Innovation of Chemistry & Materials for Sustainability

Volume 1, Issue 1, Jul-Dec **2024**

REVIEW ARTICLE

ISSN (Print): 3049 – 2548
ISSN (Online): 3049 – 0146

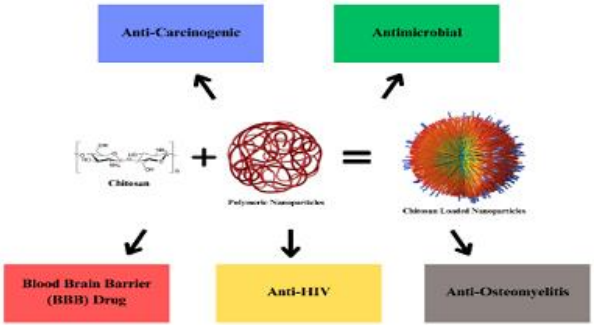
Composites of Chitosan for Biomedical Applications

Ayanjeet Chowdhury[1], Samyak Dhale[1], RBK Dinesh Kumar[1], Andrew Biju John[1], Jaya Lakkakula[1,2]* and Nilesh S. Wagh[1,3]*

[1]Amity Institute of Biotechnology, Amity University Maharashtra, Mumbai, 410206, India
[2]Centre for Computational Biology and Translational Research, Amity University Maharashtra, Mumbai, 410206, India
[3]Centre for Drug Discovery and Development, Amity University Maharashtra, Mumbai, 410206, India

*Correspondence: spencerjaya@gmail.com; waghnil@gmail.com

Abstract: Chitosan (CS) is a cationic polysaccharide that consists of jumble distributed units of N-acetyl-D-glucosamine (acetylated unit) and β-(1→4)-linked D-glucosamine (deacetylated unit). CS has gained significant importance in the field of biomedicine due to its non-toxicity, biodegradability, and biocompatibility properties. It has numerous potential applications, including in the development of bandages that can reduce bleeding and serve as antibacterial agents, as well as DDSs that can transport medication across the skin and BBB. CS can be used alone or in amalgamation with antibiotics and extracts to create antimicrobial wound dressings that are effective in treating infections. Overall, CS and its derivatives hold great promise for biomedical implicatives, particularly in wound healing and DD. Due to its qualities, CS-based NPs are being studied as possible DDS against diseases like Leishmaniasis, Bacterial Diseases, and Cancer. Throughout the chapter we will have an overview of these properties of CS, their possible applications in the biomedical field and their possible role against these diseases.



Keywords: *chitosan, drug delivery system (DDS), blood brain barrier, cancer, antimicrobial*

Contents

1. Introduction

Chitosan, a biopolymer derived from chitin, has gained significant interest in biomedicine due to its unique properties. Composed of *β*-(1→4)-linked D-glucosamine and *N*-acetyl-D-glucosamine, it is biocompatible, biodegradable, and non-toxic, making it an excellent candidate for various biomedical uses. This review focuses on chitosan composites and their diverse biomedical applications, highlighting their biochemical and biomedical significance.[1]

Chitosan has strong antimicrobial properties because of its polycationic nature,[2] which permits it to interact with negatively charged microbial membranes, leading to cell death. Its antimicrobial activity extends to bacteria, fungi, and viruses, making it valuable in wound dressings, antimicrobial coatings, and drug delivery systems.[3]

In antiviral research, chitosan has shown promising results against HIV by inhibiting viral replication and preventing the virus from binding to host cells. This makes chitosan a potential tool for developing new therapies to control viral load and prevent HIV progression.[4]

Chitosan also exhibits anti-osteomyelitis properties, addressing the challenge of delivering therapeutic agents to infected bone sites. Chitosan-based composites can deliver antibiotics directly to affected areas, promoting localized treatment and enhancing bone healing.[5]

Ayanjeet Chowdhury born and raised in Chennai, Inida. He completed his B.Tech. degree in Biotechnology from Amity University Maharastra, Mumbai. He is currently pursuing his final year M. Tech. in biotechnology from the same university. His areas of interest in biotechnology for future sustainability.

Samyak Dhale was born and raised in Mumbai, India. He completed his B.Tech. degree in Biotechnology from Amity University Maharastra, Mumbai and is currently pursuing PG Diploma in Archaeology in same institute. Samyak is building a career in Archeogenomics.

RBK Dinesh Kumar was born in Mumbai. He earned his B. Tech. degree in Biotechnology from Amity University Maharashtra, Mumbai. He was associated with Chaitanya Doshi, CEO of Kore AMMR (Additive Manufacturing and Medical Reconstruction) Pvt. Ltd for his B. Tech Project focused on developing microfluidic devices for cancer research and drug testing. Presently, Dinesh is employed as a Research Engineer at Kore AMMR Pvt. Ltd.

Andrew Biju John was born and broughup in Mumbai. He received his B.Tech. degree in Biotechnology degree from Amity University Maharastra, Mumbai. He did his B.Tech project with Dr. Sudhir Singh BARC, Mumbai on Validation and expression analysis of selected curcumin biosynthesis. Currently he is working as a Research Trainee at ACTREC.

Dr. Jaya Lakkakula completed her Ph.D. at the University of Johannesburg, South Africa, and is a recipient of the UJ-Commonwealth Bursary award. She is currently serving as an Assistant Professor at Amity University Maharastra, Mumbai. Her expertise lies in the green synthesis of nanoparticles, drug delivery, and the development of smart nanosensors, with a particular focus on cancer therapy.

Dr. Nilesh S. Wagh earned his Ph.D. in Biotechnology from Swami Ramanand Teerth Marathwada University in Nanded, Maharashtra, India. He is currently an Assistant Professor at Amity University Maharastra, Mumbai. His research focuses on plant phytochemicals and their molecular interactions, with contributions to medicine, agriculture, and functional foods through numerous articles and book chapters.

http://doi.org/10.63654/icms.2024.01076 *Innov. Chem. Mater. Sustain.* **2024**, *1*(1), 076-092
Received: 25 June 2024; Revised: 12 September 2024; Accepted: 14 December 2024; Published (online): 25 December 2024

Another key property of chitosan is its anticarcinogenic potential.[6] It has been shown to inhibit cancer cell growth through mechanisms such as apoptosis induction, cell proliferation inhibition, and metabolic disruption. These attributes make chitosan a potential adjuvant in cancer therapies, either alone or with standard treatments.[7]

Chitosan is also a promising material for drug delivery across the blood-brain barrier (BBB).[8] The BBB restricts the passage of most therapeutic agents, making treatment of neurological disorders difficult. Chitosan-based nanoparticles can cross the BBB, enabling effective drug delivery for diseases like Alzheimer's, Parkinson's, and brain tumors.[9]

The versatility of chitosan extends to forming composites with other materials, enhancing its properties and expanding its applications.[10] These composites can be designed to improve mechanical strength, control degradation, and boost biological activity. Combining chitosan with polymers, ceramics, and metals has led to innovations in tissue engineering, wound healing, and regenerative medicine.[11]

This review provides an in-depth overview of chitosan composites in biomedical applications, discussing their synthesis, characterization, and interaction with biological systems. It also explores the potential of chitosan composites to advance medical science, based on recent research and development.

2. Properties of Chitosan Nanoparticles

Chitosan nanoparticles have garnered considerable interest in biomedical research and applications due to their exceptional physicochemical properties and biocompatibility. These nanoparticles, typically ranging in size from 1 to 1000 nanometers, are produced through various methods such as ionic gelation, emulsion crosslinking, and self-assembly techniques.[12] The synthesis of chitosan nanoparticles involves the interplay of chitosan's amino groups and crosslinking agents or polyanions, resulting in a stable and uniform nanoscale structure. One of the critical attributes of chitosan nanoparticles is their size and surface charge, which significantly influence their interaction with biological systems. The small size of these nanoparticles allows for enhanced cellular uptake, improved tissue penetration, and increased surface area for functionalization with therapeutic agents or targeting ligands. Furthermore, the positive surface charge of chitosan nanoparticles, due to the protonation of amino groups in acidic environments, facilitates their interaction with negatively charged cell membranes, enhancing cellular internalization and bioavailability.

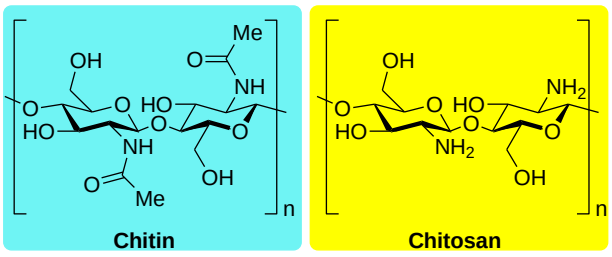


Figure 1: *The chemical structure of Chitin and Chitosan*

In addition to their size and charge, chitosan nanoparticles exhibit excellent biodegradability and biocompatibility, making them suitable for a wide range of biomedical applications (**Figure 1**).[13] The degradation of chitosan nanoparticles occurs through enzymatic hydrolysis by lysozymes and other enzymes present in the human body, leading to the formation of non-toxic, bioresorbable byproducts such as glucosamine and N-acetylglucosamine. This biodegradability ensures that chitosan nanoparticles do not accumulate in tissues, reducing the risk of long-term toxicity and adverse effects. Moreover, chitosan is well-tolerated by the human body, exhibiting minimal immunogenicity and cytotoxicity. This inherent biocompatibility, coupled with its ability to be chemically modified, allows for the creation of tailor-made chitosan nanoparticles with specific properties and functionalities. These modifications can enhance the solubility, stability, and targeting capabilities of chitosan nanoparticles, further broadening their application in drug delivery, gene therapy, and tissue engineering.[14]

One of the most notable properties of chitosan nanoparticles is their mucoadhesive nature, which arises from the interaction between the cationic chitosan and the negatively charged mucin found in mucus layers.[15] This mucoadhesive property prolongs the residence time of chitosan nanoparticles at mucosal surfaces, enhancing the absorption and bioavailability of encapsulated drugs or therapeutic agents. This feature is particularly advantageous in the delivery of drugs via mucosal routes such as oral, nasal, ocular, and vaginal administration. Additionally, chitosan nanoparticles possess inherent antimicrobial activity, attributed to their ability to disrupt microbial cell membranes and inhibit the growth of bacteria, fungi, and viruses. This antimicrobial property not only makes chitosan nanoparticles effective in preventing infections but also enhances their potential as carriers for antimicrobial agents, providing a synergistic effect.[16] Furthermore, chitosan nanoparticles have been extensively studied for their ability to facilitate the transport of drugs across biological barriers, including the blood-brain barrier (BBB), gastrointestinal tract, and pulmonary epithelium. This property is crucial for the development of targeted and efficient drug delivery systems, enabling the treatment of various diseases and conditions that are otherwise challenging to address with conventional drug formulations.

