and can be fluorescently labeled. By collecting several coplanar cross sections, a three-dimensional reconstruction of the inspected object is possible.

Chemical Analysis

Electron spectroscopy for chemical analysis (ESCA) is used to determine the surface chemistry of the microspheres. ESCA determines the atomic composition of the surface. Degradation of the polymeric matrix carrier system is determined by the Fourier Transform Infrared Spectroscopy (FTIR). The surface of the microspheres is investigated measuring total attenuated reflectance (ATR). The surface carboxylic acid residue is measured by using radioactive glycine. The radioactive glycine conjugate is prepared by reaction of 14C-glycine ethyl ester hydrochloride with the microspheres. The radioactivity of conjugate is measured using scintillation counter. Surface associated amino acid residue is determined by the radioactive 14C- acetic acid conjugate. The carboxylic acid residue is measured through the liquid scintillation counter and hence the amino acid residue can be determined indirectly.

Drug Loading

The capture efficiency or the drug loading of the microspheres or the percent entrapment can be determined by allowing washed microspheres to lyse the lysate is then subjected to the determination of active compound by suitable method. The percent encapsulation efficiency is calculated using following equation:

% Entrapment = (actual content / theoretical content) x 100

Magnetic Properties

Magnetic properties of nanocomposite particles were characterized by using vibrating sample magnetometer (VSM). The magnetic moment of each dried magnetic particles measured over a range of applied fields between -800and +800Gauss with a sensitivity of 0.1 emu/g. the prepared samples can be characterized by weight or volume in VSM. The dry samples are weighed (0.075 g), while the fluids are injected into the sample holder (~ 0.05 ml). In this system, when a magnetic sample is placed between two coils of an electromagnet creating a uniform magnetic field gradient, the applied field induces the magnetic domains to line up with the field through dipole interactions. As the magnetic field is increased, number of domains will be also enhanced until the particles reach saturation levels. During magnetic field alignment, the particles undergo a sinusoidal motion and produce an electrical signal in a set of stationary pick-up coils. This signal is proportional to magnetic moment, vibration amplitude and vibrational frequency. After the measurements, magnetic saturation values of the materials are calculated for each sample by dividing the saturation magnetization by the weight of samples.

Thermo gravimetric Analysis

Differential scanning calorimetry and other gravimetric methods are used to determine the extent of interaction of polymers with magnetite and such other magnetic materials. Moreover the stability of ferrous and ferric ions can be assessed by thermogravimetric methods.

Stability Measurements

Stability measurements can be performed by using separation analyser (e.g. LUMiFuge). Measurements are made in glass tubes at accelerated velocities from 50to 300rpm. The slope of sedimentation curve can be used to calculate sedimentation velocity and stability data can be found.

ξ - Potential measurements

ξ - Potential measurements can be made using an instrument like Zetasizer 2000. The zeta potential is measured at different pH values and stability of magnetic particles can be predicted

4. ANTIMICROBIAL ACTIVITY OF MAGNETIC NANOPARTICLES

Magnetic nanoparticles (MNPs) commonly consist of magnetic elements such as iron, nickel, cobalt and manganese or zinc, and most often by ferrite as magnetite (Fe_3O_4) or maghemite (γ-Fe_2O_3). When these nanoparticles when dispersed in colloidal solutions constituted magnetic fluids (MFs), stable suspensions of magnetic nanoparticles in inorganic or organic solvent carrier. In these solutions, the particle-liquid interactions are so strong such that their magnetic behaviors are transmitted to the liquid as a whole [87].

