World Journal of
Experimental Biosciences

Volume 7, Number 2: 09 -17
ISSN: 2313-3937
2019

Review article

Biomedical Applications of Chitosan Nanoparticles: A Comprehensive Review

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ABSTRACT

Chitosan nanoparticles (CSNPs) are a versatile polyfunctional mechano-chemical scaffold used to advance biomedicine through long-term retention in cells and tissues after delivery. Because CSNPs have multiple properties that facilitate drug delivery, including being biodegradable, biocompatible, cationically charged, mucoadhesive, possessing some inherent biological activity, and easily penetrating mucosal membranes, they are an important part of polymeric nanocarriers for drug delivery. This article offers a comprehensive and critical review of the physical and chemical properties of CSNPs and highlights various biomedical applications, such as serving as a delivery vehicle for medicine via (i) oral, (ii) nasal, (iii) pulmonary, (iv) ocular, (v) anticancer, (vi) gene therapy, (vii) vaccine adjuvants, (viii) tissue repair or wound healing, (ix) tissue regeneration, (x) antimicrobial activity, (xi) clinical studies against pathogens, (xii) cellular uptake and intracellular transport mechanisms, mucoadhesion, and the paradox of mucosal penetration, (xiii) development of safety and toxicology profiles from in vitro and in vivo preclinical studies, (xiv) surface modifications such as PEGylation and receptor-ligand conjugation for cell-specific targeting, and (xv) barriers to translating CSNPs from laboratory research to clinical use, along with future directions like delivering CRISPR/Cas9, developing theranostics, and microfluidic manufacturing of CSNPs. CSNPs consistently improve bioavailability, enhance cellular uptake, increase accumulation in tumors, prolong drug release, and reduce pathogen loads, often outperforming both free and conventional drugs across all delivery routes and medication types studied. Nevertheless, challenges such as manufacturing scale-up, raw material variability, limited correlation models between *in vitro* and *in vivo* data, and an evolving regulatory environment for polymeric nanomedicines impede clinical translation. This review critically discusses these obstacles and proposes strategies for the next generation of chitosan-based nanomedicine.

Keywords: Anticancer; Chitosan nanoparticles; Drug delivery; Gene delivery; Mucoadhesion; Nanomedicine.

Citation: Ghafil JA. (2019) Biomedical Applications of Chitosan Nanoparticles: A Comprehensive Review. *World J Exp Biosci* **7**:9-17. https://doi.org/10.65329/wjeb.v7.02.02

Received July 29, 2019; Revised August 30; Accepted September 19, 2019; Published September 28, 2019.

1. INTRODUCTION

For the last few years, pharmaceutical scientists have adopted a new way of thinking about how well a drug will work, not only based on its pharmacological activities but also on how it gets from where it is made to the site of action (biodistribution) and how quickly it reaches that site (pharmacokinetics). Standardly used dosage forms, such as pills (oral) and liquids (intravenous), are not developed with differences among cell and tissue types in mind [1].

This can lead to an increased risk of systemic exposure, dose-limiting side effects, or insufficient delivery of the medicine to the intended area (subtherapeutic). Nanomedicine, particularly the specific application of polymeric nanoparticle drug delivery, is one focus of attempts to overcome these limitations; by developing carriers at the nanoscale which will both protect (encapsulate), deliver (transport), target (distribute), and release therapeutic agents in a controlled spatiotemporal manner [2, 3].

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Various synthetic and natural polymers have been studied for the production of nanoparticles, among them are both poly (lactic-co-glycolic acid) (PLGA) and Poly (ethylene glycol) (PEG) plus albumin, gelatin and other types of polysaccharides. The most significant attention by researchers and businesses has been directed towards chitosan. Chitosan is a semi crystalline, linear polysaccharide that consistsed of D-glucosamine and N-acetyl-D-glucosamine, which is derived through the partial or complete deacetylation of chitin, is made from the exoskeleton of shells of marine invertebrates (shrimps, crabs, and krills) or from the cell wall of mushrooms, for example, most of the world's chitin supply comes from invertebrate sea food products. The global total amount produced each year exceeds 6000 Metric tonnes, so it is readily available as well as a very inexpensive choice for use as a biopolymer in pharmaceuticals [4].

The appeal of chitosan as a nanoparticle-forming polymer is multifactorial. First, the primary amine groups (pKa ≈ 6.2–6.5) confer cationic charge at physiological and acidic pH, enabling spontaneous electrostatic complexation with anionic nucleic acids, negatively charged cell membranes, and mucin glycoproteins — a property that underpins both gene delivery and mucoadhesion [5]. Second, chitosan is both biodegradable and biocompatible; it undergoes enzymatic degradation by lysozyme and bacterial chitinases to non-toxic oligosaccharides and glucosamine monomers that are incorporated into glycosaminoglycan biosynthetic pathways [6]. Third, the free amine and hydroxyl functional groups provide abundant sites for chemical modification, facilitating the conjugation of targeting ligands, PEG chains, imaging agents, and stimuli-responsive linkers. Fourth, chitosan's well-documented ability to transiently open epithelial tight junctions, mediated by interactions with the claudin and occludin transmembrane proteins of the zonula occludens complex, provides a means to enhance paracellular absorption of macromolecular therapeutics at mucosal surfaces [7].

Bulk chitosan is converted to nanoparticles that have even greater properties than the bulk form. Converting chitosan from macro to nanoscale creates an increase in surface area-to-volume ratio of two to three orders of magnitude, thus increasing the interaction with biological membranes, mucus layers, and microorganisms on the surface of the nanoparticles [8]. The size of nanoparticles (normally ranging from 50-600nm) will have a major impact on the distribution of tissues, selection of cellular uptake pathways, and the in vivo behavior of the nanoparticles. A nanoparticle will primarily undergo internalization via clathrin-mediated endocytosis in non-phagocytic cells, while smaller than 100nm nanoparticles will use caveolae-mediated processes for internalization, and larger than 500nm nanoparticles will be rapidly phagocytosed by macrophages and dendritic cells [9]. This phenomenon is utilized to deliver vaccines. Additionally, the mucoadhesive and permeation-enhancing properties of chitosan are further enhanced at the nanoscale due to increased contact surface area and faster interaction kinetics with mucosal surfaces [10].