2.1 Anti – Carcinogenic

Chitosan nanoparticles have attracted considerable attention in cancer research due to their potent anti-carcinogenic properties, which hold promise for developing more effective and targeted cancer therapies. These nanoparticles exhibit a multifaceted mechanism of action against cancer cells, including the induction of apoptosis, inhibition of cell proliferation, and disruption of tumor cell metabolism.[17] Apoptosis, or programmed cell death, is a crucial process that is often dysregulated in cancer cells, allowing them to proliferate uncontrollably. Chitosan nanoparticles can trigger apoptosis by activating caspase enzymes and promoting the discharge of cytochrome c from mitochondria, which are key steps in the apoptotic pathway. Additionally, chitosan nanoparticles can interfere with the cell cycle of cancer cells, arresting their progression in critical phases such as the G2/M phase, thereby inhibiting cell division and growth. This cell cycle arrest is often mediated through the modulation of cyclin-dependent kinases (CDKs) and other regulatory proteins that are essential for cell cycle progression.[18]

Moreover, chitosan nanoparticles can disrupt the metabolic processes of cancer cells, which are typically characterized by high rates of glycolysis and altered energy metabolism, known as the Warburg effect. By interfering with these metabolic pathways, chitosan nanoparticles can reduce the energy supply to cancer cells, impairing their growth and survival. Metabolic disruption is often achieved through the inhibition of key enzymes involved in glycolysis and oxidative phosphorylation, resulting in reduced ATP production and elevated levels of reactive oxygen species (ROS) within the cancer cells. The upraised ROS levels can persuade oxidative stress, further damaging cellular components and promoting cell death. This multifaceted approach not only targets cancer cells directly but also create an unfavorable environment for their survival and proliferation.[19]

The surface properties of chitosan nanoparticles also play a crucial role in their anti-carcinogenic efficacy. Functionalization of chitosan nanoparticles with specific ligands, such as folic acid, peptides, or antibodies, can enhance their targeting capabilities, allowing for selective delivery to cancer cells while sparing healthy tissues. This targeted delivery is particularly important in minimizing the side effects associated with conventional chemotherapy, which often affects both cancerous and non-cancerous cells. The enhanced permeability and retention (EPR) effect is a phenomenon in which nanoparticles selectively accumulate in tumor tissues due to their sieve-like vasculature and poor

lymphatic drainage, further aids in the selective targeting of chitosan nanoparticles. Once localized in the tumor microenvironment, the nanoparticles can release their therapeutic payload in a controlled manner, maximizing the anti-cancer effects while reducing systemic toxicity.[20]

Furthermore, chitosan nanoparticles can serve as carriers for delivering a range of anti-cancer agents, including chemotherapeutic drugs, siRNA, and genes, enhancing their therapeutic efficacy. The encapsulation of chemotherapeutic drugs within chitosan nanoparticles can improve their solubility, stability, and bioavailability, while also protecting them from premature degradation. This encapsulation also allows for a controlled and sustained drug release, maintaining therapeutic concentrations at the tumor site for extended periods. In gene therapy, chitosan nanoparticles can facilitate the delivery of genetic material into cancer cells, promoting the expression of tumor-suppressor genes or silencing oncogenes. The non-viral nature of chitosan nanoparticles makes them a safer alternative to viral vectors, reducing the risk of immunogenicity and insertional mutagenesis.

For example, Jaiswal *et al.* (2019) synthesized a methyl methacrylate (MMA) modified chitosan (CS) conjugate (CSMMA) through a Michael addition reaction, aiming to create a novel biopolymer for gene and drug delivery.[21] The resulting conjugate exhibited a highly porous framework, confirmed by FT-IR, ^{1}H NMR, X-ray diffraction spectrometry, and SEM analysis. The CSMMA demonstrated significant potential as a gene delivery agent, showing good transfection efficacy in various mammalian cancer cell lines (A549, HeLa, and HepG2). Curcumin-loaded CSMMA nanoparticles were prepared and characterized for drug delivery applications. These nanoparticles achieved maximal entrapment efficiency of up to 68% and exhibited pH-sensitive drug release, with more rapid release at pH 5.0 compared to physiological pH. This study underscores the potential of CSMMA nanoparticles in targeted drug delivery systems, improving the bioavailability and therapeutic effectiveness of encapsulated drugs (**Figure 2**).[21]

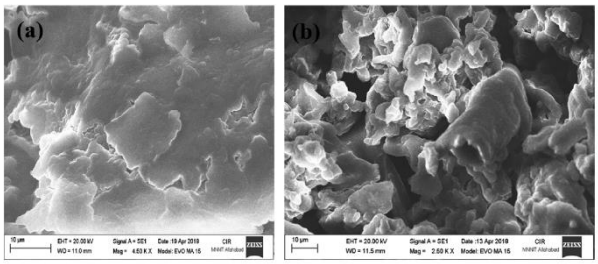


Figure 2: *SEM image of (a) chitosan (b) chitosan conjugated with methyl methacrylate [Represented with permission from Ref [5].*

Another study conducted by Subramanian *et al.* (2006), explored the use of chitosan nanoparticles for the delivery of phytochemicals in chemotherapy, emphasizing their potential in enhancing the bioavailability and therapeutic efficacy of poorly soluble compounds.[22] The study demonstrated that chitosan nanoparticles could effectively encapsulate and release various phytochemicals, such as curcumin, resulting in improved anti-cancer activity. The nanoparticles exhibited controlled release properties, maintaining a sustained release of the encapsulated drugs over an extended period. This approach not only improved the solubility and stability of the phytochemicals but also allowed for targeted delivery to cancer cells, thereby enhancing the therapeutic outcomes and reducing side effects associated with conventional chemotherapy. This research highlights the versatility of chitosan nanoparticles in developing efficient drug delivery systems for cancer treatment.[22]

Furthermore, Vivek *et al.* (2013) investigated the use of pH-responsive chitosan nanoparticles as carriers for tamoxifen in breast cancer therapy.[23] The study found that these nanoparticles could significantly improve the delivery and efficacy of tamoxifen, a commonly used anti-cancer drug. The chitosan nanoparticles were synthesized through ionic gelation, providing a stable and biocompatible platform for drug delivery. In vitro studies demonstrated enhanced cellular uptake and cytotoxicity against breast cancer cells, attributed to the pH-sensitive release mechanism of the nanoparticles. This pH-responsive behavior ensured that the drug was preferentially released in the acidic microenvironment of the tumor, maximizing its therapeutic effect while minimizing systemic toxicity. This research underscores the potential of chitosan nanoparticles in enhancing the targeted delivery and efficacy of anti-cancer drugs.[23]

Kim and co-workers (2014) evaluated polyethylenimine-grafted polyamidoamine (PAMAM) dendrimers for gene delivery, focusing on their efficiency and cytotoxicity. The study synthesized a novel gene delivery system by conjugating PAMAM dendrimers with low molecular weight polyethylenimine (PEI) through a Michael addition reaction. The resulting nanoparticles showed high gene transfection efficiency and low cytotoxicity in vitro. The researchers attributed these properties to the enhanced buffering capacity and reduced aggregation of the nanoparticles. The study highlighted the potential of these modified PAMAM dendrimers in delivering genetic material effectively and safely, paving the way for their application in gene therapy and other biomedical fields.[24–26]

Park *et al.* (2006) investigated the use of glycol chitosan nanoparticles for the delivery of doxorubicin, a widely used chemotherapeutic agent.[27] The nanoparticles were synthesized through self-assembly and characterized for their size, surface charge, and drug loading capacity. In vivo studies demonstrated that these nanoparticles could efficiently accumulate in tumor tissues, enhancing the therapeutic efficacy of doxorubicin while reducing its systemic toxicity. The glycol chitosan nanoparticles provided a stable and biocompatible platform for drug delivery, with the potential to improve the therapeutic outcomes of chemotherapy. This research highlights the advantages of using chitosan-based nanoparticles for targeted drug delivery in cancer treatment, offering a promising strategy for enhancing the efficacy and safety of chemotherapeutic agents.[27]

In the investigation by Hattori and co-workers (2002), the focus was on the evaluation of the anticarcinogenic properties of chitosan and its derivatives. The study utilized real-time zymography and reverse zymography techniques to detect the activities of matrix metalloproteinases (MMPs) and their inhibitors. Chitosan, a biopolymer derived from chitin, was modified to enhance its therapeutic efficacy. The results demonstrated that chitosan derivatives could effectively inhibit MMPs, which are enzymes involved in the degradation of the extracellular matrix and are often associated with cancer metastasis. By inhibiting MMP activity, chitosan derivatives could potentially prevent the invasion and spread of cancer cells. This study provided significant insights into the molecular mechanisms through which chitosan exerts its anticarcinogenic effects, highlighting its potential as a therapeutic agent in cancer treatment.[28]

In a subsequent study, Zhang and Zhao (2015) explored the preparation, characterization, and evaluation of β-chitosan nanoparticles loaded with tea polyphenol-Zn complexes. These nanoparticles were designed to enhance the delivery and efficacy of bioactive compounds in cancer therapy. The research demonstrated that the β-chitosan nanoparticles significantly improved the stability and bioavailability of the tea polyphenol-Zn complex. Moreover, the in vitro cytotoxicity assays revealed that these nanoparticles exhibited potent anticarcinogenic activity against various cancer cell lines. The study concluded that β-chitosan nanoparticles could serve as an effective delivery system for natural polyphenols, providing a promising approach for cancer prevention and treatment.[29]

Deshpande's team (2017) investigated the use of zinc-complexed chitosan/TPP nanoparticles as a micronutrient nanocarrier suited for foliar application.[30] The study aimed to assess the anticarcinogenic potential of these nanoparticles when used to deliver zinc, an essential micronutrient with known anticancer properties. The findings indicated that the chitosan/TPP nanoparticles effectively delivered zinc to target sites, enhancing its bioavailability and anticarcinogenic activity. The in vitro studies showed that zinc-complexed

chitosan nanoparticles were capable of inhibiting the cancer cells proliferation and inducing apoptosis. These results underscore the potential of chitosan-based nanoparticles in enhancing therapeutic efficacy of micronutrients in cancer treatment.[30]

Rodrigues *et al.* (2012) focused on the biocompatibility of chitosan carriers with applications in drug delivery, particularly in the context of cancer therapy. The study evaluated the interaction of chitosan-based nanoparticles with biological systems, including their cytotoxicity, cellular uptake, and anticarcinogenic effects. The results showed that chitosan nanoparticles exhibited minimal cytotoxicity while effectively delivering anticancer drugs to target cells. The nanoparticles facilitated sustained drug release, enhancing the therapeutic outcomes in cancer treatment. This research highlighted the potential of chitosan carriers in improving the efficacy and safety of cancer therapeutics, emphasizing their role in advanced drug delivery systems (**Figure 3**).[31]

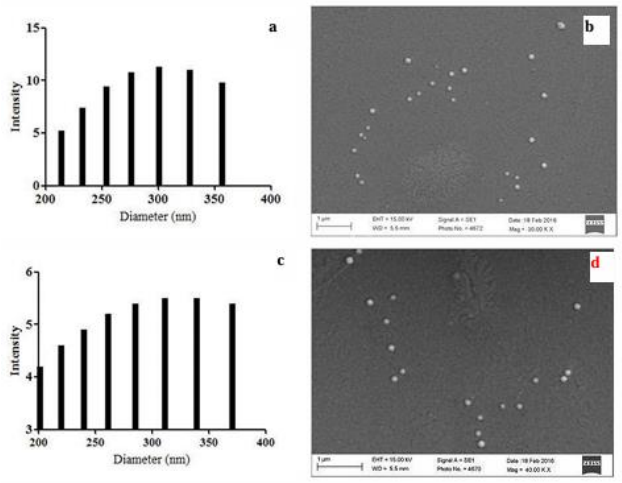


Figure 3: *Characterization of Zn-loaded chitosan nanoparticles. Size distribution of nanoparticles a: CNP, c: Zn-CNP; SEM image of nanoparticles showing spherical morphology, b: CNP and d: Zn-CNP (scale bar 10 µm). [Reprinted with permission from Ref 16]*

Again, Wang and co-workers (2004) examined the preparation, characterization, and antimicrobial activity of chitosan-Zn complexes. While the primary focus was on antimicrobial properties, the study also explored the potential anticarcinogenic effects of these complexes. The research demonstrated that chitosan-Zn complexes exhibited significant inhibitory effects on the growth of cancer cells, suggesting their potential use in cancer therapy. The study provided a comprehensive analysis of the physicochemical properties of chitosan-Zn complexes and their biological activities, offering valuable insights into their application as multifunctional therapeutic agents in both antimicrobial and anticancer treatments.[32]

Chang, Sekine, Chao, Hsu, and Chern (2017) examined the impact of chitosan on cancer progression, particularly focusing on its association with Wnt signaling in colon and hepatocellular carcinoma cells.[33] Their study revealed that chitosan significantly promoted cancer cell progression and stem cell properties. The researchers found that chitosan activates the Wnt/β-catenin signaling pathway, leading to the increased expression of stem cell markers such as CD44 and CD133, and enhancing the cancer stem cell-like properties of colon and hepatocellular carcinoma cells. These findings suggest that while chitosan is beneficial in many biomedical applications, its potential to enhance tumor progression through Wnt signaling necessitates caution when considering it for cancer therapy. The study emphasized the need for a thorough understanding of chitosan's biological interactions to mitigate its pro-tumorigenic effects.