Magnetic nanoparticles bind with the drugs, proteins, enzymes, antibodies, or nucleotides and can be directed to an organ. Although MNPs are mostly used in cancer research and treatment, new antibiotic coated magnetic nanoparticles intended as magnetically controllable agents for the recovery of bacteria loaded tissues and organs. The conjugation of antimicrobial drugs with magnetic nanoparticles can merge the best properties of both, generating an improved antimicrobial nanoparticle, and enhance therapeutic effectiveness of antimicrobial drugs in the treatment of infectious diseases. Antimicrobial activity of MNPs was studied by Grumezescu and collaborators which revealed activity of different antibiotics for the synergistic effect of the synthesized water dispersible magnetic nanocomposites against Gram-positive and Gram-negative bacterial strains [88]. Similarly, Dong and co-workers have shown that the combination of barbituric acid based N-halamine with magnetic nanoparticles reveals higher biocidal activity than the bulk powder barbituric acid-based N-halamine [89]. Nevertheless, magnetic nanoparticles are recognized by macrophages of phagocyte system and are eliminated from the body. In order to improve biocompatibility, to reduce toxicity and to ensure non-immuno-genicity, particles have been encapsulated (e.g., with chitosan, dextran, lactic acid). In addition it consists of iron, which acts to maintain the operation of essential metabolic pathways present in living organisms [52, 90]. For pathogenic microorganisms, especially P. brasiliensis, the ability to acquire iron is critical for the establishment of infection, so that the ability to capture this element from the host is considered to be a virulence factor [91]. Moreover, studies show that P. brasiliensis, both the yeast and mycelial forms requires iron for metabolic reactions [92]. Cano and colleagues demonstrated that the restriction of iron was one of the mechanisms for inhibiting the transformation of yeast in activated macrophages in the form of conidia subsequently yeast growth within macrophages [93]. Similarly, studies with chloroquine, a drug which affects the metabolism of iron in macrophages decreases the survival of the yeast P. brasiliensis in macrophages by interfering with the acquirement of iron by the fungus [94, 95, 96]. Further studies also demonstrate antimicrobial effect of zinc oxide (ZnO) nanoparticles. Vani *et al* studied that they can be used effectively for the control of microorganisms and the prevention of infections caused by Staphylococcus aureus [97]. The antibacterial activity of ZnO nanoparticles also has been studied by Jones *et al* [98]. Reports suggest that these nanoparticles have a potential application as bacteriostatic agent in visible light and might be useful for controlling the spread as well as infection of a variety of bacterial strains.

Nanomaterials for Antimicrobial activity

Nanomaterials as antibacterials complementary to antibiotics are highly promising and are gaining large interest as they might be proves beneficial to fill the gaps where antibiotics commonly fail. This includes combating multidrug-resistant mutants and biofilm Antimicrobial nanoparticles currently used are classified as follows.

Inorganic Nanoparticles: These include Metals and metal oxides which are used for their extremely potent antibacterial effect. Metal oxide nanoparticles demonstrate bactericidal properties through generation of reactive oxygen species (ROS) even though some are effective due to their physical structure and release of metal ion. E.g. Silver (Ag), iron oxide (Fe3O4), titanium oxide (TiO2), copper oxide (CuO) and zinc oxide (ZnO).

Organic nanoparticles: Polymeric nanoparticles kill microorganisms either by releasing antibiotics, antimicrobial peptides, and antimicrobial agents or by contact-killing cationic surfaces such as quaternary ammonium compounds, alkyl pyridiniums, or quaternary phosphonium. E.g. Poly-*ε*-lysine, Quaternary Ammonium Compounds, Cationic Quaternary Polyelectrolytes, N-Halamine Compounds, Polysiloxanes.Benzoic Acid, Phenol, and p-Hydroxy Benzoate Esters, Triclosan.

The description, mechanism, properties and Antimicrobial spectrum of Inorganic Nanoparticles is summarized in the table 2 and table 3.

Table 2. The description, mechanism, properties and antimicrobial spectrum of inorganic nanoparticles.

Inorganic Nanoparticles	Nanoparticle description/Mechanism	Antimicrobial spectrum
Silver (Ag)	The antimicrobial effectiveness of Ag nanoparticles, was reported to be size-dependent [99]. Ag nanoparticles produced "pits" in the cell wall of *E. coli*, by increasing the membrane permeability and inactivating the respiratory chain [100, 101]. In another work it has been reported that Ag ion can inhibit and disrupt protein structure by binding to thiol and amino groups since Ag has an affinity for sulfur	Ag nanoparticles used as an effective antimicrobial agent against bacteria, fungi, and viruses [114]. In medicine, Ag compounds are generally applied to treat burns, wounds, and a variety of infectious diseases [115, 116, 117].

