Chitosan-mediated fabrication of metal nanocomposites for enhanced biomedical applications

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Abstract

Hybrid materials based on metals and natural polymers are a promising class of nanocomposites; there is an increasing interest in metal nanoparticles (NPs) due to some fascinating characteristics associated with their nanosizes such as optical, conducting, catalytic, mechanical, sensing and superparamagnetic properties. Despite these favorable properties, the natural tendency of NPs for aggregation, high reactivity due to surface charges, and high rate of toxicity are limiting their applicability in biomedical sector. Chitosan, a naturally available amino polysaccharide biopolymer obtained from the exoskeleton of crustaceans (crabs and shrimp) and cell walls of fungi, displays unique polycationic, porous, chelating, bioadhesive and film-forming properties. The in-built characteristics of chitosan biopolymer can be utilized to alter the negative shades of metal NPs, thereby enhancing the applications in many different areas. The incorporation of chitosan significantly affects the steric stabilization of metal colloids, creates extra functional groups for biomolecule conjugation, renders the NPs suitable for bio-markers, protects metal ions from further oxidation/reduction by means of polymer coordination and has a control over toxicity. Thus by taking advantage of the additional features offered by the combination of chitosan and metal NPs, this report is designed to provide an overview about the metal NPs type, synthesis and applications in bioengineering and biomedical sector. Starting with the influencing properties due to their combination, we further reviewed the literature related to chitosan and metal NPs applicable for medicine with a specific focus on cancer diagnosis and treatment, advanced drug delivery, tissue engineering and surgical aids, to mention some.

Keywords: Chitosan, metal nanoparticles, composites, biopolymers, biomedical applications.

Introduction

Some interdisciplinary approaches between different branches of science and engineering disciplines allows for the creation of hybrid nanomaterials with expanded applications. As an example, the integration of chemistry with micro- and nanofabrication technologies allows for the transformation of complex probes containing biologically active molecules such as enzymes, antibodies, nucleic acids and cells into compact and more efficient [**1-2**]. Also, the application of such composite technology in biomedical sector offers potentials for the manufacturing of intelligent biomaterials in small sizes, integration of labile biological components into high throughput testing instruments, facilitates for the generation of bio-compatible implant devices and theranostics which allows for the simultaneous diagnosis as well as therapy [**3**]. This technology makes use of the principles and techniques from cell and developmental biology, materials science and mechanical engineering (**Fig. 1**) [**3-5**]. Since the construction principles used often originated from synthesis chemistry, and the options available for biomolecule fabrication includes direct assembly, enzymatic assembly and self-assembly. The various precisely controlled physicochemical properties of biological molecules such as charge density, hydrophobicity and self-assembling capabilities are the key driving factors for their use in composite formation. For example, natural polymers are polycationic, DNA is anionic, lipids are amphiphilic and depending on the charge of amino acid sequence, proteins show a range of hydrophobicity [**4-5**].

Among many different classes of biological materials, the material with high primary amine content such as chitosan is selected the most, as this biopolymer confer important functional properties that can be exploited for its bonding with metal nanomaterials (Fe, Cu, Ti, Ag, Au, Pt and Zn). Since the inclusion of these metals with unique features of optical, magnetic, biocompatible, and sensing properties with that of chitosan biopolymer provides novel opportunities for the creation of hybrid systems containing multiple application sites within a single molecular probe. For example, the lab-on-a-chip (for DNA analysis) devices, the microcapsules or vesicles

prepared by ‘‘smart’’ materials for combined chemotherapy and biomagnetic probes which allows early disease diagnosis and treatment are the significant achievements from these technologies [**6-7**]. Further in biomedical sector, the composites formed from chitosan and metal nanoparticles (NPs) addresses the challenges in biopharmaceuticals, implant devices, disease diagnostic tools and tissue engineering appliances. In that way, the biopolymer composite technology allows for an increased understanding of the complex underlying processes and disease mechanisms linked to bio-nanomaterials. In addition, the polymer-metal composites also enables the manipulation of multi-composite materials that results in unique properties of strength, hardness, reduced friction and increased biocompatibility [**6-8**]. Hence, by keeping in view of the advanced features offered by chitosan and metal NPs, this review is aimed to bring some of the insights about the enriched properties offered by these composites with a special focus directed towards biomedical sector. Starting with different kinds of metal NPs applicable for the formation of chitosan composites, we also addressed the present and future perspectives of the nanocomposites linked to biomedical and biotechnological applications.

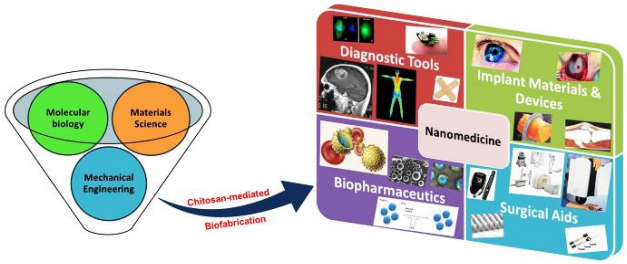

Fig. 1. Schematic representation of the engineering of biopolymer based technologies for the construction of biomedical devices and for the advancement of nanomedicine.

Chitosan

Chitin is the second most abundant polysaccharide next to cellulose and is the most abundant nitrogen rich organic compound found in nature. Chitin is a linear polymer composed of *β*-(1,4)-linked *N*-acetyl glucosamine, a water insoluble polymer. Its deacetylated form called chitosan, the amino polysaccharide copolymer of 1,4 D-glucosamine and N-acetyl glucosamine is soluble in water and at acidic pH conditions. Typically chitin and chitosan are essentially the same polymer but arbitrarily with defined degrees of deacetylation (DD), *i.e.* the term chitosan is applied only if DD>40%. Many different kinds of devices can be fabricated with chitosan due to its promising physico-chemical, biological and mechanical characteristics which have wide range of applications in engineering, medicine, agriculture and pharmaceutical sectors [**9-10**]. Also, a range of chemistries essential to biofabrication can be employed by taking advantage of chitosan’s reactive amines which includes graft polymerization, functionalization, cross-link formation to confer elasticity and also reactions under high pH conditions [**3**]. With the integration of chitosan towards biofabrication technology, the functions of chitosan can be broadened by its grouping with others possessing significant optical, magnetic, biocompatible and sensing characteristics [**11-15**].

Metal nanoparticles (NPs)

In recent years, metal NPs have attracted intense research interest due to their concerned properties and advancing applications in various sectors of biomedicine, optics, sensing and catalysis [**16**]. The commonly applied metal NPs for various applications include silver (Ag), iron (Fe), gold (Au), titanium (Ti), zinc (Zn), copper (Cu) and platinum (Pt). In biomedicine, combining the fundamental properties of metal NPs with that of the unique properties of biomaterials turns out to be an efficient approach for the production of novel antibacterial materials with improved functional properties, materials with active biological sensing, “smart” delivery systems with dynamic drug targeting and controlled release, and engineered material implants. Within metals, another special category is magnetic NPs that has been applied to biomedical fields, including enhancing magnetic resonance imaging (MRI), magnetically targeted drug delivery, magnetic intercellular hyperthermia *in vivo*, cell sorting, and tissue engineering.

Chitosan and metal NPs fabrication

Assembly of nano-scale objects into hybrid composites is an area of tremendous interest. Most of these hybrid composites are NPs connected by organic materials such as natural polymers (**Fig. 2**). It is recognized that chitosan due to its well-known characteristics of sorption, possess superior chelating properties compared to any other natural polymers and it chelates metals by five to six time better than chitin. The amino group and in some instances the hydroxyl group on chitosan skeleton acts as an electron donor, participates in electrostatic interactions and are responsible for the stabilization of chitosan-metal complexes [**17-18**].

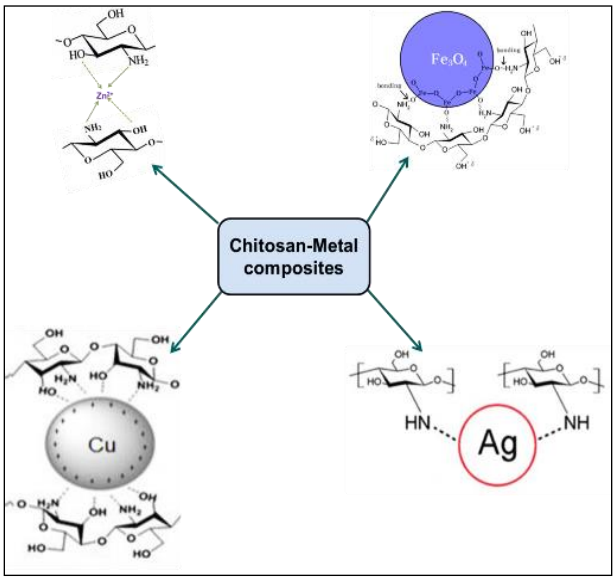

Fig. 2. Schematic representation of formation of nanospheres, nanocapsules or hydrogels of chitosan and metal nanocomposites which makes the use of reactive groups of chitosan polymer.