Tharanathan and co-workers (2004) provided a comprehensive overview of the modifications of chitosan and their vast potential applications, including anticancer properties.[34] Their review highlighted the structural versatility of chitosan, which allows for various chemical modifications enhancing its functional properties. Notably, they discussed the potential of chitosan derivatives to exhibit selective cytotoxicity towards cancer cells while sparing normal cells. This selective cytotoxicity is attributed to the interaction of chitosan with the negatively charged cell membranes of cancer cells, leading to increased permeability and subsequent apoptosis. Their findings underscore the importance of optimizing chitosan's chemical modifications to maximize its anticancer efficacy while minimizing potential side effects. This paper provides a foundational understanding of chitosan's multifaceted role in cancer treatment, supporting the development of more effective chitosan-based therapeutic strategies.

Examining the effectiveness of chitosan nanoparticles as gene carriers, Yang (2015) fabricated a bidirectional hypoxia-responsive shRNA expression vector for colorectal-specific gene therapy. This study highlighted the potential of chitosan-based nanoparticles in delivering genetic material to target cells with high efficiency and low toxicity. The PEI/chitosan-TBA blend system developed by the researchers showed effective transfection in the HT-29 cell line, indicating that this hybrid system could be a promising carrier for gene delivery in vivo. The vector specifically knocked down gene expression under hypoxic conditions in colon cancer cells, inducing cell cycle arrest and increasing apoptosis. This study suggests that chitosan-based nanoparticles can be tailored for specific gene therapy applications, providing a new avenue for colorectal cancer treatment with reduced side effects compared to conventional therapies.[35]

Chen (2015) explored the use of hierarchical targeted hepatocyte mitochondrial multifunctional chitosan nanoparticles for anticancer drug delivery.[36] Their study focused on enhancing the delivery and efficacy of anticancer drugs specifically to hepatocellular carcinoma cells. The multifunctional chitosan nanoparticles were designed to target hepatocytes selectively and deliver drugs directly to the mitochondria, improving therapeutic outcomes. The nanoparticles exhibited a high drug-loading capacity, stability, and targeted delivery, leading to increased drug accumulation in cancer cells and reduced systemic toxicity. This innovative approach underscores the potential of chitosan nanoparticles in improving the precision and effectiveness of cancer treatments, particularly for hepatocellular carcinoma, by ensuring that therapeutic agents are delivered specifically to cancer cells while minimizing adverse effects on healthy tissues.[36]

Gao *et al.*[37] (2010) investigated the use of chitosan-linked polyethyleneimine (PEI) in gene transfection systems to enhance the delivery of genetic material into cancer cells. The study found that the chitosan-PEI hybrid system provided high gene transfection efficiency with low cytotoxicity, making it a suitable candidate for gene therapy applications. The PEI/chitosan blend system effectively facilitated the delivery of therapeutic genes into colon cancer cells, leading to significant tumor-suppressive activity. This research highlights the potential of chitosan-based hybrid systems in overcoming the limitations of current gene delivery methods, such as high toxicity and low efficiency. The findings suggest that chitosan can be engineered to create more effective and safer gene delivery vehicles, advancing the field of gene therapy for cancer treatment.[37]

Collectively, these studies highlight the diverse and significant potential of chitosan nanoparticles in cancer treatment. While Chang (2017) pointed out the risk of chitosan promoting cancer progression through Wnt signaling,[33] Harish Prashanth and Tharanathan (2007) and Yang (2015) emphasized its selective cytotoxicity and effective gene delivery capabilities. Chen *et al.*[35] (2015) and Gao *et al.*[37] (2010) further demonstrated the precision targeting and low toxicity of chitosan-based systems. These findings suggest that while chitosan holds promise for cancer therapy, its application must be carefully tailored to harness its benefits while mitigating potential risks. The diverse approaches and outcomes in these studies reflect the complex interplay between chitosan's biochemical properties and its interactions

with cancer cells, underscoring the need for continued research and optimization.

In comparing these studies, it becomes evident that the functional modifications of chitosan are crucial in determining its therapeutic efficacy. The ability to modify chitosan to enhance its selectivity and reduce toxicity, as shown by Harish Prashanth and Tharanathan (2004), is a critical factor in its application in cancer therapy.[34] The targeted delivery systems developed by Chen (2015) and Yang (2015) highlight the potential of chitosan to improve the precision of cancer treatments. However, the study by Chang (2017) serves as a cautionary reminder of the complexities involved in using chitosan, particularly its potential to enhance tumor progression if not properly controlled.

Overall, these studies collectively underscore the need for a nuanced approach in developing chitosan-based cancer therapies. The promising results in targeted delivery and gene transfection indicate that chitosan nanoparticles can significantly enhance the efficacy of cancer treatments. However, the potential risks, such as those identified by Chang (2017), must be carefully managed through precise engineering and thorough understanding of chitosan's interactions with cancer cells. The continuous advancements in chitosan research are opening new possibilities for more effective and safer cancer therapies, highlighting the importance of interdisciplinary collaboration in this field.

In conclusion, the exploration of chitosan nanoparticles in cancer therapy reveals a promising yet complex potential. The benefits of targeted delivery, selective cytotoxicity, and low toxicity offer significant advancements in cancer treatment. However, the potential risks necessitate a careful and well-informed approach to harness the full therapeutic potential of chitosan. Future research should focus on optimizing chitosan modifications and understanding its interactions with biological systems to develop safe and effective cancer therapies. The integration of these findings into clinical practices holds the promise of improving patient outcomes and advancing the fight against cancer.

Overall, the anti-carcinogenic properties of chitosan nanoparticles, combined with their ability to deliver a range of therapeutic agents, present a powerful tool in the fight against cancer. Their multifaceted mechanisms of action, targeting capabilities, and potential for controlled drug release position them at the forefront of next-generation cancer therapeutics, offering hope for more effective and less toxic treatment options.

2.2 Antimicrobial

Chitosan nanoparticles have been extensively studied for their antimicrobial properties, making them a valuable tool in combating a wide range of pathogens, including bacteria, fungi, and viruses.[38] The antimicrobial activity of chitosan is mainly due to its polycationic nature, which permits it to interact with the negatively charged membranes of microbial cells. This interaction leads to the disruption of the integrity of the cell membrane, causing leakage of intracellular contents and ultimately resulting in cell death. Additionally, chitosan nanoparticles can penetrate microbial cells, binding to DNA and inhibiting RNA and protein synthesis, further enhancing their antimicrobial efficacy. The size and surface charge of chitosan nanoparticles can be finely tuned to optimize their interaction with different types of microorganisms, enhancing their effectiveness. Research has shown that smaller nanoparticles with higher surface charge densities exhibit stronger antimicrobial activities due to their increased surface area and higher interaction potential with microbial cells.[39] Moreover, chitosan nanoparticles can be functionalized with various antimicrobial agents, such as silver nanoparticles, antibiotics, and essential oils, to create synergistic effects and enhance their spectrum of activity.

The versatility of chitosan nanoparticles extends to their ability to form coatings and films on medical devices, implants, and wound dressings, providing long-lasting antimicrobial protection and preventing biofilm formation. This is particularly important in clinical settings where device-associated infections are a significant concern. Furthermore, chitosan nanoparticles exhibit inherent biocompatibility and biodegradability, creating them safe for use in various biomedical applications. Their non-toxic nature ensures that they do not cause adverse effects when applied to human tissues, making them suitable for usage in wound healing, tissue engineering, and drug delivery systems. The sustained release of encapsulated antimicrobial agents from chitosan nanoparticles provides prolonged protection against infections, reducing the need for frequent application and minimizing the risk of resistance development. Recent studies have highlighted the potential of chitosan nanoparticles in addressing the global challenge of antibiotic resistance.

By enhancing the efficacy of existing antibiotics and reducing the required dosage, chitosan nanoparticles can help mitigate the spread of resistant strains and extend the useful life of current antimicrobial agents. The ability of chitosan nanoparticles to target and disrupt biofilms, which are often resistant to conventional antibiotics, further underscores their potential in managing chronic and recalcitrant infections. Moreover, the incorporation of chitosan nanoparticles into food packaging materials has shown promise in extending the shelf life of fragile goods by obstructing microbial growth and preventing spoilage. This application not only improves food safety but also reduces food waste, contributing to sustainable practices. The broad-spectrum antimicrobial activity, combined with the customizable properties of chitosan nanoparticles, positions them as a versatile and powerful tool in the fight against infectious diseases. As research continues to uncover new functionalizations and applications, chitosan nanoparticles are poised to play a pivotal role in advancing antimicrobial strategies in both medical and non-medical fields. The ongoing development of chitosan-based antimicrobial systems promises to deliver innovative solutions for infection control, enhancing public health and safety on a global scale.

The chitosan and its derivatives antimicrobial properties have been widely studied, demonstrating significant potential in various biomedical applications. Chitosan's ability to inhibit a broad spectrum of microorganisms, including bacteria, fungi, and viruses, is attributed to its unique chemical structure and physicochemical properties. This biopolymer's effectiveness as an antimicrobial agent is influenced by numerous factors, such as its molecular weight, degree of deacetylation, and the presence of functional groups that can interact with microbial cell membranes. The studies discussed in this section highlight the diverse mechanisms through which chitosan exerts its antimicrobial effects and its potential applications in clinical settings.

Mohamed and Al-Mehbad (2013) synthesized novel chitosan hydrogels cross-linked with terephthaloyl thiourea, which exhibited significant antibacterial and antifungal activities.[40] The study found that these hydrogels had a broad spectrum of antimicrobial action against both Gram-positive and Gram-negative bacteria, as well as several fungal species. The cross-linking with terephthaloyl thiourea enhanced the mechanical strength and stability of the chitosan hydrogels, making them suitable for use in various biomedical applications, including wound dressings and drug delivery systems. The antimicrobial mechanism was primarily attributed to the ability of chitosan to disrupt microbial cell membranes, causing the leakage of cellular contents and ultimately resulting in cell death. The study also highlighted the biocompatibility of hydrogels, which is crucial for their safe application in medical treatments.