Inorganic Nanoparticles	Nanoparticle description/Mechanism	Antimicrobial spectrum
	and nitrogen [101]. In conclusion it was suggested that silver NM are photocatalytic [102] and can induce ROS [103, 104, 105].	
Titanium Oxide (TiO_2)	TiO_2 nanoparticles are photocatalytic. Their toxicity is induced by visible light, near-UV or UV [11] as well as it stimulates ROS burst. The ROS damage the membrane, DNA, and many other macromolecules and functions of the bacterial cell [13].	TiO2 nanoparticles kill both Gram-positive and Gram-negative bacteria [118]. It is also effective against various viral species and parasites [81, 82, 119]. TiO_2 is effective against many bacteria including spores of *Bacillus* [120], which is the most resistant organism known.
Zinc Oxide. *(*ZnO)	ZnO nanoparticles damage bacterial cells by two pathways [106, 107, 108] • by binding to membranes and disrupting their potential and integrity • by inducing ROS production In addition Zn nanoparticles are weak mutagenics [109].	ZnO nanoparticles inhibit the growth of methicillin-sensitive *S. aureus* (MSSA), methicillin-resistant *S. aureus* (MRSA), and methicillin-resistant *S. epidermidis* (MRSE) strains. They are effective bactericidal agents against such bacteria which were not affected by the drug-resistant mechanisms of MRSA and MRSE [121, 122]. Zinc oxide (ZnO) NM are of relatively low cost and effective in size dependency [123] against a wide range of bacteria [124, 125] such as *Klebsiella pneumonia* [126], *Listeria monocytogenes*, *Salmonella enteritidis* [106], *Streptococcus mutans,Lactobacillus* [127], and *E. coli* [106, 107] with low toxicity to human cells [128].
Iron Oxide and Gold. Fe_3O_4 nanoparticles and gold (Au)	Fe_3O_4 in its bulk form and Au are normally considered inert and lack antimicrobial properties. However they can be tailored to induce antimicrobial properties when synthesized as nanosize particles. Antibacterial activity of Au-NM is enhanced by binding to nonantibiotic molecules such as amino-substituted pyrimidines [110] and citrate, which along with light energy, induce ROS production and mutations used in therapy against cancer cells [111].	Au nanoparticles and nanorods are bactericidal when photothermally functionalized [129]. Au-NM bound to antibiotics such as ampicillin [130, 131], vancomycin [132], the antibacterial enzyme lysozyme [133] and other NM [134] were bactericidal to many multidrug-resistant pathogens, as well as penicillin and vancomycin resistant bacteria.
Copper oxide (CuO)	Antibacterial efficacy of CuO is fairly weak as compared to that of Ag or ZnO. Copper oxide (CuO) NM exert their antibacterial activity [112, 113] by membrane disruption and ROS production	Cu NM are effective against *B. subtilis* and *B. anthracis* [135, 136].
Magnesium oxides (MgO)	Magnesium (Mg) is used in various NM in the form of MgO or MgX_2 (e.g., MgF_2) [11, 137]. MgO nanoparticles induces ROS production and also directly inhibit essential enzymes of the bacteria [13].	Nano-MgO particles show efficient antimicrobial activity against bacteria (both Gram-positive and Gram-negative), spores, and viruses.
Superparamagnetic iron oxide (SPION)	It is relatively new advancement for using magnetic particles that cause local hyperthermia in the presence of a magnetic field [137]. Moreover they can be coated by other NM such as Ag and Au and their magnetic effect can be utilized to penetrate and destroy biofilms	Superparamagnetic iron oxide nanoparticles enhances efficacy of antibiotics against antibiotic-resistant biofilms

Inorganic Nanoparticles	Nanoparticle description/Mechanism	Antimicrobial spectrum
	[138, 139, 140, 141]. Bactericidal effect of aluminum NM is relatively mild and they work only at high concentrations [11, 142] unless in combination with other NM such as Ag [143]. The mechanism of action of aluminum NM, as recently shown for *E. coli*, is by diffusion and accumulation inside the cells, causing pit formation, perforation, and membrane disorganization, leading to cell death [144].	
Aluminium (Al)		Aluminum nanoparticles are used as antimicrobial agent against *E. coli*

Table 3. The description, mechanism, properties and antimicrobial spectrum of organic nanoparticles [145]