The exponential growth in the body of research literature on chitosan-based nanoparticles (CSNPs) has occurred since the landmark studies of Calvo et al. (1997) exploring ionotropic gelation to form CSNPs, using sodium tripolyphosphate as an ionic gelation agent; and of Baghdan et al. (2018) investigating the use of chitosan/DNA nanoparticles (CS-DNAs) to transfer genetic material via nanoparticle-mediated gene transfection [11,12]. PubMed contains over 4000 original research articles and reviews published on chitosan nanoparticles, demonstrating the extensive range of uses examined with those CSNPs, including: oral delivery of insulin and additional macromolecular therapies; targeted delivery of drugs to the brain via intranasal routes that bypass the blood-brain barrier; pulmonary delivery of anti-tuberculosis therapies; delivery of therapeutics to the ocular surface with prolonged precorneal residence time; drug delivery directed toward tumors based on the enhanced permeability and retention (EPR) effect; siRNA-mediated oncogene suppression; mucosal vaccination strategies; and use of CSNPs as scaffolding for wound healing. Notwithstanding this extensive compilation of preclinical data, the advancement of CSNP-based nanomedicine into clinical practice has been sluggish, with only a very limited number of CSNP-based formulations having reached the clinical trial stage; this disparity between preclinical and clinical translational progress in CSNP-based nanomedicine is likewise worthy of critical review [13].

It includes an account of the fundamental physical and chemical properties of chitosan and the different methods used in the manufacture of CSNPs, a complete range of biomedical uses and antimicrobial characteristics of CSNPs, various aspects of cellular biology and safety/toxicology, surface modification techniques, and an honest assessment of present-day limitations and evidence-supported future directions. It also provides quantitative performance data to enable comparisons between research investigations and to put the relative potential of CSNP-based nanomedicines into context.

2. PHYSICOCHEMICAL PROPERTIES OF CHITOSAN

Three primary physicochemical parameters that affect the properties of chitosan nanoparticles include molecular weight, degree of deacetylation, and the distribution of acetylated and deacetylated units along the polymer backbone (sequence distribution pattern). These parameters are interdependent; therefore, they have profound effects on solubility, viscosity, charge density, biological activity, and degradation kinetics [14].

The degree of deacetylation affects the number of free amino groups available for protonation or chemical modification. Chitosan has a net positive charge below its pKa (~6.2–6.5) and is soluble in aqueous acidic media and has the potential to form electrostatic interactions with negatively charged molecules. As such, due to protonation-dependent solubility, chitosan can undergo a pH-responsive sol-gel transition, making it useful for in situ gelling formulations at physiological pH [15].

Chitosan with a high degree of deacetylation (>85%) typically has a higher charge density, better mucoadhesive properties, greater antibacterial activity, and more effective complexation with nucleic acids, but additionally can be more cytotoxic when present at high concentrations—illustrating the need to find a balance between biological activity and safety for specific applications [6].

Molecular weight can impact viscosity, entanglement between polymer chains, stability of nanoparticles when exposed to the environment, and degradation of polymers in vivo; however low molecular weight chitosan (<50 kDa) can produce nanoparticles with increased solubility in water, decreased viscosity facilitating ease of processing, and increased rate of biodegradation compared to high molecular weight chitosan. In contrast to low MW chitosan, the use of HMW chitosan will produce robust mechanically stable nanoparticles with enhanced mucoid-hesive properties as a result of deeper penetration into the

mucus gel network; however, HMW chitosan also has the disadvantages of being more viscous and degrading more slowly than LMW chitosan. In light of these trade-offs, it may be necessary to carefully consider the type of chitosan used for each specific application/delivery route [16].

3. DEPICTION TECHNIQUES

Rigorous physicochemical and biological characterization is essential to establish structure-property-activity relationships for CSNP formulations and to support regulatory submissions. Dynamic light scattering (DLS) and nanoparticle tracking analysis (NTA) provide hydrodynamic diameter, PDI, and particle concentration in suspension. Zeta potential, measured by electrophoretic light scattering, is a key predictor of colloidal stability and surface interaction behavior, generally indicating electrostatically stabilized systems. Transmission electron microscopy (TEM) and atomic force microscopy (AFM) provide high-resolution morphological data and actual (dry-state) particle dimensions, typically 10–30% smaller than hydrodynamic diameters measured by DLS due to the absence of the hydration shell [17].

Encapsulation efficiency (EE%) and drug loading capacity (DLC%) are determined by indirect methods following separation of free drug (ultracentrifugation, ultrafiltration, or size-exclusion chromatography) and quantification by UV-Vis spectrophotometry, HPLC, or fluorescence spectroscopy. In vitro drug release profiles under simulated physiological conditions (SGF pH 1.2, SIF pH 7.4, simulated colonic fluid pH 6.8) characterize release kinetics and provide mechanistic insight into release mechanisms (Fickian diffusion, anomalous transport, or erosion-controlled). Fourier-transform infrared spectroscopy (FTIR) and X-ray photoelectron spectroscopy (XPS) confirm chemical cross-linking, surface functionalization, and drug-polymer interactions [18].

4. BIOMEDICAL APPLICATIONS OF CHITOSAN NANOPARTICLES

4.1. Oral Drug Delivery

The oral route of administration is still the preferred method of drug delivery due to its convenience and ease-of-use for the patient, as well as the fact that it does not involve any of the complications associated with injectable routes. However, because macromolecular therapeutics (proteins, peptides, nucleic acids) can be degraded by the effect of components of the gastrointestinal system (e.g., enzymatic degradation, acidic pH-mediated hydrolysis), macromolecules have very low oral bioavailability (usually <1%) resulting from the presence of physical mucosal barriers, as well as restricted paracellular/transcellular transport across the intestinal epithelium (Liu et al., 2018). CSNPs address these barriers simultaneously in three different ways: (1) the protective polymer matrix serves as a physical barrier and protects encapsulated macromolecules from proteolytic and nucleolytic degradation; (2) the mucoadhesive chitosan surface provides longer intestinal residence time; and (3) chitosan's established ability to open tight junctions (TJs) temporarily enhances paracellular permeability for the adsorption of macromolecules [19].

CSNPs are considered the most extensively studied method of delivering insulin orally to date, with numerous studies showing significant pharmacodynamic responses (i.e., blood glucose lowering effects) following oral administration (via CSNP) to diabetic rats and dogs. CSNPs containing insulin (200–500 nm, +25 to +35 mV) made from ionotropic gelation using TPP or polyelectrolyte complexation (PEC) with alginate or dextran sulfate were shown to have 3–15% oral bioavailability as compared to subcutaneous injection, representing a significant 10–50-fold increase in bioavailability when compared to unencapsulated insulin. Pharmacodynamic effects were also shown to be 1.5–2× longer than that seen following subcutaneous injection due to sustained mucosal absorption from the mucoadherent CSNP depot. Additionally, enteric-coated CSNPs further improved the acid stability of CSNPs during transit through the gastric tract, allowing for the retention of insulin activity and enabling pH-triggered release within the intestinal lumen [20,21].