There are many review articles available on chitosan but their focus has been primarily limited to the synthesis, properties or specific applications of chitosan and its derivatives [**9-10, 19-21**]. However in this, we present the opportunities to broaden the applications of chitosan by its fabrication with metal NPs with a particular interest towards bioengineering and biomedical sectors. An example of this is that chitosan and the metal NPs such as silver (Ag), copper (Cu), nickel (Ni), and zinc (Zn) exhibits some level of disinfection and bactericidal properties individually, however the fabrication of these metal ions into a single nanocomposite resulted in unusual and interesting biochemical properties which are particularly suitable for bioengineering and medicine **[22]**.

A number of studies focus on developing physical and chemical strategies for synthesis of metal NPs with controllable size, shape, morphology and magnetic properties. These are important factors to consider in biomedical applications of NPs. Extensive reviews address these issues and reveal the high versatility of using metal NPs for biofabrication with chitosan. In the following sections the improved properties resulting from chitosan mediated biofabrication of metal nanocomposites are reviewed.

Increased mechanical strength

The incorporation of inorganic metal NPs into chitosan's polymeric structure by a simple cross-linking reaction efficiently increases the mechanical strength in case of 3D scaffolds, microcapsules, vesicles and other hydrogel networks. This enhancement towards the mechanical properties of these composites is due to the fact that the high surface area of inorganic NPs improves the interaction and adhesion between polymer and fillers. This is in accordance with the theory of particle reinforcement where the matrix bears the load in the particle-reinforced composite materials. These inorganic NPs get uniformly dispersed into chitosan's matrix and block the movement of macromolecule chain of the polymer matrix and thus supports for the compressive mechanical strength. This particle reinforcement is a dependent of volume fraction, uniformity and size of the NPs present in chitosan's matrix and ideal reinforcement effects were observed when the particle sizes are in the range of 0.01 – 0.1 μm **[23]**. Similarly, during the tissue regeneration process using engineered chitosan constructs, the 3D scaffolds of metal NPs are responsible for increased mechanical strength by augmenting the extracellular matrix (ECM) produced by the seeded cells. However, in the case of chitosan containing magnetic NPs, the same mechanical strength is achieved through the generation of magnetoelastic functionality **[11]**.

Protection from self-aggregation

At nanometric level, metal NPs possess higher tendencies of aggregation and forms agglomerated structures, and in this way they may lose the peculiar properties associated with the nano-scale such as the optical, magnetic, fluorescence and cellular binding. Also, in some instances due to very high surface charge to volume ratios (Fe, Cu, Mn, Ag and Au), protection from self-aggregation can be difficult in solution flask and therefore, it is advisable to stabilize metal NPs while they are forming. Furthermore, to find applications of these composites in the biomedical field, both the stabilizing ligand and the reducing agent must not represent any biological hazard. Chitosan best suit to those requirements, as it can coordinate metal ions by chelation before the actual reduction and forms a polymer-metal ion complex with enhanced zeta potential values and complete control over the rate of reduction process. The formed complex can then be reduced by chitosan itself due to its mild reducing capacity without need of any additional reducing agent, resulting in the production of NPs of different shapes, sizes and a narrower particle size distribution [**24-25]**. For example, because of the strong reactivity of free electrons present on the surfaces of Cu, Fe, Au or Ag, the NPs formed with these metals are sensitive to pH, temperature, and solvents and hence, they easily tend to aggregate when used in such media. Long chain chitosan with its large number of cationic amine groups however acts as a capping agent and maintains the size uniformity after their formation in solution from steric hindrance through the increase of zeta potential of the colloidal system. The greater the zeta potential, greater will be the electrostatic repulsions between the colloidal NPs, more likely that the suspension can be stable and thus overcome the natural tendency of aggregation [**26-27**].

Improved stability

Chitosan coating improves the stability of colloidal solutions from undergoing precipitation and also helps for enhanced magnetization values of magnetic NPs in terms of preventing from unwanted oxidation by means of either chemical or biological. For example, chitosan coating onto magnetite (Fe_3O_4) allows for protection from further oxidation to hematite, as hematite has lower magnetization values compared to magnetite. Starch, however has a similar functional property to that of chitosan, it is less preferred due its high oxygen content and greater possibility that starch itself can mediate oxidation of magnetite core [**28**]. The improved colloidal stability of chitosan-modified NPs is due to the creation of positive charges onto the surfaces of Fe_3O_4 NPs, which allows for the generation of Coulomb's repulsive forces and maintains stable suspensions. In addition, these surface positive charged NPs possess good hemocompatibility *in vivo*, easily binds to the negatively charged cell membrane and also the amino group of chitosan enhances the coupling time of NPs with tissues [**29**]. As an example, the coating of sulfated chitosan onto titanium helped to form ECM-like layered structure by acting as heparin and also improved the anticoagulant potency of Ti cardiovascular implants [**30**].

Structural deformation

In general, a way that the materials are of responsive to external stimuli (smart drug delivery systems and sensory systems) due to the undergoing changes in their chemical or structural characteristics. It was established that

chitosan biomaterials can act as stimuli sensitive in according to the changes in temperature, pH, electric field, light or certain chemicals, and magnetic stimuli (for magnetic materials). Chitosan in response to the stimuli, the hydrogen bonding present between the interior molecules or chitosan chains are weakened and this improves the hydrophilic or decomposition behavior by the release of some controlled amino and hydroxyl groups in its chains, with a significant reduction of viscosity [**11, 31**]. For chitosan-mediated drug release studies, the deformation of chitosan's chemical structure in response to external stimuli is very important, as this offers the merits of modulated drug release rate and shortened tolerance time accompanying a constant drug release effects. For example, the organic/inorganic magnetic shell encapsulated drug, the aspirin-loaded chitosan-Fe_3O_4 cross-linked system exhibited interesting structural deformation characteristics on application of external magnetic field. In this, the deformation of chitosan's structure allowed for an active modulation of the release rate of aspirin [**11**]. Similarly, chitosan's temperature responsive property helped to modulate the size, shape, and the optical properties of Au nanostructures with a simple cooling treatment [**31**].

Enhanced magnetizations

For the surface modification of hyperthermic thermoseeds, chitosan is more preferred than any other natural polymers of similar kind due to its involvement towards the enhancement of saturation magnetization values while maintaining limited or no toxic responses. For example in a study, the magnetization and heating properties of Fe_3O_4 coated two different natural polymers, chitosan and starch were compared towards enhancing the magnetic and heat releasing effects through specific power loss (SPL) of Fe_3O_4 NPs. The chitosan-modified Fe_3O_4 suspension was shown a higher value of saturation magnetization (25.6 emu/g) and temperature change of 23°C than the corresponding starch-modified ones (16.4 emu/g and 12°C) **[28]**. The decrease in the saturation magnetization of starch- coated Fe_3O_4 NPs was attributed to an increased amount of polymer incorporation. However, the same functions were achieved with minimal chitosan coating and also its low molecular weight (compared to starch) and viscosity supported for a high saturation magnetization values, thereby more amount of heat release through efficient SPL **[32-33]**.

Controlled solubility

The main limitations of chitosan are its pH dependent solubility, *i.e.* soluble only at low pH conditions and immiscibility with other oppositely charged polyelectrolytes [**24**]. Since, chitosan's parameters are largely influenced by its amino and hydroxyl groups, the insoluble effects of chitosan are ascribed to its amine group deprotonation at high pH (> 6.5). Chitosan, on chelation with metal ions of Cu^{2+}, Zn^{2+} and Ag^{+} overcomes the limitations of high pH insolubility by means of increasing its overall zeta potential [**27**]. This metal chelation changes chitosan's natural structure and the surface electric charge by forming a secondary structure with altered size, surface charge, and morphology. The soluble properties of thus obtained chitosan-metal complexes are controlled by the cumulative charge, nature of counter ions, geometrical configuration, and oxidation state of the metal ion [**34**]. Also, chitosan when used alone is inherently water sensitive and exhibit relatively low stiffness and strength in moist environmental conditions. However, chitosan-based inorganic nanocomposite films obtained by the fabrication of chitosan with nano-silver, silver zeolite, and silicates are proved to decrease the water vapor permeability by 25-30%. This decreased water vapor permeability is due to the formation of orderly dispersed crystalline structure with large aspect ratios in the chitosan matrix, which forces the water vapor traveling through the film to follow a tortuous path, thereby increasing the effective path length for diffusion [**35**].