Organic Nanoparticles	Nanoparticle Description/Mechanism/ Antimicrobial Spectrum
Poly-ε-lysine	Poly-ε-lysine is a cationic homopeptide of L-lysine. *It* is effective against Gram-positive and Gram-negative bacteria. It also displays activity against spores of *B.coagulans, B. stearothermophilus*, and *B. subtilis*.
Quaternary Ammonium Compounds.	benzalkonium chloride, stearalkoniumchloride, and cetrimoniumchloride are well known disinfectants. Their antimicrobial activity is a attributed to N-alkyl chain length and lipophilicity. Compounds with alkyl chain length 12–14 of alkyls provide optimum antibacterial activity. They are effective against Gram-positive bacteria. Alkyls group with 14–16 carbon chains show better activity against Gram-negative bacteria
Cationic Quaternary Polyelectrolytes	*Cationic Quaternary Polyelectrolytes* are acrylic or methacrylic derivatives. Many of these are synthesized from commercial methacrylic monomers such as 2-(dimethylamino) ethyl methacrylate. These polymers provide wide structural versatility by the alteration of hydrophobicity, molecular weight, surface charge, and other parameters.
N-Halamine Compounds.	N-halamine compounds contain one or more nitrogen-halogen covalent bonds which are formed by halogenation of imide, amide, or amine groups, which provide stability and slow release free active halogen species into the environment. These oxidizing halogens promote the direct transfer of an active element to the biological target site or through dissociation to free halogen in aqueous media. These reactive free halogens lead to inhibition or inactivation of a microbial cell.
Polysiloxanes.	*Polysiloxanes* exhibit high antibacterial activity against both *Escherichia coli* and *Staphylococcus aureus.*
Benzoic Acid, Phenol, and p-Hydroxy Benzoate Esters	The stereo electronic effect of the phenyl group is amajor contributing factor for antimicrobial activity of p-hydroxyphenyl acrylate derivatives. Benzaldehyde is well known for its bactericidal, fungicidal, and algaecidal activities. Benzaldehyde containing methyl methacrylate polymers have been synthesized and tested against *Bacillus macroides, Pseudomonas aeruginosa*, and *Dunaliella tertiolecta*. Polymers show fivefold inhibition of algae growth compared to acid-glass control surfaces.
Polymeric Nanosized Antimicrobials.	Polymeric nanosized antimicrobial agents are known to have long-term antimicrobial activity: they are nonvolatile and chemically stable, can bind to the surface of interest, and hardly permeate through biological membranes such as the skin.

Table 4. Functionalized magnetite nanoparticles to treat infections.

Micro-organism	Description of MNPs
	MNPs to Inhibit Yeasts
Candida albicans	The functionalized magnetite nanoparticles with 20 nm maximum diameter were prepared by a precipitation method, using oleic acid as surfactant, and *Rosmarinus officinalis* essential oil (NPs-EO) as an antimicrobial agent [147]. The suspended core-shell nanoparticles were used to coat a catheter by applying a magnetic field on the nanofluid. The essential oil was adsorbed in a secondary covering treatment. The fungal adherence ability on the catheter was examined in multiwell plates. The main observed components of *R. officinalis* were 40.596% eucalyptol, 11.389% camphor, 10.19% caryophyllene, and 18.42% α-pinene. The catheter coated with NPs-EO showed a distinct *C. albicans* biofilm inhibitory effect in a time dependent manner, for up to 72 h, as the viable cell counts (VCCs) revealed. *C. albicans* biofilms grown on the nanomodified surfaces diminished with up to approximately 85% as compared to uncoated catheters.