Mohseni (2019) conducted a comparative analysis of wound dressings incorporating silver sulfadiazine and silver nanoparticles. They evaluated the in vitro and in vivo antimicrobial efficacy of these dressings against common wound pathogens. The incorporation of silver nanoparticles into chitosan-based dressings significantly enhanced their antimicrobial properties. Silver nanoparticles are known for their potent antimicrobial activity, and when combined with chitosan, they provide a synergistic effect that enhances the overall antimicrobial performance. The study demonstrated that these dressings effectively reduced bacterial load and promoted faster wound healing in animal models. The authors proposed that the combination of chitosan and silver nanoparticles could serve as a highly effective antimicrobial

wound dressing, offering both infection control and improved healing (**Figure 4**).[41]

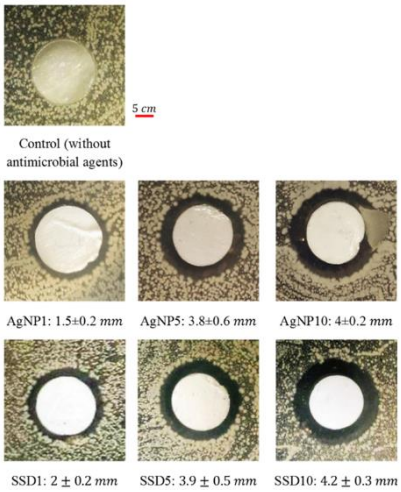


Figure 4: *Antimicrobial characterization of wound dressings against Staphylococcus aureus using the inhibition zone measurement technique (n=5). [Reprinted with permission from 36].*

Moura (2011) developed in situ forming chitosan hydrogels via ionic and covalent co-crosslinking, aiming to create a versatile and effective antimicrobial material. These hydrogels demonstrated excellent antimicrobial activity against wide range of bacterial strains, including both Gram-positive and Gram-negative bacteria. The dual cross-linking approach not only improved the mechanical properties and stability of the hydrogels but also enriched their antimicrobial efficacy. The ionic and covalent bonds in the hydrogels provided a robust structure that could be applied in various biomedical fields, including tissue engineering and drug delivery. The study concluded that these hydrogels are promising candidates for applications requiring strong antimicrobial action combined with biocompatibility and biodegradability.[42]

Munoz-Bonilla (2019) reviewed the development of bio-based polymers with antimicrobial properties, focusing on sustainable materials like chitosan. They discussed various strategies for enhancing the antimicrobial properties of chitosan, including chemical modifications and the incorporation of metallic nanoparticles. The review highlighted the versatility of chitosan as an antimicrobial agent and its potential in developing environmentally friendly and effective antimicrobial materials. The authors emphasized that chitosan's biocompatibility and biodegradability make it an attractive option for medical applications, particularly in areas where conventional antimicrobial agents may pose environmental and health risks.[43]

Nandi (2009) explored local antibiotic delivery systems for treating osteomyelitis, with a particular focus on chitosan-based carriers. Their research demonstrated that chitosan could be effectively used to deliver antibiotics directly to the infection site, thereby enhancing the local concentration of the drug and reducing systemic side effects. The study found that chitosan-based delivery systems provided sustained release of antibiotics, maintaining therapeutic levels over extended periods. This approach not only improved the efficacy of the treatment but also minimized the development of antibiotic resistance by ensuring consistent drug exposure. The authors concluded that chitosan-based antibiotic delivery systems hold great promise for the treatment of chronic bone infections like osteomyelitis.[44]

Keaton Smith (2010) explored the potential of chitosan films loaded with antibiotics as a treatment for infections associated with bone fracture fixation devices. The study primarily focused on methicillin-resistant Staphylococcus aureus (MRSA), a significant concern in musculoskeletal wound treatment. Chitosan films with varying degrees of deacetylation (DDA) were evaluated for their antibiotic uptake, elution, and activity. The study found that 80% DDA chitosan films were most effective for absorbing and releasing antibiotics, maintaining mechanical integrity and adhesive strength when applied to fracture fixation devices. The films effectively eluted antibiotics, which were active against S. aureus, demonstrating the potential of chitosan films as a complementary clinical treatment to reduce or prevent infections in musculoskeletal injuries.[45]

Monteiro (2015) investigated the antibacterial activity of gentamicin-loaded liposomes immobilized on electrospun chitosan nanofiber meshes (NFM). The study highlighted the importance of chitosan as a support structure for binding liposomes, which provided a sustained release of gentamicin. Disk diffusion and broth dilution assays demonstrated the effectiveness of gentamicin release from the liposomes immobilized on the chitosan NFM in inhibiting the growth of S. aureus, E. coli, and P. aeruginosa. These results suggest that the developed nanostructured delivery system could be used in local applications to eradicate pathogens involved in infections, showing promising performance for wound dressing applications.[46]

Peng (2011) reported on the development of a polycationic antimicrobial hydrogel derived from dimethyldecylammonium chitosan grafted with poly(ethylene glycol) methacrylate. This hydrogel demonstrated excellent antimicrobial efficiency against P. aeruginosa, E. coli, S. aureus, and Fusarium solani. The suggested mechanism of antimicrobial activity involved the attraction and disruption of microbial membranes by the hydrogel's nanopores, leading to microbe death. The hydrogel was also found to be biocompatible with rabbit conjunctiva and showed no toxicity to epithelial cells or the underlying stroma. This study highlighted the potential of chitosan-based hydrogels for use in preventing infections associated with medical implants and devices.[47]

A study by Shao (2016) explored the antimicrobial properties of chitosan/silver sulfadiazine (CS/AgSD) composite sponges designed for wound dressing applications. The composite sponges exhibited a broad spectrum of antibacterial activity without significant cytotoxicity, as confirmed by MTT viability assay and fluorescence staining technique on HEK293 cell lines. The findings demonstrated the potential application of CS/AgSD composite sponges in antimicrobial wound dressing materials, emphasizing the importance of natural polymers like chitosan in biomedical applications due to their biocompatibility, biodegradability, and antimicrobial properties.[48]

Again, a study by Zhang (2019) evaluated the antimicrobial efficacy of chitosan-based composite films incorporating different types of nanoparticles. The films showed significant antibacterial activity against various pathogens, with enhanced mechanical properties and thermal stability. The incorporation of nanoparticles such as silver, copper, and titanium dioxide into the chitosan matrix enhanced the antimicrobial activity and mechanical strength of the films, making them suitable for use as antimicrobial wound dressings and other biomedical applications. The study highlighted the versatility of chitosan in forming composites with nanoparticles to enhance its inherent antimicrobial properties.[49]

The study conducted by Lal (2016) examined the antibacterial performance of Schiff base chitosan against various microbial strains including *Aspergillus niger*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Escherichia coli*. The results demonstrated a notable inhibitory effect of Schiff base chitosan on fungi and gram-positive bacteria, whereas its outcome on gram-negative bacteria was less pronounced. The study highlighted that the antibacterial properties of chitosan derivatives are influenced by factors such as environmental pH and molecular weight (MW) of chitosan. Specifically, the agar diffusion experiment revealed that the antibacterial activity against gram-negative bacteria increased with an increase in MW up to a threshold, beyond which the activity decreased. This was particularly evident when the MW reached 30,000, showing a decline in antibacterial efficacy. The evaluation of antifungal efficacy through food toxicology testing indicated that the antibacterial efficiency of Schiff base chitosan could exceed 70%, demonstrating its potential as an effective antimicrobial agent.[50]

In a study by Piegat (2021), the antibacterial activity of N–O acylated chitosan derivatives (CH-LA) was evaluated against Escherichia coli, Helicobacter pylori, and Staphylococcus aureus using various methods including microdilution, agar immersion, and disk diffusion. The results indicated that the environmental pH and concentration of CH-LA significantly influenced the antibacterial activity. The acylated chitosan derivatives showed improved antibacterial activity against the tested bacteria, with the highest concentration and acidic environment (pH = 5) yielding survival rates of 22%, 23%, and 8% for Escherichia coli, Helicobacter pylori, and Staphylococcus aureus, respectively. This confirmed that gram-positive bacteria, like Staphylococcus aureus, are more sensitive to CH-LA than gram-negative bacteria such as Helicobacter pylori and Escherichia coli. These findings underscore the potential of N–O acylated chitosan derivatives as effective antimicrobial agents, particularly against gram-positive bacteria.[51]

Considering these papers together, a comprehensive overview reveals a diverse range of methodologies and outcomes regarding the antimicrobial properties of chitosan and its derivatives. The studies collectively demonstrate that chitosan's antimicrobial activity is affected by its molecular weight, degree of deacetylation, and the presence of functional groups. Additionally, environmental factors such as pH and the type of microorganism significantly impact chitosan's efficacy. The use of chitosan in combination with other antimicrobial agents or modifications, such as quaternization or acylation, generally enhances its antimicrobial properties, making it a versatile and effective biopolymer for various applications.

A notable trend across the studies is the higher efficacy of chitosan derivatives against gram-positive bacteria compared to gram-negative bacteria. This is endorsed to the differences in the cell wall structures of these bacteria, with gram-positive bacteria having a thicker peptidoglycan layer that is more susceptible to disruption by chitosan. The studies also highlight the potential of chitosan derivatives in various applications, including food preservation, medical treatments, and environmental protection. The antimicrobial activity of chitosan-coated films, hydrogels, and nanoparticles has been shown to be effective in controlling microbial growth, thereby prolonging the shelf life of food products and enhancing the efficacy of medical treatments.

In conclusion, the collective findings from these studies underscore the significant prospective of chitosan and its derivatives as antimicrobial agents. The variations in antimicrobial activity based on molecular weight, degree of deacetylation, and functional modifications highlight the importance of optimizing these parameters for specific applications. The ability of chitosan to interact synergistically with other antimicrobial agents further enhances its efficacy, making it a promising candidate for a extensive range of applications in food safety, medical treatments, and environmental protection. Future research should focus on addressing the challenges related to the stability and practical applications of chitosan in various industries to fully realize its potential.

2.3. Blood Brain Barrier (BBB) Drug Carrier

The Blood-Brain Barrier (BBB) is a selectively permeable barrier that safegurds the brain from potentially harmful substances in the bloodstream while permitting the passage of essential nutrients.[52] This barrier, formed by endothelial cells, tight junctions, and astrocyte end-feet, presents a noteworthy challenge for transporting therapeutic agents to the brain to treat central nervous system (CNS) disorders. Traditional drug delivery methods frequently fail to reach sufficient drug concentration in the brain because of the restrictive properties of the BBB.[53] Chitosan nanoparticles have emerged as a promising solution for overcoming this obstacle, owing to their unique physicochemical properties and ability to be functionalized for targeted delivery. Chitosan, a biopolymer derived from chitin, exhibits biocompatibility, biodegradability, and low toxicity, making it suitable for various biomedical applications. Its polycationic nature allows for the interaction with the negatively charged components of the BBB, enhancing its permeability and facilitating drug transport. The versatility of chitosan nanoparticles lies in their ability to be modified with ligands, peptides, or antibodies that can target specific receptors on the BBB, thereby improving the selectivity and efficiency of drug delivery to the brain.[54]

One of the primary benefits of using chitosan nanoparticles for BBB drug delivery is their competency to encapsulate a extensive range of therapeutic agents, such as small molecules, peptides, proteins, and nucleic acids. This encapsulation protects the therapeutic agents from degradation and premature clearance, ensuring that a higher concentration reaches the brain.[55] Additionally, the surface modification of chitosan nanoparticles with targeting ligands including transferrin, lactoferrin, and antibodies against specific BBB receptors can significantly enhance their uptake by brain endothelial cells through receptor-mediated transcytosis. This targeted tactic not only improves the efficiency of drug delivery but also minimizes potential side effects by reducing systemic exposure. Furthermore, chitosan nanoparticles can be engineered to exhibit controlled and sustained release of the encapsulated drugs, maintaining therapeutic levels in the brain over extended periods and reducing the frequency of administration.