Beyond insulin, CSNPs have demonstrated improved oral bioavailability for cyclosporin A (immunosuppressant, 38% vs. 9% for Sandimmune solution), paclitaxel (anticancer, 8.7-fold increase in AUC vs. Taxol), amphotericin B (antifungal, 3.2-fold increase), metformin, acyclovir, and heparin. The Caco-2/HT-29 co-culture intestinal model and triple co-culture models (Caco-2/HT-29/Raji B lymphocytes) are widely employed as in vitro surrogates for intestinal absorption prediction, with CSNPs consistently demonstrating 3–10-fold greater apparent permeability (Papp) compared to free drug controls [22].

4.2. Nasal Drug Delivery

The nasal route has many desirable advantages for delivering drugs both locally (allergic rhinitis, nasal polyps, sinusitis) as well as systemically. Some of these benefits include rapid onset of drug action, greater bioavailability as the first-pass effect is avoided, and a direct pathway (i.e., the olfactory bulb) for administration of medication to the brain [23].

Chitosan is a natural polysaccharide with excellent mucoadhesive properties that allow for prolonged retention of drugs within the nasal cavity (half-life of ~30–90 minutes for chitosan nanoparticles vs. ~15 minutes for liquid formulations), thus enabling sustained drug absorption through the highly vascularized submucosal space. Utilization of the nose-to-brain direct drug transport pathway (which uses olfactory and trigeminal nerve axons to transport drugs) along with transcytosis across the olfactory epithelium into the olfactory bulb may represent an important mechanism through which CSNPs facilitate CNS drug delivery, potentially circumventing the blood-brain barrier (BBB) [24].

In several rat studies comparing CSNP-encapsulated rivastigmine (for Alzheimer's disease), lorazepam (for status epilepticus), and ondansetron (for emesis) administered intranasally versus intravenously, dramatically higher brain tissue concentrations as well as greater pharmacological effects were observed in rats receiving intranasally administered drugs (Patel et al., 2012). Radiolabelled tracing revealed that concentrations in the olfactory bulb were between 10–50 times greater than plasma concentration, confirming direct nose-to-brain transport. Following administration of CSNPs to children as a vaccine against influenza, enhanced mucosal IgA titers, serum IgG responses, and T-cell-mediated immunity were observed compared to administration of the free antigen [25].

4.3. Anticancer Drug Delivery

Nanoparticles are still widely studied and remain focused on treating various types of cancer. Chemotherapy, photodynamic therapy, and immune modulators are three types of cancer

treatments extensively researched using nanoparticles. Cancer drugs can either be passively deposited into a tumor using the enhanced permeability and retention (EPR) effect or actively delivered using targeting reagents on the nanoparticle surface to attach to specific cancer cell receptors [26].

CSNPs with doxorubicin (DOX) are the best researched for breast, lung, liver, and colorectal cancers and have demonstrated 2–10 times lower IC50 values than DOX alone in numerous cancer cells that have developed multidrug resistance (MDR). The mechanism by which CSNPs overcome P-glycoprotein (P-gp)-mediated efflux appears to involve interference with P-gp ATPase activity and alteration of the intracellular membrane environment. CSNPs loaded with paclitaxel (PTX), gemcitabine, 5-fluorouracil (5-FU), curcumin, and cisplatin have demonstrated superior in vitro and in vivo tumor-growth-inhibiting properties than their respective free drugs. Patients treated with PTX-loaded deacetylated CSNPs experienced 3.4 times more drug accumulation within their MCF-7 xenograft model and 2.8 times greater inhibition of tumor growth than treatment with Taxol, due to improved EPR accumulation and prolonged drug release (Zhang et al., 2010).

4.4. Gene Delivery and Transfection

Gene delivery through non-viral means requires that nucleic acids be condensed into nano-sized complexes, be protected from nuclease degradation, be taken up by the cell, escape from the endosome, and be released to the nucleus (for DNA) or into the cytosol (for siRNA/mRNA). Chitosan, due to its cationic nature at physiological pH, can spontaneously complex with anionic nucleic acids (pDNA, siRNA, antisense oligonucleotides, mRNA) at N:P ratios ranging from 2:1 to 20:1 to form compact polyplexes (nanocomplexes) of 100–400 nm in size with zeta potentials of +10 to +30 mV [28]. Chitosan/siRNA nanoparticles for RNA interference-based therapy have shown particular promise for silencing oncogenes (VEGF, BCL-2, K-RAS, STAT3), inflammatory genes (TNF-α, IL-1β), and antiviral targets (HIV reverse transcriptase, influenza hemagglutinin) in vitro and in vivo. Hepatic VEGF silencing by galactosylated chitosan/anti-VEGF siRNA nanoparticles (targeting the asialoglycoprotein receptor on hepatocytes) achieved 78% VEGF mRNA knockdown in liver tissue of HCC-xenograft mice, accompanied by significant tumor growth retardation [29].

4.5. Vaccine Delivery and Adjuvancy

Chitosan nanoparticles are effective adjuvants for vaccine use and antigen delivery because they provide both an intrinsic immunostimulatory effect and the ability to provide a depot of particulate antigens (which mimic the size of disease-causing bacteria) thus facilitating efficient uptake of antigens by macrophages and dendritic cells. The immunological mechanisms through which chitosan acts as an adjuvant include: (1) engagement of TLR2, TLR4, and the NLRP3 inflammasome pathways in macrophages; (2) activation of the complement system via the alternative pathway; (3) NLRP3-driven IL-1β and IL-18 production leading to Th1/Th17 differentiation; and (4) increased antigen cross-presentation to CD8+ T cells via class I MHC molecules, critical for cytotoxic T lymphocyte development [30].

CSNP-adjuvanted subunit vaccines against Influenza (HA and NA antigens), Hepatitis B surface antigen, and Pneumococcal polysaccharides antigens have been shown to elicit 3–10 times greater antibody titers, demonstrate a higher avidity index, and provide a longer duration of immune response than similar vaccines formulated with aluminum adjuvants in mouse models. When administered mucosally (intranasal, oral, or pulmonary), CSNPs are able to generate both mucosal secretory IgA responses (critical for preventing pathogen entry through mucosal surfaces) as well as systemic IgG responses, overcoming a primary limitation of conventional parenteral vaccines. The needle-free delivery modality also makes CSNP vaccines particularly advantageous for mass immunization programs in resource-limited settings [30-32].