Micro-organism	Description of MNPs
Candida tropicalis	The functionalized magnetite NPs with *R. officinalis* essential oil coated onto a catheter also showed a distinct inhibition against *C. tropicalis* [147] compared to uncoated catheter. At 48 and 72 h the number of the VCCs was higher. Such antimicrobial agents of essential oils into MNPs can be used as biofilms of prosthetic devices against pathogenic microorganisms but also as nanocarriers for treating infectious diseases [148].
Candida krusei	MNPs have great potential in the treatment of *Candida krusei* infections. Essential oils have been stabilized with MNPs and used successfully in the prevention of fungal biofilms. In particular, a core/shell/coated-shell hybrid nanobioactive system composed from *Anethum graveolens* essential oil-magnetic nanoparticles was obtained by a modified Massart method. CLSM on coated and uncoated coverslips colonized with *C. krussei Y5* strain revealed a rare adherence of yeast cells on the coated surface, whereas a thin biofilm with an internal canalicular structure was developed on the uncoated surface [149]. Other recent study reports the modulation of *C. krusei* infections by MNPs [150] has been made where microbial interactions are influenced by interfacial forces, such as the electrostatic field, that plays a significant role on the interaction between MNPs and microbial surfaces [151].
Candida glabrata	*A. graveolens* essential oil-MNPs nanoparticles were used to prevent the growth of *C. glabrata*. *C. glabrata* inoculated coupons produce a monostratified biofilm, homogenously distributed on the uncoated as well as on the coated coverslip surface [151].
Saccharomyces cerevisiae	Recently, fatty acid-functionalized magnetite nanostructures were used for the investigation of *in vitro* microbial biofilms developed on different substrata, using *S. cerevisiae* strain. The results are very promising, highlighting the importance of magnetic nanoparticles in the inhibition of fungal biofilms. The CLSM technique was used to obtain images of uncovered and nanoparticles-oleic acid covered glass-slips and the growth of *S. cerevisiae* was monitored after 24, 48 and 72 h. The cover slips coated with oleic acid-MNPs showed higher microbial colonization inhibition compared to the uncoated surfaces [149].
	MNPs to Inhibit Bacteria
Escherichia coli	Magnetite nanoparticles (MNPs) coated with chitosan and grafted with cephalosporins that show great antibacterial drug properties against E. coli [147]. The magnetic chitosan microspheres were prepared by wet chemical precipitation of Fe^{2+} and Fe^{3+} ions in solution with chitosan and hydroxide. The tested antibiotics were cefepime, ceftriaxone, cefuroxime and cefoperazone. The cephalosporins were well encapsulated into the iron ore chitosan microspheres, holding their properties and being advantageous to traditional delivery of the drug since by exploitation of this magnetite/chitosan approach, the minimal inhibitory concentration was reduced from 2 to 7.8 times for the *E. coli* tested strains. Polyacrylamide doped MNPs (10–20 nm) were reported to show wonderful bactericidal properties, particularly in the elimination of microbes from water [152]. The superoxide and hydroxide radicals made by iron chemical compound appear to be the reason for microorganism harm, since it should lead to oxidative stress, damage of proteins, membranes and deoxyribonucleic acid [152]. Modified MNPs with sodium poly(γ-glutamic acid) (PGA) were found to lower the MIC of the commercial antibiotics linezolid and cefaclor against E. coli, as compared with solutions of the plain antibiotics. For the E. coli ATCC 8739 strain, the PGA-coated MNPs showed a lower MIC worth (<0.5 μg/mL) than linezolid (16 μg/mL) and cefaclor (8 μg/mL), however higher MIC values for the CaPGA-coated MNPs (128 μg/mL). it had been found that the coated PGA-MNPs don't reduce the MIC values for the E. coli O157:H7 strain compared to business antibiotics, however nonetheless they showed a particular inhibition against all tested strains. In another work, MNPs stabilized with thioglycerol showed effective inhibition against E. coli. Such stabilized iron oxide nanoparticles have potential applications within the medical specialty field, chiefly as antimicrobial agents. Dextran and disaccharide coated MNPs (with diameter of 5.8 and 7.3 nm respectively) showed good inhibition against E. coli ATCC 25922 strain, particularly at concentrations between 0.01–0.625 mg/mL [153]. This study conjointly disclosed that dextran is more effective as a coating medium relating to bactericidal activity compared to sucrose. Further the authors have demonstrated that parameters like hydroxyl group compounds, oxygen generated species, reduced size of nanoparticles and usage of specific sugars for the microbic enzymatic conversion play important roles in the germicidal bactericidal and mechanism.
Staphylococcus aureus	Magnetite NPs cross-linked with chitosan made grafted with 2 selected aminoglycoside were conjointly reported [154]. The chitosan-magnetite NPs synthesised by the co-precipitation technique and coated with aminoglycosides antibiotics (kanamycin and neomycin) proved to possess exceptional antibiotic activity against S. aureus strains. The concentration of both antibiotic kanamycin and neomycin utilized in the magnetite NPs was significantly less than the one without NPs that was necessary to stop the growth of *S. aureus*. Approximately the concentration of kanamycin or neomycin used with chitosan-magnetite NPs needed to stop the growth of *S. aureus* was half the amount of these antibiotics required without MNPs. The reason of this exceptional antimicrobial activity was the higher surface area to volume ratio of the MNPs and hence the greater available surface of the antibiotic that was in contact with the microorganisms but also the control release ratio. Recently, Grumezescu *et al*. found that spherical magnetites containing eugenol and prepared by the precipitation technique had excellent anti-adherence activity against S. aureus biofilm formation [155]. To prepare such NPs, 3-hydroxybutyric acid-co-3-hydroxyvaleric acid, polyvinyl alcohol and eugenol were used because the organic phaset to organize the emulsion, that when sonication, water addition, chloroform evaporation and centrifugation gave the magnetites. The MNPs with eugenol were fabricated by matrix assisted pulsed laser evaporation (MAPLE). The size of the MNPs was less than 10 nm and the microbiology resulted demonstrated that such MNPs with eugenol had very good anti-biofilm activity against *S. aureus*. MNPs coated with chitosan-carboxymethylcellulose were found to have improved antibiotic activity once incorporated with best-known antibiotics [156]. Such Fe_3O_4/chitosan-carboxymethylcellulose MNPs were found to boost significantly (2%–10%) the effectivity and drug delivery of penicillins, macrolides, aminoglycosides, rifampicines and quinolones categories against S. aureus. Thus, such MNPs may be used as potential carriers of antibiotics by enhancing their effectiveness without being cytotoxic or influencing the HCT8 eukaryotic cell cycle.