Recent studies have demonstrated the efficacy of chitosan nanoparticles in delivering a variety of therapeutic agents across the BBB for the treatment of neurological disorders including Alzheimer's disease, Parkinson's disease, brain tumors, and stroke. For instance, chitosan nanoparticles loaded with anti-Alzheimer's drugs have shown improved drug bioavailability and therapeutic efficacy in animal models, resulting in enhanced cognitive function and reduced amyloid-beta plaques in the brain. Similarly, chitosan-based delivery systems have been successful in transporting chemotherapeutic agents to brain tumors, increasing drug accumulation at the tumor site and inhibiting tumor growth. The ability of chitosan nanoparticles to cross the BBB and deliver drugs effectively opens new avenues for treating CNS disorders that are currently difficult to manage with conventional therapies.[55]

The safety profile of chitosan nanoparticles further supports their potential as BBB drug carriers. Studies have shown that chitosan and its derivatives exhibit low cytotoxicity and immunogenicity, making them well-tolerated in both in vitro and in vivo models. The biodegradability of chitosan ensures that the nanoparticles are gradually decomposed into non-toxic byproducts, minimizing the risk of long-term buildup and harmful effects. Additionally, the ease of production and scalability of chitosan nanoparticles make them a cost-effective option for large-scale drug delivery applications.

In the study by Wohlfart (2012), the transport of drugs across the BBB using nanoparticles was investigated, highlighting the effectiveness of chitosan nanoparticles in enhancing drug delivery to the brain. The authors demonstrated that the mucoadhesive properties of chitosan significantly improve the retention time of the nanoparticles at the nasal mucosa, facilitating the transport of encapsulated drugs to the brain via the olfactory and trigeminal nerve pathways. This non-invasive delivery route bypasses the BBB, which is a major obstacle in CNS drug delivery. Additionally, the study found that the positive charge of chitosan nanoparticles enhances their interaction with the negatively charged cell membranes, endorsing cellular uptake and enhancing the drug concentration in the brain.

These findings underscore the potential of chitosan nanoparticles in delivering therapeutics for treating neurological diseases such as gliomas, Alzheimer's disease, and Parkinson's disease.[56]

A study by Freiherr (2013) focused on the nasal delivery of insulin using chitosan nanoparticles for the treatment of Alzheimer's disease. The authors explored the potential of intranasal administration of insulin-loaded chitosan nanoparticles to improve cognitive function in Alzheimer's patients. The results exposed that the nanoparticles significantly increased the bioavailability of insulin in the brain, leading to improved cognitive performance in animal models. The study also highlighted the ability of chitosan

nanoparticles to protect insulin from degradation in the nasal cavity and enhance its transport across the nasal epithelium into the brain. These findings suggest that chitosan nanoparticles could be a viable strategy for delivering therapeutic proteins to the brain, providing a non-invasive and effective treatment option for neurodegenerative diseases.[57]

Chen (2017) investigated the use of vitamin E succinate-grafted chitosan oligosaccharide nanoparticles for delivering paclitaxel to brain tumors. The study demonstrated that the nanoparticles effectively delivered paclitaxel to glioma cells, resulting in significant inhibition of tumor growth. The authors attributed the enhanced delivery and therapeutic efficacy to the mucoadhesive properties of chitosan, which increased the retention time of the nanoparticles at the tumor site, and the antioxidative properties of vitamin E, which provided additional protection to the encapsulated drug. The findings of this study underscore the prospective of chitosan-based nanoparticles in enhancing the delivery and efficacy of chemotherapeutic agents for treating brain tumors. [58]

In another study, Md (2013) developed bromocriptine-loaded chitosan nanoparticles for the purpose of treating Parkinson's disease via the nose-to-brain delivery route. The pharmacokinetic and pharmacodynamic evaluations indicated that the nanoparticles provided a sustained release of bromocriptine, leading to prolonged therapeutic effects and improved bioavailability in the brain. The study also demonstrated that the nanoparticles significantly reduced the frequency and severity of Parkinsonian symptoms in animal models. These results highlight the potential of chitosan nanoparticles in delivering dopamine agonists directly to the brain, offering a promising approach for managing Parkinson's disease.[59]

Zhao (2017) explored the use of a nano-in-nano polymer-dendrimer system based on chitosan for the controlled delivery of multiple drugs to the brain and the system was designed to encapsulate; co-deliver chemotherapeutic agents and gene therapy vectors to brain tumors. The study demonstrated that the chitosan-based nanosystem effectively delivered therapeutic payloads to glioma cells, resulting in enhanced apoptosis and reduced tumor growth. The authors emphasized the importance of the chitosan matrix in providing stability, controlled release, and targeted delivery of the encapsulated agents, which collectively improved therapeutic outcomes. This study highlights the versatility and efficacy of chitosan nanoparticles in complex drug delivery systems for treating brain tumors (Refer **Figure 5**).[60]

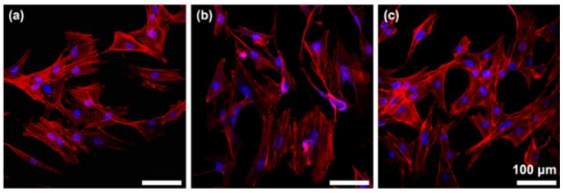


Figure 5: *CLSM images of osteoblasts adhered to (a) native Ti, (b) TNT and LBL substrates after 2 days culture with staining of actin (red) and nucleus (blue)[Reprinted with permission from 57].*

Meng (2016) investigated the use of Pluronic F127 and D-alpha-Tocopheryl Polyethylene Glycol Succinate (TPGS) based mixed micelles for targeted drug delivery across the BBB. Their study focused on the encapsulation of a hydrophobic drug, docetaxel, within these mixed micelles and evaluated their stability, drug release profile, and cellular uptake. The results demonstrated that the mixed micelles had a high drug loading capacity and could sustain drug release over an extended period. Additionally, in vitro studies using brain endothelial cells showed that these micelles significantly enhanced the cellular uptake of docetaxel compared to free drug solutions. The authors also reported that the micelles could reduce the cytotoxicity of docetaxel to non-target cells, suggesting a more targeted delivery mechanism. There are in vivo experiments further confirmed that the micelles could effectively deliver docetaxel to the brain, resulting in improved therapeutic outcomes in a glioma mouse model.[61]

The study by Rip (2014) explored the pharmacokinetics and brain delivery capabilities of glutathione PEGylated liposomes. The research aimed to improve the delivery of antioxidant enzymes to the brain to treat neurodegenerative diseases. The PEGylation of liposomes with glutathione allowed for the enhanced crossing of the BBB, leveraging the natural transport mechanisms of glutathione. Their findings showed that these modified liposomes had increased stability in the bloodstream and prolonged circulation times. In vivo studies in rats demonstrated that the glutathione PEGylated liposomes could efficiently deliver encapsulated enzymes to the brain, significantly reducing oxidative stress markers. This study provided strong evidence for the potential of PEGylated liposomes as a viable strategy for delivering therapeutic proteins and enzymes to the brain, offering a promising approach for treating diseases characterized by oxidative damage.[62]

In the research conducted by Salvalaio (2016), the focus was on developing targeted polymeric nanoparticles for delivering high molecular weight molecules to the brain in lysosomal storage disorders. The study utilized nanoparticles made from poly(lactic-co-glycolic acid) (PLGA) and polycaprolactone (PCL) that were functionalized with specific ligands to target receptors on brain endothelial cells. Their results indicated that these nanoparticles could cross the BBB efficiently and deliver therapeutic enzymes directly to the lysosomes of brain cells. This targeted delivery significantly reduced the accumulation of storage material in the brain, demonstrating a potential therapeutic approach for lysosomal storage disorders. The study also highlighted the role of surface functionalization in enhancing the specificity and efficiency of nanoparticle-mediated drug delivery to the brain.[63]

Dalpiaz (2012) explored the conjugation of zidovudine and ursodeoxycholic acid to improve the delivery of antiretroviral drugs across the BBB. This study aimed to overcome the limitations of current antiretroviral therapies that have poor penetration into the CNS, leading to suboptimal treatment of HIV-associated neurocognitive disorders. The conjugation strategy utilized the prodrug approach, enhancing the lipophilicity of zidovudine to facilitate its transport across the BBB. The in vitro and in vivo studies validated that the conjugated prodrug could successfully cross the BBB and release the active drug within the brain tissue. This approach significantly improved the concentration of zidovudine in the brain, suggesting a potential method for enhancing the efficacy of antiretroviral therapies in treating CNS manifestations of HIV.[64]

Khan (2024) investigated the potential of chitosan-based polymeric nanoparticles for gene delivery across the BBB. The study involved the synthesis of chitosan-GFP nanoparticles using a complex coacervation method, yielding particles around 260 nm in size. The in vitro transfection efficiency was evaluated using HEK293 and U87 MG cell lines, showing higher (53%) transfection efficiency compared to the commercially accessible transfection reagent CTR-6 (27%). The in vivo studies on BALB/c mice demonstrated the nanoparticles' ability to cross the BBB and deliver the GFP gene effectively, indicating their potential as gene delivery vehicles for CNS disorders. The study concluded that chitosan nanoparticles could be promising candidates for gene therapy, given their biocompatibility, low cytotoxicity, and efficient transfection capabilities.[65]

Banerjee (2002) focused on the preparation, characterization, and biodistribution of ultrafine chitosan nanoparticles. Using a modified ionotropic gelation technique, the researchers synthesized nanoparticles with an average size of 100-200 nm. Biodistribution studies in mice revealed significant accumulation of nanoparticles in the brain, suggesting efficient crossing of the BBB. The study also highlighted the nanoparticles' stability and low cytotoxicity, making them suitable for long-term therapeutic applications. The results validated that the chitosan nanoparticles could be efficiently utilized for targeted drug delivery in brain cancer therapy, providing a non-invasive alternative to conventional delivery methods.[66]

Li (2021) explored chemo-physical approaches to enhance the in vivo performance of targeted nanomedicine, focusing on chitosan nanoparticles. The study emphasized the

importance of nanoparticle surface modification, such as PEGylation and ligand attachment, to improve BBB permeability and target specificity. The researchers demonstrated that functionalized chitosan nanoparticles exhibited improved cellular uptake and extended circulation time, leading to better-quality therapeutic outcomes in brain tumor models. The findings suggested that such modifications could significantly enhance the delivery efficiency and therapeutic efficacy of chitosan nanoparticles in treating CNS disorders.[67]