4.6. Ocular Drug Delivery

There are numerous barriers to effective ocular drug delivery: nasolacrimal drainage removes over 80% of a topically instilled dose within 5 minutes; corneal epithelial tight junctions restrict paracellular transport; and conjunctival blood vessel absorption can cause systemic side effects. CSNPs overcome these barriers through their adhesive properties to corneal and conjunctival mucin (extending precorneal residence time from <5 minutes to >30 minutes), maintenance of drug concentration at the corneal surface, and direct enhancement of corneal penetration [33].

Corneal concentrations achieved after a single instillation of cyclosporin A-loaded CSNPs were 4.2 times greater than those achieved with free drug, with therapeutic levels maintained for 12 hours versus only 4 hours for the equivalent commercial formulation. Other validated ocular applications include acyclovir-loaded CSNPs for Herpes Simplex Keratitis, levofloxacin CSNPs for bacterial conjunctivitis, and latanoprost CSNPs for glaucoma, all demonstrating superior precorneal retention. For intravitreal injection targeting the posterior segment (AMD, diabetic retinopathy), CSNPs provided sustained drug delivery lasting 30–90 days compared to 1–3 days for free drug, substantially reducing injection frequency and cumulative procedural risk [34].

4.7. Wound Healing and Tissue Regeneration

Chitosan provides a unique mechanism of action for enhancing wound healing through hemostasis (activation of platelets, aggregation of red blood cells, and stabilization of fibrin), antimicrobial barrier function, stimulation of macrophage cytokines to stimulate fibroblast proliferation and migration, collagen synthesis and remodeling, and provision of a biodegradable scaffold for granulation tissue. Sustained local delivery of growth factors and antibiotics or silver nanoparticles can be achieved using CSNPs within hydrogel, electrospun, and film dressing materials [35].

Yoo et al. (2013) demonstrated that incorporating epidermal growth factor (EGF) within CSNP/hydrogel composites delivered a wound closure rate 35% greater and collagen deposition 2.1-fold greater than CSNP/hydrogel alone in a diabetic rat wound model, highlighting particular promise for addressing impaired wound healing in diabetes mellitus. CSNP scaffolds for bone tissue engineering containing synthetic hydroxyapatite and osteogenic growth factors (BMP-2, rhBMP-7) supported osteoblast differentiation, mineralization, and new bone formation in animal models of critical-sized calvarial defects, resulting in increased bone area fraction [36].

4.8. Pulmonary Drug Delivery

CSNPs administered by inhalation enable treatment of local pulmonary diseases directly through airway and alveolar epithelial access, and permit systemic drug absorption through the

large alveolar surface area. Critical aerodynamic properties determining lung distribution include the aerodynamic diameter, mass median aerodynamic diameter (MMAD), fine particle fraction (FPF), and geometric standard deviation (GSD). CSNPs can be produced as microparticle carriers by spray-drying or spray-freeze-drying for dry powder inhaler (DPI) or nebulizer formulations [37].

Spray-dried mannitol/CSNP composite microparticles with MMADs between 2.1 and 3.4 μm and FPFs between 42% and 65% exhibited good alveolar deposition and increased drug retention in the lung compared to solution formulations. CSNPs containing anti-tuberculosis drugs (rifampicin, isoniazid, pyrazinamide, ethambutol) achieved macrophage drug concentrations 8–15 times higher than free drug, allowing for reduced dosing frequency in TB guinea pig models — a significant advantage given the lengthy and complex treatment regimens required for drug-sensitive and drug-resistant TB [38].

5. ANTIMICROBIAL APPLICATIONS OF CHITOSAN NANOPARTICLES

5.1. Antibacterial Activity

The antibacterial properties of CSNPs exhibit a significantly higher level of activity than bulk chitosan on a mass basis, as a result of their two-orders-of-magnitude greater surface area-to-volume ratio. CSNPs possess antibacterial activity against numerous clinically important pathogens including methicillin-resistant *Staphylococcus aureus* (MRSA), multidrug-resistant *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and drug-resistant *Mycobacterium tuberculosis* (Table 1). The multi-targeting bactericidal mechanism involves: electrostatic adhesion to bacterial surfaces causing membrane depolarization and formation of large membrane pores (evidenced by leakage of K^+, Mg^{2+}, nucleotides, and proteins); inhibition of cell wall biosynthetic enzymes; and disruption of nucleic acid–protein complexes [39]. CSNPs are small enough to penetrate into bacterial biofilms through diffusion-limited percolation, achieving bactericidal concentrations in biofilm regions inaccessible to conventional antibiotics.

Combinations of CSNPs and antibiotics demonstrate synergistic activity through: increased membrane permeability allowing higher intracellular antibiotic concentrations; protection of labile antibiotics from beta-lactamase and efflux enzymes; sustained antibiotic delivery above the MIC; and biofilm matrix disruption. The fractional inhibitory concentration index (FICI) for CSNP/ciprofloxacin combinations against P. aeruginosa biofilms was 0.28 ± 0.04, indicating strong synergism (FICI < 0.5), versus 1.0 for either agent alone. Table 1 summarizes antimicrobial efficacy data against key clinical pathogens [40].

6. MECHANISMS OF CELLULAR INTERNALIZATION AND INTRACELLULAR TRAFFICKING

6.1. Endocytic Pathways

For the rational design of nanoparticles to maximize cytosolic/nuclear drug delivery and minimize lysosomal degradation, it is necessary to understand the internalization mechanisms of CSNPs. Several endocytic pathways have been identified. Clathrin-mediated endocytosis is the primary pathway for CSNPs in non-phagocytic cells (Caco-2, HeLa, MCF-7, HEK293), with 60–80% inhibition of CSNP uptake observed upon clathrin-mediated endocytosis inhibition with chlorpromazine. Caveolae-mediated endocytosis also participates in cellular uptake of CSNPs of 50–100 nm in epithelial cells. Macrophages and dendritic cells utilize macropinocytosis as a means to internalize CSNPs as part of their baseline pinocytic activity [41]. Following endocytic capture, CSNPs are trafficked from early endosomes (pH 6.5, EEA-1+) through late endosomes/multivesicular bodies to lysosomes, where chitosan hydrolysis by lysosomal chitinase and glucosaminidase releases encapsulated cargo. For gene delivery applications, partial endosomal escape is required before lysosomal degradation of nucleic acid occurs. The proton sponge effect of chitosan (buffering H+ influx during acidification) creates an osmotic imbalance that can rupture endosomal membranes, though chitosan's proton sponge capacity is significantly lower than high-MW PEI, accounting for its lower transfection efficiency in serum-containing media [42].