Increased biocompatibility and reduced cytotoxicity

It was observed that chitosan coating enhances the biocompatibility and reduces the cytotoxicity effects originating from the surface of metal NPs without compromising the aqueous solubility. The metal NPs due to their small size, high surface area and mechanical strength, even though exhibit properties that can be potential for biomedical field, the high toxicity levels are concerning their use for a majority of clinical applications. For example in tissue engineering, materials such as collagen, gelatin, starch, albumin, fibrin, poly-glycolic acid, and poly-lactic acid though able to construct 3D scaffolds and maintains biocompatibility, they tend to degrade easily by becoming mechanically weaker *in vivo*. The use of engineered constructs made-up of chitosan-incorporated metal NPs (Fe_3O_4, TiO_2, ZnO and Cu) however increases the mechanical strength by augmenting the ECM produced by the seeded cells during the process of tissue regeneration. The randomly located N-acetyl-glucosamine units of chitosan are functionally similar to that of glycosaminoglycans, a major component of ECM [**23, 34, 36-37**]. Also, the increased zeta potential values of the engineered constructs facilitates for a higher affinity with those of negatively charged biological membranes and promote many other associated functions, such as proliferation, differentiation, migration, metabolism and adhesion to ECM.

Upregulation of cells

Studies shows that surface modification of metal NPs by chitosan accelerates cell invasion, binding of fibroblast growth factor (FGF), activates macrophages, helps for antitumor immunity development, facilitates tissue regeneration capacity, and enhances de novo synthesis of ECM [**38-39**]. In general, the uptake of NPs by cells is a two-step process, *i.e.* a binding step onto the cell membrane followed by an internalization step. Since, the adsorption of NPs onto the cell membrane in a binding step are majorly governed by the electrostatic interactions; an increased zeta potential value of chitosan-coated metal NPs leads to stronger electrostatic interactions of these NPs with that of the cell membrane and thus occurrence of higher

internalization. For example, when compared with pure chitosan or naked Cu(II) NPs, the Cu(II)-loaded chitosan NPs were reported to show greater cytotoxic effects and antitumor activity towards BEL7402, BGC823, and Colo320 cancer cells. The operated chelation theory and higher surface charge density (by means of zeta potential) of Cu-loaded chitosan NPs, led to a superior affinity of the positively charged amino groups of chitosan with those of negatively charged tumor cell membranes and greater amounts of internalization. Using the same principle of enhancing the electrostatic interactions by means of chitosan coating, polar drugs also transported across epithelial surfaces. These chitosan-loaded drugs stay in circulation for longer periods, resulting in a better release and availability of those drugs at the targeted sites [**39**].

Enhanced photothermal effects

Nanostructures obtained by the fabrication of chitosan along with optically active Au, Ag, Mn, Zn or Ti exhibit unique absorption properties due to a very broad in-plane SPR band that extends well into the NIR region, makes them particularly attractive for optical applications. The NIR absorption of such metal nanostructures are promising for biomedical applications of inducing hyperthermia in tumors by lasers, laser-activated drug release, active drug targeting using lasers and heat absorption in special equipment [**25, 31, 40**-**41**]. With the remarkable features of metal NPs such as their chemical inertia, stability under physiological conditions, chemical versatility and high laser irradiance, the fabricated hybrid devices replace organic dyes in biomedicine, allows for the integration of nanotechnology and biotechnology leading to major advances in medical diagnostics, and molecular and cell biology [**41**]. Using the principle of photo activation, the nanocomposite of chitosan-Au nanorods were readily bonded to biological tissues and generated substantial functional photothermal effects in connective tissues due to the triggering of embedded Au with a NIR laser device. This laser-activatable adhesion via photothermal conversion formed new H-bonding with tissues due to chitosan and thus makes it to be suitable for wound dressing and tissue repair. However, chitosan also maintains Au nanorods to be in thermal equilibrium with the dispersion medium, which minimizes unnecessary overheating of Au and prevents detrimental side-effects such as fragmentation of Au nanorods and irreversible target damage and therefore, serves as ideal candidates for light-induced hyperthermia and localized drug release **[25]**.

Catalytic activity

It has been reported that chitosan is more than a stabilizing agent and that the metal precursors (such as $AgNO_3$ or $HAuCl_4$) can be reduced to zero/monovalent ions (Ag or Au) by chitosan itself. Chitosan as catalyst is thus widely used in obtaining various materials, including Au^0, Ag^0, Pd^0, Co^{2+}, Cu^{2+}, Os(VIII), and bimetal of Pd^0–Ni^0 [**42**]. The reducing ability of chitosan is due to its polysaccharide chemical structure, which can hydrolyze in an aqueous acid to give D-glucosamine (glucose derivative) and also the $–CH_2–OH$ groups of chitosan to act as reducing groups during the formation of metals NPs. Chitosan offers a much slower reduction and thus greater degree of control over the production, along with highly stable, reproducible and monodisperse nanostructures [**40**].

Another advantage of using chitosan is that while simultaneously acting as catalyst for reduction, it also serves as a surfactant and protects the formed NPs from self-aggregating.

Since the metals like Au, Ag are electropositive and can be reduced easily by weak reducing agent like chitosan and the reductive sites in chitosan molecule are more easily accessible to the ions undergoing reduction. Also, the use of chitosan as catalyst creates a mild, biofriendly medium that allows for the product formation in various shapes of films, gels, and beads by the direct interaction of the polar groups (such as –OH and/or $–NH_2$) of chitosan with particle surfaces. The reduction temperature, concentration, molecular weight and ionic gelation (by TPP) of chitosan strongly influence the size, shape and morphology of the forming metal nanostructures [**43**-**44**].

Antibacterial and antifungal efficiency

Chitosan-mediated metal NPs fabrication significantly enhances the antibacterial and antifungal efficiencies by means of increased electrostatic interactions and zeta potential values, compared to those of chitosan NPs alone or metal ions of Ag^+, Mn^{2+}, Fe^{2+}, Cu^{2+}, Ti^{2+} and Zn^{2+}. Since, metal ions are referred to as "super acid" as they strongly accept electrons than H^+ and the formed chitosan-metal ion chelation increases the positive charge density of chitosan. This increased charge density (zeta potential) leads to an enriched adsorption of polycationic amines onto the negatively charged bacterial cell surface causing enhanced growth inhibition [**45**].

The physiological effects and delocalizing mechanism displayed as in case of up regulation of cells are operating here also and the interactions by means of zeta potential are directly proportional to the increased amount of antibacterial activity.

For example in a study by Du *et al*, on loading of chitosan NPs possessing a zeta potential value of +51.37 mV with that of positively charged metal ions of Ag^+, Cu^{2+}, Zn^{2+}, Mn^{2+} and Fe^{2+} individually, increased the zeta potentials to +92.05 mV, +88.69 mV, +86.65 and +75.74 mV, and +71.42 mV, respectively. The antibacterial activities against various gram-positive and gram-negative bacteria are following the same order to that of zeta potential values, *i.e.* Ag^+ loaded chitosan NPs being the highly efficient and Fe^{2+} loaded chitosan being the lowest (but still higher than that of chitosan NPs).

These enhanced effects are attributed to the stability of these metal ion-loaded chitosan NPs in aqueous nanosuspensions. It is proved that a minimum zeta potential of ±30 mV is required to generate a physically stable nanosuspension solely stabilized by electrostatic repulsions. Also, to generate ideal inhibitory effects, metal ions of high charge intensity and the molar ratio of chitosan to metal above 1:1 are required [**45**-**46**].

Biomedical applications of chitosan-mediated metal nanocomposites fabrication

Hybrid materials or composites fabricated by using chitosan and metal NPs have been investigated by various research groups. The main applications are summarized in **Fig. 3**. The following section describes the several combinations and biomedical uses of various metal NPs incorporated chitosan matrices.

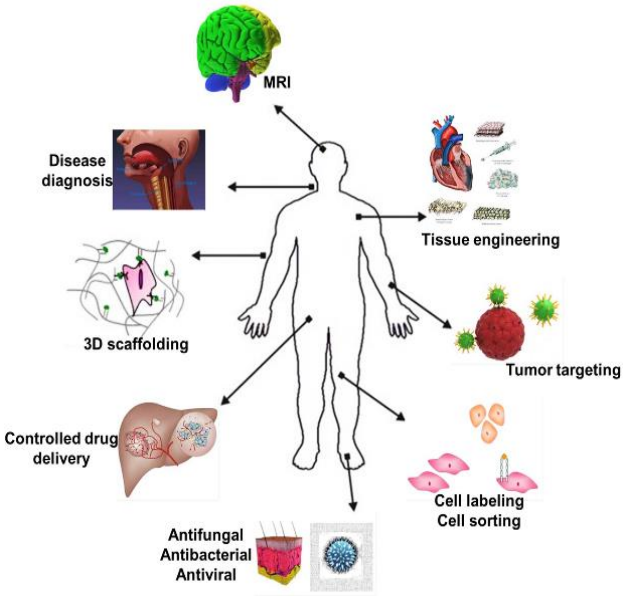

Fig. 3. The biomedical applications of chitosan-metal nanocomposites.