Carradori (2018) evaluated antibody-functionalized polymer nanoparticles for promoting memory recovery in a transgenic mouse model resembling Alzheimer's disease. The study utilized chitosan nanoparticles conjugated with antibodies targeting amyloid-beta plaques, a hallmark of Alzheimer's disease. The in vivo experiments showed that the functionalized nanoparticles crossed the BBB and reduced amyloid-beta levels in the brain, leading to significant improvements in cognitive function. These results underscored the potential of chitosan nanoparticles as a platform for developing targeted therapies for neurodegenerative diseases.[68]

Caprifico (2020) investigated the use of functionalized chitosan nanocarriers for overcoming the BBB in the treatment of glioblastoma. The study synthesized chitosan nanoparticles modified with various ligands, including transferrin and lactoferrin, to enhance BBB penetration and target glioma cells. The in vitro and in vivo analyses confirmed that the modified nanoparticles showed higher transfection efficiency and better targeting capabilities compared to unmodified nanoparticles. The researchers concluded that functionalized chitosan nanoparticles could provide a promising approach for delivering therapeutic agents to treat brain tumors effectively.[69]

Comparing these studies, it is evident that chitosan nanoparticles possess a versatile and adaptable platform for drug delivery across the BBB. The primary advantage lies in their biocompatibility and ease of functionalization, allowing for targeted delivery and improved therapeutic outcomes. For instance, while Khan et al. demonstrated the basic transfection capabilities of chitosan nanoparticles, Banerjee et al. and Li et al. highlighted the importance of nanoparticle size and surface modifications, respectively, in enhancing delivery efficiency. Carradori and Caprifico further extended these findings by demonstrating the potential of functionalized nanoparticles in treating specific CNS disorders, such as Alzheimer's disease and glioblastoma.

The studies collectively suggest that the efficiency of chitosan nanoparticles can be significantly improved through surface modifications and functionalization. The ability to conjugate ligands, such as antibodies or proteins, to the nanoparticles enhances their targeting capabilities, allowing for more precise delivery of therapeutic agents to the brain. This targeted strategy improves therapeutic efficacy while minimizing potential side effects associated to non-specific drug distribution.

Conclusively, the research reviewed underscores the promising potential of chitosan-based nanoparticles as drug carriers across the BBB. Their inherent properties, combined with the possibility of functionalization, make them fit for a extensive range of therapeutic applications targeting the CNS. Future research should concentrate on optimizing the surface modifications and understanding the long-term effects of these nanoparticles in clinical settings. The development of such advanced nanocarriers holds the promise of revolutionizing the treatment of neurological disorders, offering safer and more effective therapeutic options.

In conclusion, chitosan nanoparticles represent a promising platform for overcoming the challenges associated with BBB drug delivery. Their unique properties, including biocompatibility, biodegradability, and the ability to be functionalized for targeted delivery, make them suitable for transporting a wide range of therapeutic agents to the brain. As research in this field continues to advance, chitosan nanoparticles hold great potential for improving the treatment outcomes of various CNS disorders, offering hope for more effective and less invasive therapeutic options.

2.4 Anti - HIV

Chitosan nanoparticles have garnered attention as a potential vehicle for the delivery of anti-HIV drugs, offering several advantages over traditional drug delivery systems.[70] The management and treatment of HIV/AIDS require the consistent and controlled delivery of antiretroviral drugs to maintain therapeutic levels, reduce viral load, and prevent drug resistance. Chitosan, a biopolymer derived from chitin, is characterized by its biocompatibility, biodegradability, and non-toxic nature, making it suitable for various biomedical applications. The cationic nature of chitosan allows it to form nanoparticles through ionic gelation with anionic cross-linkers, resulting in stable, biocompatible drug delivery systems. These chitosan nanoparticles can encapsulate a extensive range of therapeutic agents, such as hydrophilic and hydrophobic drugs, peptides, and nucleic acids, protecting them from degradation and facilitating their controlled release. The surface of chitosan nanoparticles can be modified with targeting ligands, enhancing their ability to selectively deliver drugs to HIV-infected cells and tissues, such as macrophages and lymphoid organs, where the virus persists and replicates.

The unique properties of chitosan nanoparticles, including their mucoadhesive nature, enable them to adhere to mucosal surfaces, providing a prolonged residence time and enhancing drug absorption. This characteristic is particularly beneficial for the delivery of antiretroviral drugs via mucosal routes, such as intranasal or intravaginal administration, which are critical entry points for HIV transmission. By facilitating localized drug delivery, chitosan nanoparticles can enhance the bioavailability of antiretroviral drugs at the site of viral entry, potentially preventing the establishment and spread of infection. Additionally, the ability to engineer chitosan nanoparticles for sustained and controlled release of encapsulated drugs ensures a consistent therapeutic effect, dropping the frequency of drug administration and enhancing patient adherence. This sustained release mechanism is crucial in maintaining effective drug concentrations in the body, thereby reducing the risk of viral rebound and the development of drug-resistant strains.

Research has demonstrated the potential of chitosan nanoparticles in enhancing the efficacy of various antiretroviral drugs, such as reverse transcriptase inhibitors, protease inhibitors, and integrase inhibitors. These nanoparticles can boost the solubility and stability of poorly soluble drugs, improve their penetration across biological barriers, and provide targeted delivery to HIV reservoirs. The versatility of chitosan nanoparticles also permits for the co-delivery of multiple therapeutic agents, offering a synergistic effect that can enhance overall treatment efficacy. For instance, the co-encapsulation of antiretroviral drugs with anti-inflammatory agents or immune modulators can address both viral suppression and immune restoration, providing a comprehensive approach to HIV treatment. The ability to deliver siRNA or CRISPR/Cas9 components using chitosan nanoparticles further expands their potential in gene therapy applications aimed at targeting and eliminating HIV proviral DNA from infected cells.

Moreover, the safety profile of chitosan nanoparticles supports their potential use in long-term HIV therapy. Studies have shown that chitosan and its derivatives exhibit minimal cytotoxicity and immunogenicity, making them well-tolerated equally in vitro and in vivo models. The biodegradability of chitosan ensures that the nanoparticles are gradually broken down into non-toxic byproducts, minimizing the risk of long-term buildup and adverse effects. The scalable and cost-effective production of chitosan nanoparticles also makes them an attractive option for widespread use in HIV treatment, particularly in resource-limited settings where access to advanced therapies is often restricted.

Chitosan nanoparticles (CNPs) have been investigated extensively for their potential applications in anti-HIV therapies. One notable study by Ashish Dev and colleagues at the Amrita Centre for Nanosciences and Molecular Medicine explored the preparation of poly(lactic acid)/chitosan (PLA/CS) nanoparticles for delivering the antiretroviral drug Lamivudine. The study highlighted the nanoparticles'-controlled drug release behavior, which is

critical for maintaining therapeutic drug levels over an extended period. The researchers characterized the nanoparticles using dynamic light scattering (DLS), scanning electron microscopy (SEM), and Fourier transform infrared spectroscopy (FTIR). They found that the drug release rate was inferior in acidic pH related to alkaline pH, and this is attributed to the repulsion between hydrogen ions and cationic groups in the polymeric nanoparticles. Furthermore, cytotoxicity assays demonstrated that the PLA/CS nanoparticles were biocompatible, with no significant toxicity observed in fibroblast cell lines, thus presenting them as a promising carrier system for controlled anti-HIV drug delivery.[71]

Another significant study by Ji Sun Park and Yong Woo Cho focused on the cellular uptake and cytotoxicity of paclitaxel-loaded glycol chitosan (GC) self-assembled nanoparticles. These nanoparticles demonstrated efficient drug delivery capabilities due to their ability to form stable self-assembled structures in aqueous solutions. The study utilized flow cytometry and confocal microscopy to investigate the endocytosis and exocytosis of fluorescein isothiocyanate (FITC)-conjugated GC nanoparticles. It was observed that the nanoparticles were internalized through adsorptive endocytosis and distributed in the cytoplasm, but not the nucleus. The paclitaxel-loaded nanoparticles effectively arrested cancer cell growth by causing cell cycle arrest in the G2-M phase. These findings underscore the potential of GC nanoparticles in delivering hydrophobic drugs like paclitaxel with high efficiency and minimal cytotoxicity.[72]

The third study, conducted by L.N. Ramana, examined the protein adsorption properties of saquinavir-loaded chitosan nanoparticles. This research is crucial as it addresses the issue of immune recognition of nanoparticles in vivo, which can affect their therapeutic efficacy. The study found that saquinavir-loaded chitosan nanoparticles exhibited lower protein adsorption compared to blank chitosan nanoparticles, attributed to the reduced surface charge after drug loading. This reduction in protein adsorption is beneficial as it can potentially decrease the immune system's recognition and clearance of the nanoparticles, enhancing their circulation time and effectiveness in delivering antiretroviral drugs. Additionally, confocal microscopy and flow cytometry analyses confirmed the high cellular uptake and efficient intracellular delivery of the drug-loaded nanoparticles.[73]

A study by H.Y. Nam investigated the mechanism of cellular uptake and intracellular behaviour of hydrophobically modified glycol chitosan nanoparticles. The research focused on the interactions between glycol chitosan and cell membranes, emphasizing the nanoparticles' ability to enter cells via adsorptive endocytosis. The study revealed that a significant portion of the endocytosed nanoparticles were exocytosed, especially during the early stages of endocytosis, indicating that exocytosis is a critical barrier for intracellular drug delivery. The in vitro cytotoxicity assays confirmed that paclitaxel-incorporated GC nanoparticles were poweful in arresting cancer cell growth, suggesting their potential as a delivery system for anticancer drugs with controlled release properties.[74]

Another comprehensive study by J.H. Park evaluated the use of glycol chitosan nanoparticles for gene delivery, specifically targeting brain tumors. The research utilized GFP-conjugated chitosan nanoparticles to transfect U-87 MG (human glioblastoma) cell lines, assessing the efficiency of transfection through in vitro and in vivo assays. The study demonstrated successful transfection and minimal cytotoxicity compared to conventional gene delivery vehicles. The findings indicated that glycol chitosan nanoparticles could serve as effective carriers for gene therapy, particularly in overcoming the blood-brain barrier, which is a significant challenge in treating brain tumors.[75]

Efavirenz-loaded chitosan nanoparticles (EFV-CNP) have been studied extensively for their potential in enhancing the delivery and bioavailability of anti-HIV drugs. Rozana (2020) synthesized EFV-CNP using an ionotropic gelation method, achieving particle sizes around 104 nm with a zeta potential of -30.7 mV, which indicates good colloidal stability. The entrapment efficiency and loading capacity of EFV in the chitosan nanoparticles were 91.09% and 38.71%, respectively. In vitro release studies showed a sustained release of EFV, with 69.05% of the drug released over 24 hours in phosphate buffer at pH 7.4. This controlled release profile suggests that EFV-CNP could significantly improve the bioavailability and therapeutic efficacy of EFV, particularly by maintaining drug levels within the therapeutic window for extended periods.[76]

Mallikarjuna (2013) explored the preparation of chitosan-based biodegradable hydrogel microspheres for the controlled release of Valganciclovir hydrochloride (VHCl), another anti-HIV drug. Using an emulsion-crosslinking method, the study achieved microspheres with smooth surfaces and average particle sizes ranging from 297 µm to 412 µm. The encapsulation efficiency varied between 67.03% and 80.13%, depending on the formulation parameters. In vitro release studies showed non-Fickian or anomalous release behavior, indicating that the drug release from the microspheres is governed by a combination of diffusion and polymer relaxation mechanisms. The study highlighted the potential of chitosan microspheres for sustained drug delivery, which could diminish dosing frequency and develop patient compliance.[77]