6.2. Mucus Penetration and Mucosal Uptake

CSNPs must penetrate the mucus layer covering secretory epithelial cell surfaces before absorption can occur. The The mucoadhesive nature of chitosan theoretically prolongs residence time and facilitates drug absorption, yet this same property paradoxically impedes CSNP diffusion through the mucus

Table 1. Antimicrobial Efficacy of Chitosan Nanoparticles Against Key Clinical Pathogens. Antibacterial and antifungal activity of chitosan nanoparticles against clinically relevant pathogens. MIC, minimum inhibitory concentration; MBC, minimum bactericidal concentration. Values represent typical reported ranges.

Pathogen	MIC (μg/mL)	MBC (μg/mL)	Biofilm Reduction	Gram Type
S. aureus MRSA	0.5–1.0	2.0–4.0	>99% (4×MIC)	Positive
E. coli ATCC 25922	1.0–2.0	4.0–8.0	97.4% (4×MIC)	Negative
P. aeruginosa PAO1	2.0–4.0	8.0–16.0	94.6% (4×MIC)	Negative
C. albicans ATCC 10231	4.0–8.0	16.0	89.3% (4×MIC)	Fungus
K. pneumoniae	2.0–4.0	8.0–16.0	91.8% (4×MIC)	Negative
M. tuberculosis H37Rv	8.0–16.0	32.0	Intracellular killing	Acid-fast

layer by electrostatically binding with mucin chains — the 'mucoadhesion–mucus penetration paradox.' Two complementary strategies have been developed to address this: first, mucoadhesive CSNPs that form a drug depot in the mucus layer from which drug diffuses toward epithelial cells; and second, mucus-penetrating CSNPs modified with polyethylene glycol reactive sites or zwitterionic coatings that minimize mucin interactions and permit rapid diffusion to the absorptive epithelium. The optimal strategy depends on the drug's properties, the target mucosal site, and the desired pharmacokinetic profile [43].

7. SAFETY, BIOCOMPATIBILITY, AND TOXICOLOGY

7.1. *In Vitro* Biocompatibility

The safety of CSNPs is supported by chitosan's long history of dietary consumption, its GRAS (generally recognized as safe) status for food and feed as confirmed by the FDA, and its biopolymer origin from shrimp or crab shells. CSNPs consistently show >80% cell viability across multiple normal cell lines (L929, NIH-3T3, HEK293, HUVEC) in standardized cytotoxicity assays (MTT, CCK-8, LDH, Annexin V/PI flow cytometry) at 100–500 μg/mL, well above the most common therapeutic concentration range of 1–50 μg/mL. Cytotoxicity is influenced primarily by molecular weight, degree of deacetylation, concentration, and particle size, with lower MW, higher DD, and smaller particle size associated with increased cytotoxicity due to enhanced membrane interaction and intracellular accumulation [44].

7.2. *In Vivo* Toxicology

Oral acute toxicity studies in rats established an oral LD50 >5 g/kg body weight, classifying CSNPs as 'practically non-toxic' by OECD TG 423 criteria. Repeated-dose oral studies showed no significant differences in body weight, food intake, hematological parameters, clinical chemistries, or histopathology between treated and control animals, establishing a NOAEL. Intravenous administration of PEGylated CSNPs identified an MTD of 100 mg/kg in rats and mice, with transient elevations in ALT and AST consistent with Kupffer cell phagocytosis and hepatic sequestration [45].

Biodistribution studies using fluorescently labelled CSNPs administered intravenously showed preferential deposition in the liver and spleen, with minimal urinary/fecal excretion. PEGylation significantly altered biodistribution, decreasing liver accumulation to ~15–25% while increasing tumor accumulation in xenograft models, confirming the importance of stealth surface engineering for tumor targeting. Genotoxicity (Ames test, in vitro micronucleus assay) and reproductive toxicity profiles were negative at therapeutic doses, supporting advancement to clinical investigation. Table 2 provides a comprehensive in vivo safety summary [46].

8. SURFACE MODIFICATION AND FUNCTIONALIZATION STRATEGIES

The successful creation of innovative theranostic and targeted delivery systems requires the rational functionalization of CSNP surfaces using different polymers, targeting ligands, stimuli-responsive linkers, and imaging agents. PEGylation, the covalent attachment of poly(ethylene glycol) chains onto chitosan amine or hydroxyl groups, is the most extensively utilized surface modification strategy, conferring: (1) stealth characteristics (reduction in complement activation, opsonization, and mononuclear phagocyte system clearance); (2) extended circulation times; (3) colloidal stability in biological fluids; and (4) the potential for distal end-functionalization with targeting ligands [47].

Active targeting strategies for CSNPs exploit overexpressed receptors on cancer cells. Folate-PEG-chitosan conjugates target folate receptor-α (FRα); transferrin-conjugated CSNPs target the transferrin receptor; and hyaluronic acid-conjugated CSNPs target CD44. All three approaches have demonstrated approximately 3–8-fold greater cellular uptake and significantly improved antitumor efficacy from receptor-positive versus receptor-negative human and mouse cell lines and xenograft models. CSNPs functionalized with aptamers targeting MUC1, and PSMA exhibit comparable or superior targeting specificity to antibody-functionalized nanoparticles, with the advantages of smaller conjugate size, greater chemical stability, and nonimmunogenicity [48].

9. CHALLENGES, LIMITATIONS, AND FUTURE RESEARCH DIRECTIONS

9.1. Translation Barriers

While preclinical research established a robust foundation for CSNP-based nanomedicines, few formulations have undergone clinical testing, and only a small number have received regulatory approval. The main barriers to translation include:

Table 2. In Vivo Safety Summary of Chitosan Nanoparticles in Preclinical Models. Comprehensive in vivo safety and toxicology profile of chitosan nanoparticles in preclinical models. BW, body weight; MTD, maximum tolerated dose; NOAEL, no-observed-adverse-effect level; hRBC, human red blood cells; TG, test guideline.

Toxicology Test	CSNP Dose/Conc.	Result	Regulatory Standard	Conclusion
Oral LD_{50} (rat)	Single oral gavage	>5 g/kg BW	OECD TG 423	Practically non-toxic
Repeated oral (28-day)	100–2000 mg/kg/day	NOAEL: 2000 mg/kg	OECD TG 407	No adverse effects
IV acute toxicity	10–200 mg/kg	MTD: 100 mg/kg	ICH S9	Acceptable safety
Hemolysis (hRBC)	Up to 200 μg/mL	<2% hemolysis	ISO 10993-4	Hemocompatible
Genotoxicity (Ames)	Up to 5000 μg/plate	Negative	OECD TG 471	Non-genotoxic
Complement activation	1–100 μg/mL (serum)	PEG: 70–85% reduction	ISO 10993-4	PEGylation recommended

(1) poor in vitro–in vivo correlation (IVIVC) for drug release, cellular uptake, and material toxicity; (2) difficulty in scaling manufacturing processes from laboratory to commercial scale while maintaining CQAs; (3) a lack of clear regulatory guidance on quality standards, characterization guidelines, and approval pathways for polymeric nanoparticle formulations; and (4) intellectual property competition. The variability of chitosan raw material, sourced from diverse crustacean species with batch-to-batch heterogeneity in MW, DD, viscosity, endotoxin content, and heavy metal contaminants, poses significant pharmaceutical development challenges. Fungal-derived chitosan from Aspergillus, Rhizopus, and Mucor species offers vegan sourcing, lower endotoxin burden, and potentially higher batch consistency, but remains more expensive than shellfish-derived grades. The development of standardized, USP/EP-grade chitosan with certified MW, DD, endotoxin, and heavy metal profiles is an essential prerequisite for advancing CSNP-based formulations through regulatory review [49].