Chitosan-silver (Ag)

The antimicrobial properties of silver has been recognized for several centuries ago (in certain Egyptian and Indian cultures), however the increasing threat of antibiotic resistance caused by the abuse of antibiotics increased interest in this element [**47**]. NPs of Ag are usually obtained by reducing silver nitrate either by $NaBH_4$, EDTA or γ-irradiation.

Other methods applied are the photochemical reduction, reduction by microwave irradiation, micelle, reverse micelle, microemulsion method, lamellar liquid crystal, aero-sol spraying and laser irradiation. In medicine, silver is well known for its distinct antibacterial activity and the general mechanism for this activity is the direct attachment of metallic silver (Ag^0) or ionic silver (Ag^+) to the cell wall followed by the disturbance of membrane permeability and cellular respiration, flow of matter is disrupted and energy indispensable for bacterial life due to its high electric conductivity is unbalanced. Also, Ag NPs can penetrate the bacteria and damage DNA and other proteins by its interaction with some of their phosphorus, sulfur containing groups [**23, 48**]. No evidence exists thus far that bacteria have developed efficient defense mechanism against the diversified action of Ag or its ions and practically no strains resistant to silver bactericidal action were found.

In order to prevent bacterial infections and to improve the quality of cosmetics, products (skincare) containing Ag NPs are continue to emerge ranging from ointment to bandages [**3, 49**].

The intercalation of Ag NPs into chitosan's polymeric structure led to many interesting results which indicate that the combination can improve the antimicrobial efficacy. Wei *et al* using chitosan-based Ag nanomaterials (of both NPs and films) observed that the composite possess high antibacterial activity against gram-positive and gram-negative bacteria. However, in comparison with pure chitosan films or active Ag salts, the Ag-impregnated chitosan films showed fast and long-lasting antibacterial effect against *Escherichia coli* (*E. coli*). These results demonstrate that chitosan-mediated Ag fabrication results in an unusual dual mechanism of action due to bactericidal effect of Ag and cationic effects from chitosan [**23**]. In another study, chitosan-tungstosilicate acid-Ag nanocomplex developed by the fabrication of tungstosilicate acid-Ag NPs (45 nm) with chitosan in the form of thin films were effective for the electro-catalytic reduction of dioxygen species. The obtained chitosan containing organic-Ag nanocomposites can find applications as implants for scavenging the free radicals formed during biochemical processes [**50**]. Other studies conducted by the fabrication of chitosan-Ag nanocomposite films promoted wound healing, infection control, and had effective action against gram-positive and gram-negative bacteria [**24, 51**].

Chitosan-iron (Fe)

Microcapsules or vesicles fabricated with chitosan and iron oxide NPs (Fe_3O_4 or γ-Fe_2O_3) of superparamagnetic behaviour (**Fig. 4**) finds unique applications as "smart" magnetic drug delivery systems, contact-facilitated drug delivery systems in clinical medicine and in other medical perspectives as magnetic contrast enhancing agents (for molecular imaging and to apply clinical hyperthermia) [**11, 52-53**].

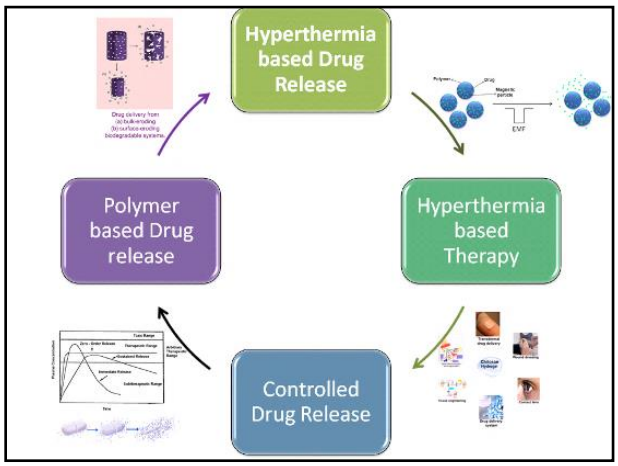

Fig. 4. Schematic representation of the unique advantages of applying chitosan-iron oxide based biodegradable polymer composites for disease diagnostics and controlled drug delivery.

The iron or iron oxide-based NPs due to their small size, high surface charge and magnetic susceptibility, possess a high rate of aggregation and agglomeration. Due to their tendency to agglomerate, the magnetic NPs lose some unusual properties associated with the nanoscale and this can be controlled by the use of suitable

ligands as surfactants. The organic surfactants such as oleic acid, oleylamine, mercaptoundecanoic acid or the polymer surfactants like polyphosphate, polyacrylate, poly(vinyl-sulfate), poly(ethylene-imine), poly(allyl-amine) can however protect these NPs from agglomeration, but the aqueous solubility and high toxicity can be a concern for majority of biomedical applications. Use of chitosan as surfactant provides sufficient water solubility, excellent biocompatibility and biodegradability, while simultaneously maintaining the superparamagnetic behaviour and crystalline nature of iron-based composites. In addition to providing those characteristics, chitosan also acts as a stable matrix by offering extra conjugating groups (–OH, $–NH_2$) that can be used for other biomolecule assembly (**Fig. 5**) [**24, 28, 52, 54**].

"Smart" magnetic drug drug delivery systems consists of stimuli responsive properties and are composed of magnetic material-based composites encapsulated or adsorbed onto a polymer. On application of external stimuli including changes in temperature, pH values, electric field, or certain chemicals, the "smart" delivery systems are capable of targeting an organ/tissue of specific interest and release its encapsulated drug or other therapeutic agents in accordance with the stimuli [**11**]. Contact-facilitated delivery system functions on the principle of localized manufacture and signal-guided delivery of drug molecules to targeted cell surfaces. By taking advantage of the standard amine group chemistry of chitosan to attach antibodies, Bentley *et al* constructed magnetic nanofactories consisting of enzymes with activatable 'pro-tags' conjugated to functionalized Fe_3O_4 NPs. In this, chitosan served as biodegradable polymer scaffold with enzyme binding surface, in addition to providing cell capture ability using its simple and reversible pH dependent property [**55**]. Similarly, in a study using Fe_3O_4-chitosan core-shell microspheres the release of aspirin was facilitated by external stimuli of AC magnetic field. Furthermore, the magnetic field induced drug release and heat conducting characteristics with these "smart" magnetic drug delivery systems could be controlled externally by varying the frequency, amplitude and time profile of the alternating current applied to the electro-magnet. Such type of delivery systems with magnetoelastic functionality can effectively interact with external magnetic field positioned at a specified area and therefore, can function as dual application probe for a simultaneous detection of cancer cells by molecular imaging and also for the treatment of diagnosed cancer cells by targeted drug delivery or heating effect [**11, 56**]. Also, chitosan modified Fe_3O_4 NPs possess enhanced magnetic susceptibility and the temperature change under alternating magnetic field was observed to be 23°C, which is very high in terms of applying for clinical hyperthermia of tumors. Since the surface modification using chitosan increased the biocompatibility and magnetic susceptibility of Fe_3O_4, chitosan-Fe_3O_4 composite can be promising to induce magnetic intracellular hyperthermia [**28**]. In a similar study, carboplatin-Fe-carbon-loaded chitosan NPs exhibited good magnetic targeting and heat releasing properties based on magnetic fluid hyperthermia. Due to the stability offered by chitosan matrix in intestinal fluids, these NPs were actively delivered to liver cancer tissue in rats by static magnetic field, and further responsible for the local heating of tumor cells on application of external magnetic field and thus facilitated the apoptosis of tumor cells [**53**].

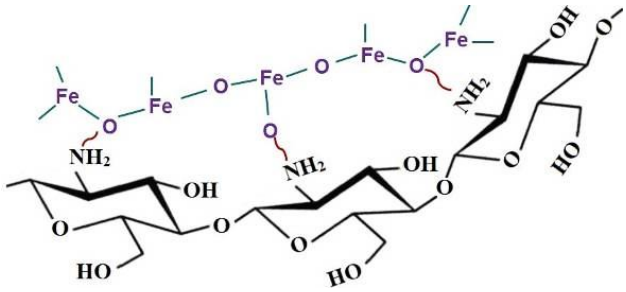

Fig. 5. Schematic representation of the formation of Fe bonding in magnetite (Fe_3O_4 or γ–Fe_2O_3) with that of $–NH_2$ groups of chitosan biopolymer.