Another study by Dev (2010) investigated the encapsulation of Lamivudine, an anti-HIV drug, within chitosan/poly(lactic acid) (PLA) nanoparticles using an emulsion solvent evaporation technique. The resulting nanoparticles had a mean size of around 200 nm and exhibited a high encapsulation efficiency. In vitro drug release analyses indicated that the nanoparticles delivered a sustained release of Lamivudine over several hours. The study suggested that the use of chitosan in combination with PLA could enhance the stability and control the release profile of hydrophilic drugs, making them suitable for HIV treatment applications.[78]

Chitosan-based nanoparticles have also been evaluated for their potential to improve the delivery of Tenofovir alafenamide (TAF). Narayanan (2017) developed spray-dried chitosan nanoparticles loaded with TAF, achieving smooth, spherical particles with optimal size and stability. The in vitro release analyses in phosphate buffer at pH 7.4 demonstrated a sustained release of TAF over 16 days, with the nanoparticles showing a higher release rate compared to the drug alone. This study underscores the potential of chitosan nanoparticles to enhance the bioavailability and therapeutic effect of TAF, particularly in the context of long-term HIV treatment.[79]

The study by Mallikarjuna (2004) further confirmed the utility of chitosan microspheres for the controlled release of anti-HIV drugs. The researchers used Fourier transform infrared spectroscopy (FTIR) and X-ray diffraction (XRD) to confirm the chemical stability of VHCl in the microspheres and the absence of drug-polymer interactions. Scanning electron microscopy (SEM) revealed smooth and spherical microspheres, while in vitro release studies showed a prolonged release of VHCl over 12 hours. The microspheres' ability to maintain a steady drug release rate highlights their potential for improving the pharmacokinetic profiles of anti-HIV drugs.[80]

Dang explored the synthesis of betulinic acid congeners as entry inhibitors against HIV-1 and bevirimat-resistant HIV-1 variants. Their study revealed that the synthesized derivatives exhibited potent anti-HIV-1 activity by directing the viral entry stage. These derivatives were found to interact specifically with the HIV-1 envelope glycoprotein gp120, which is essential for the virus to attach and enter host cells. The interaction disrupted the binding of gp120 to the CD4 receptors on host cells, effectively preventing the virus from establishing infection. The research demonstrated the importance of targeting the entry process in developing new therapeutic strategies for HIV-1, particularly for drug-resistant strains.[81]

The study by Ramana focused on the anti-HIV activity of chitosan nanoparticles conjugated with siRNA. They developed a novel chitosan-based nanocarrier system designed to deliver anti-HIV siRNA to infected cells. The chitosan nanoparticles were found to efficiently encapsulate the siRNA, protect it from degradation, and facilitate its delivery into target cells. This delivery system significantly enhanced the gene silencing efficacy of the siRNA, resulting

in a marked reduction in HIV-1 replication in vitro. The researchers concluded that chitosan nanoparticles are a promising vehicle for siRNA delivery, offering a new avenue for HIV-1 gene therapy (**Figure 6**).[82]

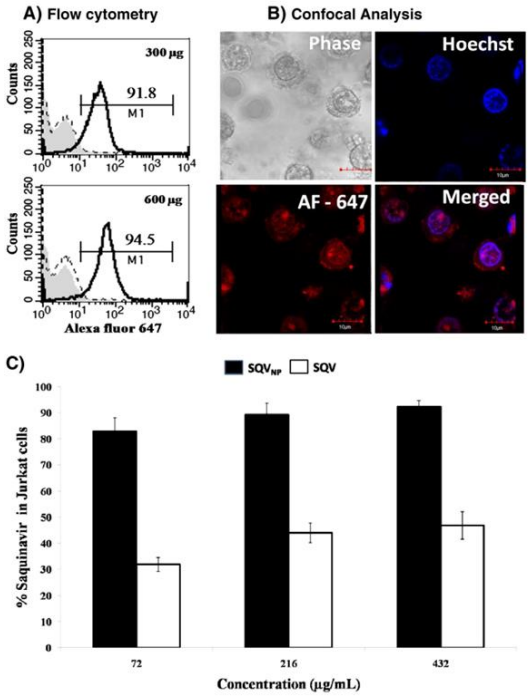


Figure 6: *Cell-targeting efficiency of the Alexa Fluor647-loaded chitosan nanoparticles in Jurkat T-cells. A)Flow cytometry : cells were treated with plain or dye-loaded (300 or 600 µg) nano particles. The gray - filled histogram represents the cells unstained, the dashed line represents the cells stained with plain chitosan particles (PC) and the solid black line represents the cells stained with Alexa fluorophore-loaded (AFL) particles. The percentage of cells stained for the fluorophore is depicted above the marker. [B] Confocal images showing delivery of Alexa Fluor 647-loaded chitosan to Jurka T-cells. Nuclear staining by Hoechst has been depicted in color blue and the red color represents Alexa Fluor 647 [C] cellular uptake of saquinavir loaded chitosan and plain saquinavir using Jurkat cell line. The dark bar represents the chitosan nanoparticles loaded with saquinavir (SQVNP) and white bar represents the plain saquinavir (SQV). [Reprinted with permission from 89].*

Narashimhan conducted a comprehensive assessment of chitosan nanoformulations as effective anti-HIV therapeutic systems. Their research focused on the development and characterization of chitosan nanoparticles loaded with saquinavir, a protease inhibitor. The study showed that the chitosan nanoparticles significantly enhanced the bioavailability and stability of saquinavir, leading to improved antiviral efficacy. Transmission electron microscopy and differential scanning calorimetry analyses confirmed the efficient encapsulation and stability of the drug within the nanoparticles. The antiviral efficacy tests demonstrated that the saquinavir-loaded chitosan nanoparticles inhibited HIV-1 proliferation more effectively than free saquinavir, highlighting their potential as a superior drug delivery system for HIV-1 treatment.[83]

Karadeniz investigated the in vitro anti-HIV-1 activity of chitosan oligomers (COS) N-conjugated with asparagine and glutamine.[84] Their study aimed to enhance the antiviral properties of COS by conjugating them with amino acids that contain amide groups. The results indicated that the conjugated COS exhibited superior anti-HIV-1 activity compared to unmodified COS. The enhanced activity was attributed to the improved solubility and interaction with the HIV-1 envelope glycoprotein gp120, which is crucial for viral entry into host cells. The study suggested that further modification of COS with different amino acids could yield even more effective anti-HIV agents, providing a basis for future research in this area.

Jaber *et al.* studied the antiviral prospective of various chitosan derivatives, including those conjugated with arginine.[85a] Their comprehensive analysis highlighted the broad-spectrum antiviral activities of chitosan-arginine derivatives, which showed efficacy against multiple viruses, including HIV-1. The review emphasized the importance of chemical modifications in enhancing the antiviral properties of chitosan, particularly through the addition of amino acids and other bioactive molecules. The findings underscored the potential of chitosan derivatives as multifunctional antiviral agents, capable of inhibiting viral entry and replication through multiple mechanisms.[85b]

When comparing all twelve studies on the anti-HIV properties of chitosan and its derivatives, several key themes emerge. Firstly, the modification of chitosan with various bioactive compounds, such as amino acids and siRNA, consistently enhances its antiviral efficacy. These modifications improve the solubility, stability, and cellular uptake of chitosan, making it a more effective therapeutic agent. Secondly, the mechanism of action for these chitosan derivatives primarily involves the inhibition of viral entry by disrupting the interaction between HIV-1 gp120 and the host cell CD4 receptors.[86] This mode of action is crucial for preventing the virus from establishing infection and highlights the potential of chitosan derivatives as entry inhibitors.

Moreover, the studies demonstrate the versatility of chitosan as a drug delivery system. Chitosan nanoparticles show great promise in encapsulating and delivering various antiviral agents, including protease inhibitors and siRNA. This ability to enhance the bioavailability and efficacy of encapsulated drugs positions chitosan nanoparticles as a valuable tool in the fight against HIV-1. Additionally, the research underscores the importance of continued exploration into different chemical modifications of chitosan to unlock new therapeutic potentials and overcome existing challenges in HIV-1 treatment.

In conclusion, the body of research on chitosan and its derivatives provides a compelling case for their use as antiviral agents against HIV-1. The studies collectively highlight the significant improvements in antiviral efficacy achieved through chemical modifications and the development of advanced delivery systems. These findings not only advance our understanding of chitosan's potential but also pave the way for future research aimed at optimizing and expanding its use in antiviral therapies. The continuous innovation in chitosan-based formulations holds promise for developing more effective and targeted treatments for HIV-1 and other viral infections.

In summary, chitosan nanoparticles offer an encouraging platform for the delivery of anti-HIV drugs, providing enhanced bioavailability, targeted delivery, and sustained release of therapeutic agents. Their unique properties, combined with the potential for surface modification and co-delivery of multiple agents, position chitosan nanoparticles as a versatile and effective tool in the fight against HIV/AIDS. Ongoing research and development in this area have the potential to improve the efficacy of HIV treatments, reduce the burden of drug resistance, and ultimately contribute to the global efforts to end the HIV/AIDS epidemic.

2.5 Anti - Osteomyelitis

Osteomyelitis, a severe bone infection caused predominantly by Staphylococcus aureus, poses significant treatment challenges due to its recalcitrant nature and the difficulty of delivering antibiotics to infected bone tissues.[87] Chitosan, a natural polysaccharide derived from chitin, has emerged as a promising candidate for developing effective anti-osteomyelitis treatments. Its biocompatibility, biodegradability, and ability to form nanoparticles make it an ideal carrier for antibiotics and other therapeutic agents.[88] Chitosan's inherent antimicrobial properties, along with its capacity to promote wound healing and bone regeneration, further enhance its potential in treating osteomyelitis. The positively charged chitosan molecules can bind to the negatively charged microbial cell membranes, disturbing their integrity and leading to cell death. Moreover, chitosan can be chemically modified to improve its solubility and functional properties, permitting for the precised and sustained release of encapsulated drugs at the site of infection.

Nanoparticle formulations of chitosan have been extensively studied for their ability to enhance the delivery of antibiotics to bone tissues. These nanoparticles can penetrate biofilms, which are complex microbial communities that protect bacteria from antibiotics and immune responses, thereby enhancing the efficacy of the encapsulated drugs. The large surface area to volume ratio of nanoparticles enables for increased drug loading and improved pharmacokinetics. Moreover, chitosan nanoparticles can be engineered to release antibiotics in a controlled manner, sustaining therapeutic drug levels over an extended period and reducing the frequency of administration. This controlled release is particularly important in treating chronic infections like osteomyelitis, where maintaining consistent drug levels is crucial for eradicating infection and preventing relapse.

Research has shown that chitosan nanoparticles can be effectively loaded with a variety of antibiotics, including vancomycin, gentamicin, and ciprofloxacin, which are commonly used in the treatment of osteomyelitis. In vitro and in vivo studies have shown that these chitosan-based formulations can enhance the antibacterial activity of the antibiotics, reduce biofilm formation, and promote bone healing. For instance, chitosan nanoparticles loaded with vancomycin have been shown to eradicate Staphylococcus aureus biofilms more effectively than free vancomycin, highlighting the potential of this delivery system in overcoming antibiotic resistance. Furthermore, chitosan's ability to stimulate osteoblast proliferation and differentiation supports its use in bone regeneration, which is critical in repairing the bone damage caused by osteomyelitis.