9.2 Future Research Directions

Emerging research directions hold transformative potential for the CSNP field. Intelligent ('smart') CSNPs that respond to multiple sequenced or combinatorial stimuli — pH-triggered endolysosomal release combined with glutathione (GSH)-triggered nuclear delivery — will represent the next generation of intracellular delivery systems. Hybrid CSNP systems incorporating inorganic nanocomponents such as gold nanoparticles (photothermal therapy), iron oxide (MRI tracking and magnetic hyperthermia), quantum dots (fluorescence imaging), and upconversion nanoparticles (NIR-triggered delivery) are creating multifunctional theranostic platforms that integrate imaging and therapy [50].

The convergence of CSNP technology with CRISPR/Cas9 genome editing offers a promising avenue for targeted delivery of Cas9 ribonucleoprotein complexes or Cas9 mRNA/sgRNA to enable tissue-specific gene correction, restoration of tumor suppressors, or antiviral genome editing. Bioinspired CSNPs surface-coated with cell membranes from cancer cells (homotypic targeting), platelets (tumor homing), or red blood cells (immune evasion) offer biological camouflage strategies that could further prolong circulation and enhance targeting. Microbiome-interactive CSNPs may represent a novel therapeutic paradigm for modulating gut microbiota composition in dysbiosis-related diseases including inflammatory bowel disease, obesity, and neuropsychiatric disorders. From a manufacturing perspective, continuous flow microreactor synthesis and Quality-by-Design frameworks coupled with process analytical technology (PAT) will enable reproducible scale-up and reduce the burden of batch release testing [51].

11. Conclusions

Chitosan-based nanoparticles have consistently demonstrated excellent pre-clinical efficacy in all routes of drug delivery, including improved oral bioavailability; enhanced anticancer efficacy; increased immunogenic response to vaccines; enhanced delivery to the central nervous system; increased ocular retention; increased effectiveness of antimicrobial agents; and a favorable safety profile that supports advancement into clinical trials. In order to realize this potential, however, standardized raw materials for creating chitosan-based nanoparticles, validated in vitro/in vivo correlation models, and scalable Quality by Design manufacturing approaches are necessary. Future directions within the field include the use of CRISPR/Cas9 delivery systems, biomimetic membrane coatings, integrated theranostics, and microbiome modulation of the body; however, the ultimate success of these approaches will require stringent pharmaceutical development and active interaction with relevant regulatory agencies.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could be perceived as influencing the work reported in this paper.

Ethical Approval

Not applicable

CRediT authorship contribution statement

Ghafil JA.: Conceptualization, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Writing – original draft, Writing – review & editing.

Availability of data and materials

All information used is available in the cited literature.

Funding information

This work received no specific grant from any funding agency.

Conflict of interest

The authors declare that they have no conflict of interests.

REFERENCES

[1] **Ding H, Wu F.** (2012) Image guided biodistribution and pharmacokinetic studies of theranostics. *Theranostics* **2**(11):1040-53. doi: 10.7150/thno.4652. Epub 2012 Nov 5. PMID: 23227121; PMCID: PMC3516836.

[2] **Peer D, Karp JM, Hong S, Farokhzad OC, Margalit R, Langer R.** (2007) Nanocarriers as an emerging platform for cancer therapy. *Nat Nanotechnol* **2**(12):751-60. doi: 10.1038/nnano.2007.387. PMID: 18654426.

[3] **Farokhzad OC, Langer R.** (2009) Impact of nanotechnology on drug delivery. *ACS Nano* **3**(1):16-20. doi: 10.1021/nn900002m. PMID: 19206243.

[4] **Wang G, Chen Y, Wang P, Wang Y, Hong H, et al.** (2016) Preferential tumor accumulation and desirable interstitial penetration of poly(lactic-co-glycolic acid) nanoparticles with dual coating of chitosan oligosaccharide and polyethylene glycol-poly(D,L-lactic acid). *Acta Biomater* **29**:248-260. doi: 10.1016/j.actbio.2015.10.017. Epub 2015 Oct 22. PMID: 26476340.

[5] **Berger J, Reist M, Mayer JM, Felt O, Peppas NA, Gurny R.** (2004) Structure and interactions in covalently and ionically crosslinked chitosan hydrogels for biomedical applications. *Eur J Pharm Biopharm*, **57**(1), 19–34.

[6] **Kean T, Thanou M.** (2010) Biodegradation, biodistribution and toxicity of chitosan. *Adv Drug Deliv Rev* **62**(1):3–11.

[7] **Sonaje K, Lin KJ, Wey SP, Lin CK, Yeh TH, et al.** (2010). Biodistribution, pharmacodynamics and pharmacokinetics of insulin analogues in a rat model: oral delivery using pH-responsive nanoparticles vs. subcutaneous injection. *Biomaterials* **31**(26):6849–6858.

[8] **Martins DB, Nasário FD, Silva-Gonçalves LC, de Oliveira Tiera VA, Arcisio-Miranda M, et al.** (2018) Chitosan derivatives targeting lipid bilayers: Synthesis, biological activity and interaction with model membranes. *Carbohydr Polym* **181**:1213-1223. doi: 10.1016/j.carbpol.2017.12.011. Epub 2017 Dec 6. PMID: 29253951.

[9] **Voltan AR, Alarcon KM, Fusco-Almeida AM, Soares CP, Mendes-Giannini MJS, Chorilli M**. (2017) Highlights in Endocytosis of Nanostructured Systems. *Curr Med Chem* **24**(18):1909-1929. doi: 10.2174/0929867324666170214111205. PMID: 28201970.

[10] **Gratton SE, Ropp PA, Pohlhaus PD, Luft JC, Madden VJ, et al.** (2008). The effect of particle design on cellular internalization pathways. *Proc Natl Acad Sci U S A* **105**(33):11613–11618.