Commercially available MRI contrasting agents such as gadolinium-based agents (Gd-DTPA, Gd-DTPA-BMA) and Feridex (Fe_3O_4) use dextran as biocompatible coating material. These Gd-based materials are suitable as T_1 contrast agents, while iron oxide-based materials are for T_2 contrasting ability. Despite the uses as T_1 contrasting agents, the aqueous solubility of dextran-coated materials and very high rate of Gd toxicity is a major concern. To overcome these limitations, results of the study conducted with chitosan-coated Fe_3O_4 NPs (67 nm) indicate a very high T_2 relaxivity, as compared with the commercial contrast agents (Gd-based). A stable colloidal aqueous suspension was achieved with chitosan through the formation of slightly positive charged Fe_3O_4 NPs by their bonding with $–NH_2$ groups of chitosan, leaving the –OH groups as free. Also, a 32% signal loss in the MRI of rabbit liver was observed after injecting the aqueous solution of chitosan-coated Fe_3O_4 NPs into the rabbit, which shows the potential of chitosan-coated materials as T_2 MRI contrast agents [**29**]. In a similar study, the superparamagnetic Fe_3O_4 and γ-Fe_2O_3 encapsulated chitosan nanospheres were proven to be good candidates for stem cell labelling applications. The cationic charges from the surface of chitosan facilitated for the adhesion of negatively charged phospholipid bilayer of Kusa O cells. Also, chitosan coating on these NPs markedly enhanced the cellular uptake with no effect on cell morphology or viability under magnetization conditions [**36**]. Similarly, magnetic force-based tissue engineering scaffolds to accelerate the repair or replacement of damaged tissues were developed by the fabrication of chitosan with magnetic NPs. Thus obtained three-dimensional (3D) biodegradable scaffolds due to the presence of magnetic force, efficiently enhanced the cell invasion, cell seeding into depth of the scaffold, increased the cell-cell interactions and helped to shorten the period of cell proliferation [**37**]. Chitosan-mediated iron oxide nanocomposites in addition to their applications as contrasting agents (MRI and hyperthermia), drug delivery systems and novel therapeutic scaffolds, also possess potential to serve as amperometric sensors. For example,

ferritin antibody immobilized Fe_3O_4-chitosan nanocomposite system, perceived to act as an immunosensor for the clinical determination of ferritin in human serum samples [**57**]. From these observations, it can be inferred that chitosan provides a stable matrix for enzyme or biomolecule assembly, protects drug-loaded microcapsules from dissociation at various pH and other external forces, provides mechanical strength to the scaffolds, helps to maintain monodispersity of NPs and contributes to enhanced magnetization values, in addition to offering biocompability for iron-based composites.

Chitosan-gold (Au)

Motivated by the unique properties of conductivity, fluorescence, surface plasmon resonance (SPR) and redox behavior of gold (Au), nanocomposites fabricated by the combination of chitosan and Au finds applications as: (1) scaffold materials for chemical and biological sensors [**26, 58-59**], (2) laser-activatable bio-adhesives for tissue repair and wound dressing [**25**], and (3) cancer diagnosis and therapeutic probes [**32, 60**]. Due to the peculiar tendency of chitosan to form films of high mechanical strength and biocompatibility, it is used for constructing ampherometric biosensors to provide a stabilizing environment for the immobilization of enzymes. In addition to chitosan's ability as capping agent, it also serves as an active catalyst during the reduction of tetrachloroauric acid ($HAuCl_4$) in order to form Au NPs, gels or films. The obtained composites exhibits surface-induced fluorescence and surface-enhanced raman scattering (SERS) [**16, 31, 40**]. This *in situ* synthesis approach further opened up new opportunities for the construction of cell-based superoxide anion ($O_2^{\bullet-}$) biosensor by making use of Au NPs-embedded chitosan composites [**59**]. In another study, using the bio-inspired gel formed from chitosan-Au composite, a sensitive electrical impedance cell sensor was constructed and applied for electrochemical monitoring of cell adhesion, proliferation and apoptosis on electrode surfaces. In that, the K562 leukemia cells immobilized onto glassy carbon electrodes showed a correlation between electron-transfer resistance of redox probe at the electrode and the concentration of K562 cells. The system demonstrated an irreversible voltammetric response and enhanced the electron-transfer resistance with a detection limit of 8.71×10^2 cells/mL [**58**]. Similarly, by taking advantage of the low reducing ability of chitosan to form Au NPs, an analytical biosensor was developed and is promising for estimating quantitatively the dynamic changes of $O_2^{\bullet-}$ in biological systems. The probe is capable of exhibiting selectivity, fast amperometric response (<5 s), wide linear range ($5.6\text{-}2.7\times10^3$ nM), low detection limit (1.7 nM), and proved to be excellent for real-time measurement of $O_2^{\bullet-}$ in a variety of *in vitro* and *in vivo* models [**26**].

By using the property of chemical inertia of Au to mediate substantial photothermal effects in connective tissues, Matteini *et al* fabricated nanocomposite bio-adhesive film using chitosan and Au nanorods. This laser-activatable adhesive film can readily bond to biological tissues by the activation of embedded composites with a near-infrared (NIR) laser device. Chitosan in this plays a critical role during post-operative healing process, which includes stimulated reorganization of tissue architecture and prevention from microbial infections along with the possibility to host and release drugs. In addition, chitosan also provides stability against aggregation and controlling over local density of Au nanorods. This laser-activatable bio-adhesion system *via* photothermal conversion of light into heat, further responsible for the formation of new bonds by the cleavage of existing bonds of chitosan onto tissues, and thus make it to be an ideal candidate for advanced tissue repair, wound dressing and localized drug release applications [**25**].

It has been observed that hybrid materials formed from a combination of Au and superparamagnetic Fe_3O_4 exhibit enhanced properties of optical, magnetic and heat releasing properties. They act as contrast enhancing agents for bioimaging of cancer cells based on their optical and magnetic properties and also treatment by direct hyperthermia or drug delivery subjected to the cancer diagnosed cells [**32**]. Similarly, chitosan capped magnetic core-metallic shell architectures prepared from superparamagnetic Fe_3O_4 core-Au shell exhibited superior optical and magnetic characteristics. The presence of chitosan on these composites allows for the fine tuning of magnetic properties of the core by generating monodispersed NPs, controls the optical properties of Au shell, and protects the composite from unwanted toxicity such as cell-induced oxidation or reduction. These hybrid materials, in addition to their uses as tumor imaging, targeted drug releasing and treatment probes, also have potential benefits in the emerging biotechnological and biomedical fields for magnetic bio separation [**32, 60**].

Chitosan-titanium (Ti)

Nanocomposites fabricated in combination with chitosan and titanium dioxide (TiO_2) has significant effects towards the photocatalytic activity, mechanical strength, chemical resistance under UV, biocompatibility, solubility in acidic/alkaline media and antimicrobial behavior. These components find applications in current biomedical sector as scaffolding materials for tissue engineering, functional antibacterial agents, in clinical dermatology as photobacteriocidal and artificial skin substituents, and ingredient of cosmetics products [**23, 30, 38, 61-65**]. For example, skin cancer mainly caused by UV radiation and among two different UV blockers, organic and inorganic, the inorganic blockers such as TiO_2 and ZnO efficiently scatter both UV-A and UV-B radiation.

The observation of significant enhancement in the antibacterial and antiviral properties of TiO_2 fabricated chitosan is due to the photocatalytic activity of TiO_2, *i.e.* upon UV light exposure, ambient oxygen and water gets decomposed onto the surface of TiO_2 to generate high reactive free radical species ($O_2^{\bullet-}$ and $^{\bullet}OH$). These radicals interact with phospholipids and other sulfur containing groups of bacteria or viruses, causing a disruption of cell membrane, which finally leads to reduction of viability [**38, 65**].

Similarly, the composites of chitosan-TiO_2 in presence of gelatin enhanced the antibacterial activity against

E. coli, *S. aureus* and accelerated the wound healing characteristics when applied to Sprauge-Dawley rats. These composites exhibited the features of low density, fine thickness, high mechanical strength, sufficient porosity, moderate water absorptivity, enhanced biodegradability, promising bactericidal (sterilization) effect, and steady immunological response, which is suitable to use this to be as artificial skin substituent [**38**]. Also, the hybrid composites of TiO_2, polyvinyl alcohol and chitosan, displayed enhanced antibacterial activities of 99 and 98 % against *S. aureus* and *E. coli*, respectively [**61**].

Zhavo *et al*. macroporous TiO_2/chitosan composite scaffolds obtained by freeze-drying technique enhanced the mechanical stability of chitosan due to TiO_2 and increased the cell wall attachment of HL-7702 hepatic cells by the biomaterial chitosan. In this, chitosan's porous architecture provided the optimal spatial conditions for seeding high-density hepatocyte cell culture and also helped to maintain long term metabolic functions of cells by means of albumin secretion. By considering the compressive strength of scaffold and resulting hepatocyte cell attachment, proliferation and functional expression, the TiO_2/chitosan composite can be promising as scaffolds for liver tissue engineering applications [**62**]. In a similar study with nanocomposites formed from multilayer coating of TiO_2 and sulfated chitosan by layer-by-layer assembly showed high anticoagulation potency and hemocompatibility as indicated by means of platelet adhesion and their activation. In view of the high mechanical strength due to TiO_2 and anticoagulant potency, mimicking the functions of ECM by sulfated chitosan, this composite can be applicable to cardiovascular implants as scaffolds of heart-valves and vascular stents [**30**].