Micro-organism	Description of MNPs
Pseudomonas aeruginosa	Magnetite NPs cross-linked with chitosan and coated with aminoglycoside antibiotics (kanamycinand neomycin) were conjointly accustomed to stop the growth of P. aeruginosa [154]. The incorporation of the NPs with these antibiotics clearly increased the antimicrobial activity of the last because of higher surface area to volume ratio and to the controlled release of the aminoglycosides. MNPs functionalized with eugenol had additionally similar anti-adherence activity against P. aeruginosa strains [155], creating them ideal candidates for developing new anti-bacterial materials. Effective against *P. aeruginosa* were conjointly the MNPs coated with chitosan-carboxymethylcellulose and incorporated with antibiotics [156]. Nanocomposites consisting of biogenic magnetite, silver NPs and chitosan exhibited MIC values between 14 to 50 mg/L against P. aeruginosa, lower the plain drugs [148]. MNPs stabilised with thioglycerol showed additionally a good inhibition against P. aeruginosa with a MIC worth of 0.047 mg/mL [157].
Enterococcus faecalis	Chifiriuc *et al*., reported the primary study making an attempt to analyze the ability of magnetite nanoparticles to enhance the anti-bacterial activity of current antibiotics against planktonic and biofilm-growing E. faecalis. The antibiotics tested with MNPs were Vancocin, penicillin and streptomycin. The results prompt that magnetite nanoparticles are often thought of effective aminoglycoside antibiotics carriers, so as to get improved methods for elimination of E. faecalis biofilms on biomedical devices or human tissues. Also, it had been found that MNPs improved the antimicrobial activity of streptomycin, both against planktonic and completely different E. faecalis cells most likely as a result of the binding impact of MNPs to bacterium and also the resulting membrane disruption. The authors seen that since this is often the primary work with the uset of MNPs for the elimination of E. faecalis from prosthetic devices or human tissues, additional work is required with the use of a variety of antibiotics to better know the inhibition mechanisms [158].

Functionalized Magnetite Nanoparticles to Treat Infections

In recent years, researchers have reported the potential of MNPs used for microbial growth inhibition, biofilm formation of many pathogenic species, such as fungi, yeasts and bacteria and also to treat specific infectious diseases [146]. The various Functionalized Magnetite Nanoparticles to Treat Infections are summarized in table 4.

4. PERSPECTIVES AND FUTURE CHALLENGES

Actually, we are experiencing the early use of MNPs in practice as drug delivery vectors and as tools for hyperthermia/thermal ablation. Magnetic drug delivery is emerging as a promising tool to treat infections and some products are already in the market. The limitation with the use of external magnetic fields will be overcome by use of internal magnets placed within the proximity of the target by minor invasive surgery [56, 158, 159, 160, 161]. The paramount therapeutic potential may be correlated with the applications involving 'intelligent' particles with a magnetic core (in order to direct the particles to the proximity of the target), a recognition layer and a therapeutic load. The challenges like development of appropriate recognition layers are difficult. In conclusion, uses of magnetic NPs by the medical specialty is not limited to drug delivery and hence new applications for magnetic NPs are possibly in magnetic resonance imaging, where contrast agents may be labeled with a recognition moiety, cell sorting/targeting, bioseparation [51].

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