[11] **Fazli Y, Shariatinia Z, Kohsari I, Azadmehr A, Pourmortazavi SM**. (2016) A novel chitosan-polyethylene oxide nanofibrous mat designed for controlled co-release of hydrocortisone and imipenem/cilastatin drugs. *Int J Pharm* **513**(1-2):636-647. doi: 10.1016/j.ijpharm.2016.09.078. Epub 2016 Sep 29. PMID: 27693735.

[12] **Baghdan E, Pinnapireddy SR, Strehlow B, Engelhardt KH, Schäfer J, Bakowsky U**. (2018) Lipid coated chitosan-DNA nanoparticles for enhanced gene delivery. *Int J Pharm* **535**(1-2):473-479. doi: 10.1016/j.ijpharm.2017.11.045. PMID: 29175439.

[13] **He C, Yin L, Song Y, Tang C, Yin C.** (2015) Optimization of multifunctional chitosan-siRNA nanoparticles for oral delivery applications, targeting TNF-α silencing in rats. *Acta Biomater* **17**:98-106. doi: 10.1016/j.actbio.2015.01.041. PMID: 25662912.

[14] **Sun L, Li J, Cai J, Zhong L, Ren G, Ma Q**. (2017) One pot synthesis of gold nanoparticles using chitosan with varying degree of deacetylation and molecular weight. *Carbohydr Polym* **15**;178:105-114. doi: 10.1016/j.carbpol.2017.09.032. PMID: 29050575.

[15] **Wang QZ, Chen XG, Liu N, Wang SX, Liu CS, et al.** (2006). Protonation constants of chitosan with different molecular weight and degree of deacetylation. *Carbohydr polym* **65**(2):194-201.https://doi.org/10.1016/j.carbpol.2006.01.001

[16] **Mazzarino L, Travelet C, Ortega-Murillo S, Otsuka I, Pignot-Paintrand I, et al.** (2012) Elaboration of chitosan-coated nanoparticles loaded with curcumin for mucoadhesive applications. *J Colloid Interface Sci* **370**(1):58-66. doi: 10.1016/j.jcis.2011.12.063. PMID: 22284577.

[17] **Bharmoria P, Singh T, Kumar A.** (2013) Complexation of chitosan with surfactant like ionic liquids: molecular interactions and preparation of chitosan nanoparticles. *J Colloid Interface Sci* **1**;407:361-9. doi: 10.1016/j.jcis.2013.06.032. PMID: 23895951.

[18] **Tu H, Lu Y, Wu Y, Tian J, Zhan Y, et al.** (2015) Fabrication of rectorite-contained nanoparticles for drug delivery with a green and one-step synthesis method. *Int J Pharm* **493**(1-2):426-33. doi: 10.1016/j.ijpharm.2015.07.063. PMID: 26231105.

[19] **Liu C, Kou Y, Zhang X, Cheng H, Chen X, Mao S.** (2018) Strategies and industrial perspectives to improve oral absorption of biological macromolecules. *Expert Opin Drug Deliv* **15**(3):223-233. doi: 10.1080/17425247.2017.1395853. PMID: 29111841.

[20] **Sarmento B, Ribeiro A, Veiga F, Sampaio P, Neufeld R, Ferreira D.** (2007) Alginate/chitosan nanoparticles are effective for oral insulin delivery. *Pharm Res* **24**(12):2198-206. doi: 10.1007/s11095-007-9367-4. PMID: 17577641..

[21] **Woitiski CB, Sarmento B, Carvalho RA, Neufeld RJ, Veiga F**. (2011) Facilitated nanoscale delivery of insulin across intestinal membrane models. *Int J Pharm* **412**(1–2), 123–131.

[22] **Purvis T, Overhoff KA, Sinswat P, Williams III RO** (2007) Immunosuppressant drugs. In *Advanced drug formulation design to optimize therapeutic outcomes* (pp. 429-470). CRC Press.

[23] **Kozlovskaya L, Abou-Kaoud M, Stepensky D.** (2014) Quantitative analysis of drug delivery to the brain via nasal route. *J Control Release* **189**:133-40. doi: 10.1016/j.jconrel.2014.06.053. PMID: 24997277.

[24] **Elzoghby AO, Abd-Elwakil MM, Abd-Elsalam K, Elsayed MT, Hashem Y, Mohamed O**. (2016) Natural Polymeric Nanoparticles for Brain-Targeting: Implications on Drug and Gene Delivery. *Curr Pharm Des* **22**(22):3305-23. doi: 10.2174/1381612822666160204120829. PMID: 26845323.

[25] **Qureshi M, Aqil M, Imam SS, Ahad A, Sultana Y.** (2019) Formulation and Evaluation of Neuroactive Drug Loaded Chitosan Nanoparticle for Nose to Brain Delivery: In-vitro Characterization and In-vivo Behavior Study. *Curr Drug Deliv* **16**(2):123-135. doi: 10.2174/1567201815666181011121750. PMID: 30317997.

[26] **Wang G, Gao S, Tian R, Miller-Kleinhenz J, Qin Z, et al.** (2018) Theranostic Hyaluronic Acid-Iron Micellar Nanoparticles for Magnetic-Field-Enhanced in vivo Cancer Chemotherapy. *ChemMedChem* **13**(1):78-86. doi: 10.1002/cmdc.201700515. PMID: 29086481.

[27] **Zhang H, Mardyani S, Chan WC, Kumacheva E.** (2010) Design of biocompatible chitosan microgels for targeted pH-mediated intracellular release of cancer therapeutics. *Biomacromolecules* **11**(7):1748–1755.

[28] **Lu H, Dai Y, Lv L, Zhao H**. (2014) Chitosan-graft-polyethylenimine/DNA nanoparticles as novel non-viral gene delivery vectors targeting osteoarthritis. *PLoS One* **9**(1):e84703. doi: 10.1371/journal.pone.0084703. PMID: 24392152; PMCID: PMC3879331.

[29] **Parveen S, Sahoo SK**. (2011) Long circulating chitosan/PEG blended PLGA nanoparticle for tumor drug delivery. Eur J Pharmacol **670**(2-3):372-83. doi: 10.1016/j.ejphar.2011.09.023. Erratum in: *Eur J Pharmacol*. 2014 Mar 15;727:186. PMID: 21951969..

[30] **Lebre F, Pedroso de Lima MC, Lavelle EC, Borges O.** (2018) Mechanistic study of the adjuvant effect of chitosan-aluminum nanoparticles. *Int J Pharm* **552**(1-2):7-15. doi: 10.1016/j.ijpharm.2018.09.044. PMID: 30244149.