Chitosan-zinc (Zn)

It is well known that both chitosan and zinc oxide (ZnO) exhibit the properties of disinfection and bactericidal, hence the mixed composites of these can be appropriate to the medical and food industry. As similar to TiO_2, ZnO also exhibits active photocatalytic properties, band-gaps, increased mechanical strength, chemical stability and antibacterial action and therefore, can serve as a dynamic ingredient in sunscreen lotions, cosmetics and paints. The mechanism of inhibitory action of ZnO towards broad spectrum of bacteria and fungi is quiet comparable to that of TiO_2, *i.e.* photocatalytic activation of ZnO forms hydrogen peroxide (H_2O_2), followed by the generation of reactive free radicals of $O_2^{\bullet-}$ and $^{\bullet}OH$, thereby penetration and disorganization of bacterial cell membrane finally leads to loss of viability [**63, 66-67**]. Chitosan's ability to form chelation with Zn is due to its amine and hydroxyl groups, and the formed complexes showed a change of molecular structure in according to the changes in molar ratios of zinc and chitosan (**Fig. 6**). Also, an improvement in the antimicrobial activity of these complexes was observed by means of increased ratio of chelation, which in turn due to increased content of zinc ions. As chitosan chelates to Zn ions, the positive charge on its amino group strengthened and as a result, the complex easily interacts with anionic components of cell surface, and thereby exhibiting higher inhibitory activities. When tested for antibacterial efficiency, chitosan-Zn complexes showed a 2-8 times and 4-16 times higher than those of chitosan and zinc sulfate, respectively [**64**].

Chitosan-Zn complexes also attracted greater potentials for their use as skin protector due to its UV blocking properties and also as medicament or nutriment, as Zn ions possess nutritional features which can be important to human health. For example, in a study conducted using chitosan-ZnO NPs of 60 nm size when deposited onto cotton fabric, enhanced the UV absorbing capacity of treated fabric and also showed significant improvement in the antibacterial behavior **[63**]. In another study of similar type, the existence of ultra-small ZnO NPs (< 2.1 nm) in chitosan-ZnO composite coated textiles led to a significant decrease towards water solubility, in addition to an increase in the antibacterial activity. These chitosan-ZnO composites therefore are advantageous for various applications where the stability of coating is of utmost importance [**68**].

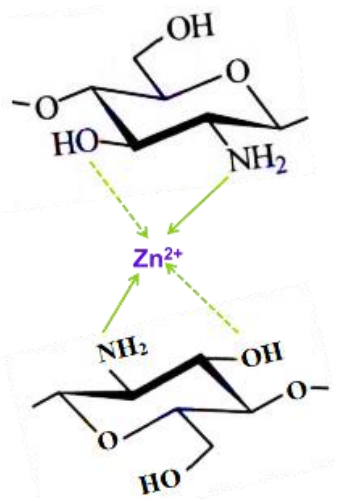

Fig. 6. Schematic representation of the formation of reasonable structure for chitosan-Zn nanocomposite [**64**].

Chitosan-copper (Cu)

It was observed that loading of copper (Cu) NPs into chitosan's matrix (**Fig. 7**) mainly influences the antimicrobial and antibacterial efficiency and the complexes formed are strongly influenced by the solution pH. For example, in a study conducted using Cu-loaded carboxymethyl chitosan NPs greatly improved the antibacterial activity by more than 30% when tested against *S. aureus* and this increased efficiency was attributed to the enhanced zeta potential of the complex [**69**]. Similarly, Cu-loaded chitosan NPs (of 257 nm) manufactured by ionic gelation were capable of increasing the zeta potential (from 96 mV to 51 mV) due to the sorption of Cu ions onto chitosan matrix and observed superior antibacterial activity against *E. coli*, *S. choleraesuis*, *S. typhimurium*, and *S. aureus*. The superior antibacterial activity of chitosan containing copper complexes was explained by chelation theory, *i.e.* upon chelation, the polarity of Cu ions gets reduced, which subsequently increases the lipophilicity of the chelates

and enhances their permeation through lipid bilayer of the cell membrane. Following the penetration of complex into the organism, the intracellular Cu(II) undergoes reduction to a Cu(I) complex by cellular oxidization and correspondingly, the Cu^{+}/Cu^{2+} couple is involved as a redox center. The O_2 and H_2O_2 produced by such redox reactions cause cytotoxic reactions by inhibition of DNA synthesis and finally lead to a destruction of cell viability. However, the continuous antibacterial activity of Cu-loaded NPs provided by the controlled and firm release of Cu ions dissociated in the small chitosan cores [**34**].

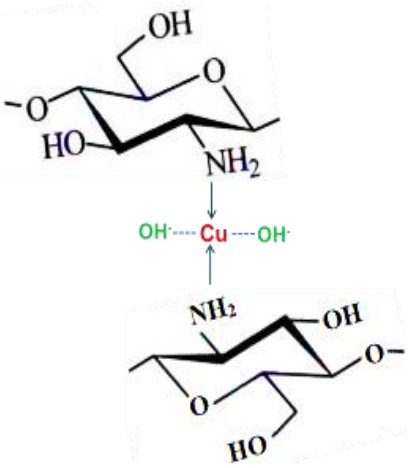

Fig. 7. Schematic for the formation of chitosan-Cu polymer composite.

Chitosan-platinum (Pt)

Other coordinated complexes fabricated using chitosan and metal NPs of platinum (Pt) have potential applications in amperometric sensors, catalysis, and as antimicrobial agents [**70-72**]. In a study, the amperometric biosensors fabricated by the electrochemical deposition of Pt NPs on to carbon nanotube-chitosan matrices confirmed to be suitable as cholesterol biosensors. Since, Pt is known for its high catalytic activity towards hydrogen peroxide electrooxidation and chitosan served as stabilizing medium for the dispersion of carbon nanotubes and subsequently allowed for an increase in the exposed surface area [**70**]. Similarly, by taking help of platinum's catalytic property, various types of nanocatalysts are constructed that includes the Pt-chitosan core-shell composites, silica-supported chitosan-Pt-Fe complex.

The Pt-chitosan nanocatalyst served as excellent nitrite sensor for the analysis of biological samples by the oxidative determination of nitrite with good reproducibility, stability and fast response, with a detection limit of $1.5x10^{-7}$ M and concentration range of $9.9x10^{-7}$ to 7.5×10^{-3} M.

In this, the amino functional groups of chitosan helped for the stabilization and increased molecular interactions with nitrite anions to accumulate them on the surface of nanocatalyst and thus contributed for enhanced catalytic activity and sensing of Pt [**71**].

However, the silica-supported chitosan-Pt-Fe complex proved to be suitable for catalyzing asymmetric hydrogenation of ketones; 2-hexanone and methyl acetoacetate [**72**].

Chitosan-quantum dots

Semiconductor NPs also called quantum dots are a prominent class of imaging agents, characterized by their unique features of nanocrystalline structure, large stokes shifts, broad absorption bands and narrow size dependent emission bands. One of the quantum dots, zinc sulphide (ZnS) when doped with manganese (Mn) isoelectronic impurities, significantly enhances the luminescent emission efficiency of ZnS nanocrystal even at room temperature by the action of Mn^{2+} ion's d-electron states as efficient luminescent centers [**15**]. The capping of quantum dots with chitosan biopolymer helps for surface passivation, ensures long time stability and luminosity. Therefore, the ZnS-doped Mn^{2+} NPs stabilized by chitosan offers excellent photoluminiscent property due to its photostability and can replace organic dyes and upon irradiation with UV light glows with an orange-red luminescence peaking at 590 nm. Since the cell walls of bacilli are anionic in nature and chitosan-functionalized ZnS:Mn^{2+} NPs with a net positive charge (at acidic pH) easily attaches and organizes on the cell walls leading to biolabeling. With its luminescent and cell wall conjugating property, the chitosan-ZnS:Mn^{2+} composite finds applications as effective biomarker and also as biological sensory probe for the detection of microorganisms, allowing rapid and "real-time" identification [**41**]. Similarly, to enhance the therapeutic potential of chemotherapy agents by tumor cell targeting while simultaneously imaging of the cancer cells, a cancer cell imaging-based targeted drug delivery system was developed. The folic acid (FA) conjugated carboxymethyl chitosan coordinated to Mn doped ZnS quantum dot (FA–CMC–ZnS:Mn) NPs constructed by a simple aqueous route synthesis and then formulated with a well-known anticancer drug, 5-Fluorouracil. The presence of folic acid helps to target the cancer cell-membrane folate receptor, chitosan derivative offers controlled drug delivery and at the same time, the ZnS:Mn^{2+} quantum dot imaging the path of this drug delivery system by fluorescence. The constructed bioprobe in addition to specific targeting and fluorescent imaging, suits also for the delivery of genes and proteins with simultaneous imaging [**15**].