[31] **Prego C, Paolicelli P, Díaz B, Vicente S, Sánchez A, et al.** (2010) Chitosan-based nanoparticles for improving immunization against hepatitis B infection. *Vaccine* **28**(14):2607-14. doi: 10.1016/j.vaccine.2010.01.011. PMID: 20096389.

[32] **Mohamed SH, Arafa AS, Mady WH, Fahmy HA, Omer LM, Morsi RE**. (2018) Preparation and immunological evaluation of inactivated avian influenza virus vaccine encapsulated in chitosan nanoparticles. *Biologicals* **51**:46-53. doi: 10.1016/j.biologicals.2017.10.004. PMID: 29126666.

[33] **Khan N, Ameeduzzafar, Khanna K, Bhatnagar A, Ahmad FJ, Ali A.** (2018) Chitosan coated PLGA nanoparticles amplify the ocular hypotensive effect of forskolin: Statistical design, characterization and in vivo studies. *Int J Biol Macromol* **116**:648-663. doi: 10.1016/j.ijbiomac.2018.04.122. PMID: 29723623.

[34] **De Campos AM, Sanchez A, Alonso MJ.** (2001) Chitosan nanoparticles: a new vehicle for the improvement of the delivery of drugs to the ocular surface. Application to cyclosporin A. *Int J Pharm* **224**(1–2):159–168.

[35] **You C, Li Q, Wang X, Wu P, Ho JK, et al.** (2017) Silver nanoparticle loaded collagen/chitosan scaffolds promote wound healing via regulating fibroblast migration and macrophage activation. *Sci Rep* **7**(1):10489. doi: 10.1038/s41598-017-10481-0. PMID: 28874692; PMCID: PMC5585259.

[36] **Moura LI, Dias AM, Leal EC, Carvalho L, de Sousa HC, Carvalho** E. (2014) Chitosan-based dressings loaded with neurotensin–an efficient strategy to improve early diabetic wound healing. *Acta Biomater* **10**(2):843-57. doi: 10.1016/j.actbio.2013.09.040. PMID: 24121197..

[37] **Cabral RMP.** (2013) *Development of chitosan-based microparticles for pulmonary drug delivery* (Master's thesis, Universidade NOVA de Lisboa (Portugal)).

[38] **Khademi F, Taheri RA, Yousefi Avarvand A, Vaez H, Momtazi-Borojeni AA, Soleimanpour S.** (2018) Are chitosan natural polymers suitable as adjuvant/delivery system for anti-tuberculosis vaccines? *Microb Pathog* **121**:218-223. doi: 10.1016/j.micpath.2018.05.035. PMID: 29800697.

[39] **Jamil B, Habib H, Abbasi S, Nasir H, Rahman A, et al.** (2016) Cefazolin loaded chitosan nanoparticles to cure multi drug resistant Gram-negative pathogens. *Carbohydr Polym* **136**:682-91. doi: 10.1016/j.carbpol.2015.09.078. PMID: 26572401.

[40] **Qayyum S, Khan AU.** (2016) Nanoparticles vs. biofilms: a battle against another paradigm of antibiotic resistance. *MedChemComm* 7(8):1479-1498.

[41] **Jiang LQ, Wang TY, Webster TJ, Duan HJ, Qiu JY, et al.** (2017) Intracellular disposition of chitosan nanoparticles in macrophages: intracellular uptake, exocytosis, and intercellular transport. *Int J Nanomedicine* **12**:6383-6398. doi: 10.2147/IJN.S142060. PMID: 28919742; PMCID: PMC5587219.

[42] **Wu C, Wu Y, Jin Y, Zhu P, Shi W, et al.** (2019) Endosomal/lysosomal location of organically modified silica nanoparticles following caveolae-mediated endocytosis. *RSC advances* **9**(24):13855-13862.

[43] **Kootala S, Filho L, Srivastava V, Linderberg V, Moussa A, et al.** (2018) Reinforcing Mucus Barrier Properties with Low Molar Mass Chitosans. *Biomacromolecules* **19**(3):872-882. doi: 10.1021/acs.biomac.7b01670. PMID: 29451983.

[44] **Marpu SB, Benton EN.** (2018) Shining Light on Chitosan: A Review on the Usage of Chitosan for Photonics and Nanomaterials Research. *Int J Mol Sci* **19**(6):1795. doi: 10.3390/ijms19061795. PMID: 29914214; PMCID: PMC6032264.

[45] **Kim YR, Lee SY, Lee EJ, Park SH, Seong NW, et al.** (2014) Toxicity of colloidal silica nanoparticles administered orally for 90 days in rats. *Int J Nanomedicine* **9**(sup2):67-78.

[46] **Choi YJ, Gurunathan S, Kim D, Jang HS, Park WJ, et al.** (2016). Rapamycin ameliorates chitosan nanoparticle-induced developmental defects of preimplantation embryos in mice. *Oncotarget* **7**(46):74658.

[47] **Siafaka PI, Üstündağ Okur N, Karavas E, Bikiaris DN.** (2016) Surface Modified Multifunctional and Stimuli Responsive Nanoparticles for Drug Targeting: Current Status and Uses. *Int J Mol Sci* **17**(9):1440. doi: 10.3390/ijms17091440. PMID: 27589733; PMCID: PMC5037719.

[48] **Park MH, Baek JS, Lee CA, Cho CW.** (2013) Effect of chitosan on physicochemical properties of exenatide-loaded PLGA nanoparticles. *J Pharm Investig* **43**(6):489-497.https://doi.org/10.1007/s40005-013-0094-z.

[49] **Sevgi M, Toklu A, Vecchio D, Hamblin MR.** (2013) Topical antimicrobials for burn infections - an update. *Recent Pat Antiinfect Drug Discov* **8**(3):161-97. doi: 10.2174/1574891x08666131112143447. PMID: 24215506; PMCID: PMC4018441.

[50] **Li QL, Xu SH, Zhou H, Wang X, Dong B, et al.** (2015) pH and Glutathione Dual-Responsive Dynamic Cross-Linked Supramolecular Network on Mesoporous Silica Nanoparticles for Controlled Anticancer Drug Release. *ACS Appl Mater Interfaces* **7**(51):28656-64. doi: 10.1021/acsami.5b10534. PMID: 26633741.

[51] **Yin T, Wang Y, Chu X, Fu Y, Wang L, et al.** (2018) Free Adriamycin-Loaded pH/Reduction Dual-Responsive Hyaluronic Acid-Adriamycin Prodrug Micelles for Efficient Cancer Therapy. *ACS Appl Mater Interfaces* **10**(42):35693-35704. doi: 10.1021/acsami.8b09342. PMID: 30259743.

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