Chitosan containing other metal NPs such as cobalt (Co^{2+}) and nickel (Ni^{2+}) complexes exhibit greater potentials as catalyst materials and antibacterial agents. The antibacterial activities are due to the easy association of high charge density chitosan-metal chelates with negatively charged bacterial cell surfaces. This activity is greatly influenced by the metal ion concentration and studies shows that, chitosan-metal chelates with a molar ratio of 1:3 are approximately 3-fold more active than the 1:1 molar ratio [**45**].

Conclusion

In this review, we highlighted the promising applications of chitosan and metal NPs fabrication with an interest towards the bioengineering and biomedical sectors. Interestingly, most of the research efforts showed that chitosan–metal complexes were superior to free chitosan

or simple metal composites. The chitosan's modification of metal nanostructures improved the quality and efficiency of metal ions by means of enhancing the stability, solubility, biocompatibility, catalytic activity and magnetization values.

There are some challenges associated with the use of metal nanostructures to the current biomedical applications. Many studies which use chitosan and metal NPs (Ag, Ti, Zn, Cu, Co and Ni) focuses only on the antibacterial and antifungal activities; while the potentials of these materials for other applications such as controlled delivery, magnetic bioseparation, tumor imaging, cancer therapy and tissue scaffolds are not thoroughly investigated. Similar to Fe, the metal ions of Co and Mn also exhibit superparamagnetism when falls under nanosizes, where they can be explored for magnetic fluid hyperthermia based therapy and drug delivery. Similarly, Pt and quantum dots (Zns:Mn) offers interesting optical and limuniscent properties that can be potential for introducing photothermal effects to the tumors while simultaneously imaging cells. Also, chitosan-mediated complexes of Cu, Ti and Ni are known for their mechanical strength and biocompatibility which can be explored as tissue engineering scaffolds. Many attempts to improve the biochemical characteristics of chitosan such as structural modification, adjustment of molecular factors by forming various derivatives are underway. The influencing applications of biomedical sector includes, early cancer detection as well as treatment, personalized medicine to cancerous diseases and diabetic, active role in tissue engineering appliances, implants and surgical aids. Overall, the combination of nanotechnology, biofabrication, biotechnology and biomedical engineering are strongly enhanced by chitosan-metal nanocomposites and future biomedical applications will benefit from this technology.

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References

1. Liu, Y.; Yang, X.; Shi, X. W.; Bentley, W. E.; Payne, G. F; *Green Polym. Chem. Biocat. Biomater*. **2007**, *1043*, 35. **DOI:** 10.1021/bk-2010-1043.ch003
2. Tiwari, A.; Ramalingam, M.; Kobayashi, H.; Turner, A.P.F. (Eds.); Biomedical materials and diagnostic devices; Wiley: USA, **2012**. **DOI:** 978-1-118-03014-1
3. Yi, H.; Wu, L. Q.; Bentley, W. E.; Ghodssi, R.; *et al. Biomacromolecules*, **2005**, *6*, 2881. **DOI:** 10.1021/bm050410l
4. Tiwari, A.; Mishra, A.K.; Kobayashi, H.; Turner, A.P.F. (Eds.); Intelligent nanomaterials; Wiley: USA, **2012**. **DOI:** 978-0-470-93879-9
5. Wu, L. Q.; Payne, G. F.; *Trends Biotechnol.* **2004**, *22*, 593. **DOI:**10.1016/j.tibtech.2004.09.008
6. Tiwari, A.; Tiwari, A. (Eds.); Nanomaterials in drug delivery, imaging, and tissue engineering; Wiley: USA, **2013**. **DOI:** 978-1-118-29032-3
7. Tiwari, A.; Tiwari, A. (Eds.) Bioengineered nanomaterials; CRC Press: USA, **2013**. **DOI:** 978-1-4665-8595-9
8. Tang, N.; Du, G.; Wang, N.; Liu, C.; Hang, H.; Liang, W; *J. Natl. Cancer Inst.* **2007**, *99*, 1004. **DOI:** 10.1093/jnci/djm027
9. Inmaculada, A.; Marian, M.; Ruth, H.; Ines, P.; *et al; Curr. Chem. Biol*. **2009**, *3*, 203. **DOI:** 10.2174/2212796810903020203
10. Hamman, J. H; *Mar. Drugs*. **2010**, *8*, 1305. **DOI:** 10.3390/md8041305
11. Gong, X.; Peng, S.; Wen, W.; Sheng, P.; Li, W; *Adv. Funct. Mater*. **2008**, *19*, 292. **DOI:** 10.1002/adfm.200801315
12. Park, J. J.; Luo, X.; Yi, H.; Valentine, T. M. *et al; Lab Chip*. **2006**, *6*, 1315. **DOI:** 10.1039/B603101C
13. Regmi, R.; Bhattarai, S. R.; Sudakar, C.; Wani, A. S.; *et al. J. Mater. Chem.* **2010**, *20*, 6158. **DOI:** 10.1039/C0JM00844C
14. Zhang, H.; Yee, D.; Wan, C; *Nanomedicine*, **2008**, *3*, 83. **DOI:** 10.2217/17435889.3.1.83
15. Mathew, M. E.; Mohan, J. C.; Manzoor, K.; Nair, S. V.; *et al; Carbohydr. Polym.* **2010**, *80*, 442. **DOI:** 10.1016/j.carbpol.2009.11.047
16. Wei, D.; Qian, W.; Shi, Y.; Ding, S.; Xia, Y; *Carbohydr. Res.* **2007**, *342*, 2494. **DOI:** 10.1016/j.carres.2007.07.001
17. Mekahlia, S.; Ouzid, B. B; *Phys. Procedia.*, **2009**, *2*, 1045. **DOI:** 10.1016/j.phpro.2009.11.061
18. Wang, X.; Du, Y.; Fan, L.; Liu, H.; Hu, Y; *Polym. Bull.*, **2005**, *55*, 105. **DOI:** 10.1007/s00289-005-0414-1
19. Bansal, V.; Sharma, P. K.; Sharma, N.; Pal, O. P.; Malviya, R; *Adv. Biol. Res.*, **2011**, *5*, 28. **DOI:** 10.1007/12_2011_137
20. Krayukhina, M. A.; Samoilova, N. A.; Yamskov, I. A; *Russ. Chem. Rev.*, **2008**, *77*, 854. **DOI:** http://dx.doi.org/10.1070/RC2008v077n09ABEH003750
21. Li'ina, A. V.; Varlamov, V. P; *Appl. Biochem. Microbiol.*, **2005**, *41*, 5.
22. Muzzarelli, C.; Muzzarelli, R. A. A; *J. Inorg. Biochem.*, **2002**, *92*, 89. **DOI:** 10.1016/S0162-0134(02)00486-5
23. Wei, D.; Sun, W.; Qian, W.; Ye, Y.; Ma, X; *Carbohydr. Res.*, **2009**, *344*, 2375. **DOI:** 10.1016/j.carres.2009.09.001
24. Travan, A.; Pelillo, C.; Donati, I.; Marsich, E.; *et al; Biomacromolecules*, **2009**, *10*, 1429. **DOI:** 10.1021/bm900039x
25. Matteini, P.; Ratto, F.; Rossi, F.; Centi, S.; Dei, L.; Pini, R; *Adv. Mater.*, **2010**, *22*, 4313. **DOI:** 10.1002/adma.201002228
26. Wang, L.; Wen, W.; Xiong, H.; Zhang, X.; Gu, H.; Wang, S; *Anal. Chim. Acta.*, **2013**, *758*, 66. **DOI:** 10.1016/j.aca.2012.10.050
27. Mu L, Feng S. S; *J. Controlled Release*, **2001**, *76*, 239. **DOI:** 10.1016/S0168-3659(01)00440-0
28. Kim, D. H.; Lee, S. H.; Im, K. H.; Kim, K. N.; *et al; Curr. Appl. Phys.*, **2006**, *6*, 242. **DOI:** 10.1016/j.cap.2006.01.048
29. Hong, S.; Chang, Y.; Rhee, I. *J. Kor. Phys. Soc.* **2010**, 56, 868. **DOI:** 10.3938/jkps.56.868
30. Li, Q. L.; Huang, N.; Chen, J.; Wan, G.; *et al. J. Biomed. Mater. Res. A.* **2009**, *89*, 575. **DOI:** 10.1002/jbm.a.31999
31. Wei, D.; Qian, W.; Shi, Y.; Ding, S.; Xia, Y. *Carbohydr. Res.* **2008**, *343*, 512. **DOI:** 10.1016/j.carres.2007.10.031
32. Mohammad, F.; Balaji, G.; Weber, A.; Uppu, R. M.; Kumar, C. S. S. R. *J. Phys. Chem. C.* **2010**, *114*, 19194. **DOI:** 10.1021/jp105807r
33. Kumar, C. S. S. R.; Mohammad, F. *J. Phys. Chem. Lett.* **2010**, *1*, 3141. **DOI:** 10.1021/jz101202a
34. Qi, L.; Xu, Z.; Jiang, X.; Hu, C.; Zou, X. *Carbohydr. Res.* **2004**, *339*, 2693. **DOI:** 10.1016/j.carres.2004.09.007
35. Rhim, J. W.; Hong, S. I.; Park, H. M.; Ng, P. K. *J. Agr. Food Chem.* **2006**, *54*, 5814. **DOI:** 10.1021/jf060658h
36. Chaleawlert-umpon, S.; Mayen, V.; Manotham, K.; Pimpha, N. *J. Biomater. Sci. Polym. Ed.* **2010**, *21*, 1515.

DOI: 10.1163/092050609X12519805626031
37. Sasaki, T.; Iwasaki, N.; Kohno, K.; Kishimoto, M.; *et al. J. Biomed. Mater. Res. A.* **2008**, *86*, 969. **DOI:** 10.1002/jbm.a.31724
38. Peng, C. C.; Yang, M. H.; Chiu, W. T.; Chiu, C. H. *Macromol. Biosci.* **2008**, *8*, 316. **DOI:** 10.1002/mabi.200700188
39. Qi, L.; Xu, Z.; Jiang, X.; Li, Y.; Wang, M. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1397. **DOI:** 10.1016/j.bmcl.2005.01.010
40. Dos Santos, D. S. Jr.; Goulet, P. J.; Pieczonka, N, P.; Oliveira, O. N. Jr.; Aroca, R. F. *Langmuir*. **2004**, *20*, 10273. **DOI:** 10.1021/la048328j
41. Warad, H. C.; Thanachayanont, C.; Tumcharern, G.; Dutta, J. *Proc. 2nd IEEE Int. Conf. Nano/Micro Eng. Mol. Syst.* January, **2007**, 16. **DOI:** 10.1109/NEMS.2007.352015
42. Mekhaev, A. V.; Pestov, A. V.; Molochnikov, L. S.; Kovaleva, E. G.; *et al. Russ. J. Phys. Chem. A.* **2011**, *85*, 1155. **DOI:** 10.1134/S0036024411070211
43. Huang, H.; Yang, X. *Biomacromolecules*, **2004**, *5*, 2340. **DOI:** 10.1021/bm0497116
44. Wei, D.; Qian, W. *Colloid. Surface. B.* **2008**, *62*, 136. **DOI:** 10.1016/j.colsurfb.2007.09.030
45. Adewuyi, S.; Kareem, K. T.; Atayese, A. O.; Amolegbe, S. A.; Akinremi, C. A. *Int. J. Biol. Macromol.* **2011**, *48*, 301. **DOI:** 10.1016/j.ijbiomac.2010.12.004
46. Du, W. L.; Niu, S. S.; Xu, Y. L.; Xu, Z. R.; Fan, C. L. *Carbohydr. Polym.* **2009**, *75*, 385. **DOI:** 10.1016/j.carbpol.2008.07.039
47. Panáek, A.; Kvitek, L.; Prucek, R.; Kolář, M.; *et al. J. Phys. Chem. B.* **2006**, *110*, 16248. **DOI:** 10.1021/jp063826h
48. Sambhy, V.; MacBride, M. M.; Peterson, B. R.; Sen, A. *J. Am. Chem. Soc.* **2006**, *128*, 9798. **DOI:** 10.1021/ja061442z
49. Wang, B.; Chen, K.; Jiang, S.; Reincke, F.; Tong, W.; Wang, D.; Gao, C. Y. *Biomacromolecules*, **2006**, *7*, 1203. **DOI:** 10.1021/bm060030f
50. Zhang, L.; Shen, Y. H.; Xie, A. J.; Li, S. K.; Li, Y. M. *J. Mater. Chem.* **2009**, *19*, 1884. **DOI:** 10.1039/B817071A
51. Lu, S.; Gao, W.; Gu, H. Y.; *Burns.* **2008**, 34, 623-628. **DOI:** 10.1016/j.burns.2007.08.020
52. Sreeram, K. J.; Nidhin, M.; Nair, B. U. *Colloid. Surf. B.* **2009**, *71*, 260. **DOI:** 10.1016/j.colsurfb.2009.02.015
53. Li, F. R.; Yan, W. H.; Guo, Y. H.; Qi, H.; Zhou, H. X. *Int. J. Hypertherm.* **2009**, *25*, 383. **DOI:** 10.1080/02656730902834949
54. Yuan, Q.; Venkatasubramanian, R.; Hein, S. *Acta Biomater.* **2008**, *4*, 1024. **DOI:** 10.1016/j.actbio.2008.02.002
55. Fernandes, R.; Tsao, C. Y.; Hashimoto, Y.; Wang, L.; *et al. Metab. Eng.* **2007**, *9*, 228. **DOI:** 10.1016/j.ymben.2006.11.004
56. Kumar, C. S. S. R.; Mohammad, F. *Adv. Drug Deliv. Rev.* **2011**, *63*, 789. **DOI:** 10.1016/j.addr.2011.03.008
57. Wang, S. F.; Tan, Y. M. *Anal. Bioanal. Chem.* **2007**, *387*, 703. **DOI:** 10.1007/s00216-006-0976-2
58. Ding, L.; Hao, C.; Xue, Y.; Ju, H. *Biomacromolecules*, **2007**, *8*, 1341. **DOI:** 10.1021/bm061224y
59. Sugunan, A.; Thanachayanont, C.; Duttaa, J.; Hilborn, J. G. *Sci. Technol. Adv. Mater.* **2005**, *6*, 335. **DOI:** http://dx.doi.org/10.1016/j.stam.2005.03.007
60. Salehizadeh, H.; Hekmatian, E.; Sadeghi, M.; Kennedy, K. *J. Nanobiotechnol.* **2012**, *10*, 1. **DOI:** 10.1186/1477-3155-10-3
61. Son, B.; Yeom, B. Y.; Song, S. H.; Lee, C. S.; Hwang, T. S. *J. Appl. Polym. Sci.* **2009**, *111*, 2892. **DOI:** 10.1002/app.29233
62. Zhao, L.; Chang, J.; Zhai, W. *J. Mater. Sci.* **2009**, *20*, 949. **DOI:** 10.1007/s10856-008-3645-5
63. Abd Elhady, M. M. *Int. J. Carbohydr. Chem.* **2012**, 1. **DOI:** http://dx.doi.org/10.1155/2012/840591
64. Wang, X.; Du, Y.; Liu, H. *Carbohydr. Polym.* **2004**, *56*, 21. **DOI:** 10.1016/j.carbpol.2003.11.007
65. Hajkova, P.; Spatenka, P.; Horsky, J.; Horska, I.; Kolouch, A. *Plasma Processes Polym.* **2007**, *4*, 97. **DOI:** 10.1002/ppap.200731007
66. Huang, Z.; Zheng, X.; Yan, D.; Yin, G.; *et al. Langmuir*, **2008**, *24*, 4140. **DOI:** 10.1021/la7035949
67. Sawai, J.; Shoji, S.; Igarashi, H.; Hashimoto, A.; *et al. J. Ferment. Bioeng.* **2008**, *86*, 521. **DOI:** 10.1016/S0922-338X(98)80165-7
68. Perelshtein, I.; Ruderman, E.; Perkas, N.; Tzanov, T.; *et al. J. Mater. Chem. B.* **2013**, *1*, 1968. **DOI:** 10.1039/C3TB00555K
69. Gu, C.; Sun, B.; Wu, W.; Wang, F.; Zhu, M. *Macromol. Symp.* **2007**, *254*, 160. **DOI:** 10.1002/masy.200750825
70. Tsai, Y. C.; Chen, S. Y.; Lee, C. A. *Sens. Actuators, B.* **2008**, *135*, 96. **DOI:** 10.1016/j.snb.2008.07.025
71. Zhu, X.; Ding, A. *Int. J. Electrochem. Sci.* **2013**, *8*, 135. **DOI:** 10.1016/j.colsurfb.2011.12.028
72. Wei, W. L.; Hao, S. J.; Zhou, J.; Huang, M. Y.; Jiang, Y. Y. *Polym. Adv. Technol*. **2004**, *15*, 287. **DOI:** 10.1002/pat.438