In addition to their antimicrobial and bone regenerative properties, chitosan nanoparticles can be functionalized with various targeting ligands to enhance their specificity for infected bone tissues. By attaching ligands that bind to receptors overexpressed on osteoclasts or infected cells, chitosan nanoparticles can achieve targeted drug delivery, minimizing off-target effects and improving therapeutic outcomes. The use of biodegradable and biocompatible materials in these formulations ensures that the nanoparticles are safely metabolized and excreted, reducing the risk of long-term toxicity.

The antibacterial efficacy of chitosan and its composites against osteomyelitis has been a focal point in recent research. Noha H. Radwan and colleagues (2020) developed chitosan-calcium phosphate composites loaded with moxifloxacin hydrochloride for preventing postoperative osteomyelitis.[89] Their study demonstrated the composites' ability to provide complete drug release over three days, inducing osteoblast differentiation and proliferation, while also reducing bacterial count, inflammation, and intramedullary fibrosis in a bone tissue specimen from an osteomyelitis-induced animal model. The in-situ generation of calcium phosphates within the composite was verified using Fourier transform infra-red spectroscopy, X-ray powder diffraction, and scanning electron microscopy. The results indicated that these composites are promising in preventing postoperative osteomyelitis, making them worthy of clinical experimentation.

Muzzarelli (2009) explored the stimulatory effect of modified chitosan on bone formation.[90] Their findings revealed that the modified chitosan significantly enhanced osteoblast activity and bone regeneration. This study emphasized the importance of chitosan's biochemical properties, particularly its ability to form complexes with various biomolecules, thereby promoting cellular adhesion and proliferation. The modified chitosan exhibited superior biocompatibility and biodegradability, creating it an ideal candidate for bone tissue engineering applications. The results demonstrated a marked improvement in bone healing, suggesting that modified chitosan could be a valuable tool in treating osteomyelitis and other bone-related infections.[91]

Beenken (2014) investigated the therapeutic efficacy of calcium sulfate pellets coated with deacetylated chitosan in treating chronic osteomyelitis. The study demonstrated that chitosan coatings significantly enhanced the elution profile of daptomycin, reducing the initial burst release and maintaining high antibiotic concentrations for extended periods. Bacteriological analysis confirmed a significant reduction in bacterial load in the treated groups compared to controls. Histopathological analysis also showed improved bone regeneration and reduced inflammation in the chitosan-coated groups. These findings suggest that chitosan coatings can improve the efficacy of localized antibiotic delivery systems, making them a promising approach for treating chronic osteomyelitis.[92]

Another study by Uskokovic and Desai (2013) focused on the *in vitro* exploration of nanoparticulate hydroxyapatite/chitosan composites as budding drug delivery podiums for osteomyelitis treatment. Their research highlighted the composites' ability to sustain antibiotic release over three weeks, promoting osteoblastic cell proliferation and differentiation while exhibiting antibacterial efficacy against Staphylococcus aureus. Despite some reduction in antibacterial activity due to chitosan addition, the overall therapeutic potential of the composites was evident. The study concluded that hydroxyapatite/chitosan composites could effectively control drug release and support bone regeneration, making them suitable for osteomyelitis treatment.[93]

Chitosan nanoparticles have appeared as a hopeful therapeutic strategy for osteomyelitis due to their inherent biocompatibility, biodegradability, and capability to enhance drug delivery efficiency. In one study, Pawar and Srivastava (2019) developed chitosan-polycaprolactone blend sponges and evaluated their potential in managing chronic osteomyelitis. The study focused on the sponges' structural properties, drug release kinetics, and antimicrobial efficacy. The chitosan-polycaprolactone blend demonstrated a porous structure conducive to tissue growth and effective drug release. When loaded with antibiotics, these sponges showed sustained drug release over several days, significantly inhibiting bacterial growth in vitro. Additionally, the study highlighted the sponges' biocompatibility, with cell proliferation assays indicating minimal cytotoxicity. The blend's mechanical properties were also deemed suitable for bone tissue engineering applications, providing the necessary support while allowing for gradual degradation and replacement by new bone tissue.[94]

Another significant contribution to the field was made by Uskokovic and Desai (2014), who investigated the potential of hydroxyapatite (HAp) and chitosan nanoparticulate composites for the controlled release of antibiotics in osteomyelitis treatment.[95] The researchers synthesized the composites via ultrasound-assisted sequential precipitation, which resulted in the formation of HAp nanoparticles embedded within a chitosan matrix. This combination aimed to balance the rapid drug release typically associated with HAp and the controlled release properties of chitosan. The study found that the composites could sustain the release of antibiotics over several weeks, effectively reducing the initial burst release. However, while the composite's drug delivery profile was promising, its antibacterial efficacy against *Staphylococcus aureus* was somewhat compromised, and higher concentrations of the composite adversely affected osteoblast proliferation and differentiation. These findings suggest a need for optimization to enhance both antibacterial and osteogenic outcomes.

The work by Radwan (2020) further explored the potential of chitosan-based scaffolds for localized osteomyelitis treatment. They developed a chitosan-calcium phosphate composite loaded with moxifloxacin hydrochloride and evaluated it’s in vitro and in vivo performance. The composite demonstrated efficient drug release, complete within three days, and promoted osteoblast differentiation and proliferation while reducing bacterial load and inflammation in an osteomyelitis-induced animal model. The study concluded that the chitosan-calcium phosphate composite is a promising candidate for preventing post-operative osteomyelitis, warranting further clinical investigation (**Figure 7**).[96]

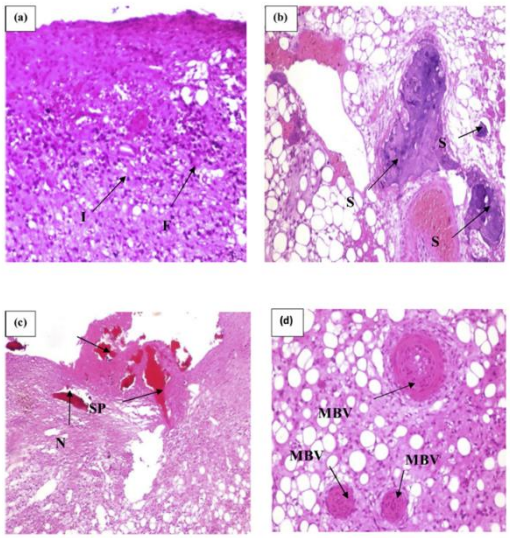


Figure 7: *Histopathological sections of animals bone tissues (x200 H&E stain): (a), (b), (c) and (d) collected from group A (positive control). I: inflammation, F: fibrosis, S: focus of sequestrum, SP: separated spicules, N: necrotic tissue, MBV: marrow blood vessels [Reprinted with permission from Ref 93].*

Cevher (2006) focused on the encapsulation efficiency and sustained release properties of vancomycin-loaded chitosan microspheres. The microspheres were prepared using a spray drying method and evaluated for their potential in treating methicillin-resistant Staphylococcus aureus (MRSA)-induced osteomyelitis. The results showed high encapsulation efficiency and a sustained release profile, with drug release influenced by the polymer-to-drug ratio. In vivo studies in a rat model demonstrated that the microspheres effectively reduced bacterial load in bone tissue compared to intramuscular injection of vancomycin, highlighting the microspheres' potential for localized antibiotic delivery in osteomyelitis treatment.[97]

In another study, Shi (2010) developed gentamicin-impregnated chitosan/nanohydroxyapatite/ethyl cellulose microsphere granules for chronic osteomyelitis therapy. These granules aimed to provide a dual-function approach by combining antibiotic delivery with bone regeneration. The composite material exhibited sustained drug release and significant antibacterial activity against Staphylococcus aureus. In vitro studies showed enhanced osteoblast proliferation and differentiation, suggesting that the composite could support bone healing while preventing infection. The study emphasized the importance of optimizing the composite's formulation to achieve a balance between antibacterial efficacy and osteogenic support.[98]

Pawar and Srivastava explored the biocompatibility and antimicrobial activity of chitosan/polycaprolactone (CH/PCL) blend sponges loaded with antibiotics for osteomyelitis treatment. Their hemocompatibility studies showed that the blend sponges caused less than 5% hemolysis, indicating good compatibility with red blood cells. The cell viability assays with L929 fibroblast and MG 63 osteosarcoma cell lines revealed that the sponges maintained high cell viability, ranging from 75 to 90%, which is essential for their potential use in biomedical applications. Additionally, the in vitro antibacterial tests against MRSA and Pseudomonas aeruginosa showed significant reductions in bacterial growth, with the blend sponges demonstrating prolonged antimicrobial activity. This study highlighted the importance of using biocompatible and effective antimicrobial sponges in managing osteomyelitis, especially in preventing infection recurrence post-surgery.[99]

Ma focused on developing a chitosan-based scaffold loaded with clindamycin to treat osteomyelitis. The scaffold's characterization showed that it possessed a porous structure, which is conducive for bone tissue regeneration. The drug release studies indicated that the scaffold could release clindamycin in a sustained manner over several weeks, maintaining therapeutic concentrations essential for eradicating bone infections. The in vivo studies in a rabbit model demonstrated significant reductions in bacterial counts and inflammation in the treated groups compared to controls. Moreover, the scaffold facilitated new bone formation, highlighting its dual role in providing antimicrobial action and supporting bone regeneration. This dual functionality makes chitosan-based scaffolds a promising candidate for osteomyelitis treatment.[100]

Hashad investigated the osteogenic and antimicrobial properties of metformin-loaded human serum albumin (HSA)/chitosan nanoparticles (MHCNPs). The study demonstrated that MHCNPs significantly enhanced the osteogenic differentiation of bone marrow mesenchymal stem cells (BMSCs) in vitro, as evidenced by increased alkaline phosphatase activity and upregulation of osteogenic genes like osteocalcin and osteoprotegerin. The nanoparticles also exhibited potent antimicrobial activity against S. aureus and E. coli, making them suitable for treating osteomyelitis where bone regeneration and infection control are crucial. The results indicated that MHCNPs could effectively promote bone healing while simultaneously preventing bacterial infections, thereby offering a comprehensive treatment approach for osteomyelitis.[101]

Considering all twelve studies, a comprehensive comparison reveals several common themes and distinctive findings. Firstly, the sustained release of antibiotics from chitosan-based formulations consistently showed prolonged therapeutic levels necessary for treating chronic osteomyelitis. This sustained release is crucial in maintaining effective drug concentrations at the infection site, thereby reducing the frequency of drug administration and improving patient compliance. Secondly, the biocompatibility of chitosan and its derivatives across various studies underscores its suitability for biomedical applications, especially in formulations requiring prolonged contact with biological tissues.

Moreover, the studies collectively highlight the dual functionality of chitosan-based materials in osteomyelitis treatment, combining antimicrobial efficacy with support for bone regeneration. This dual role is particularly advantageous in treating bone infections, where eliminating the infection and promoting bone healing are equally important. The ability of chitosan scaffolds to support new bone formation while delivering antimicrobial agents provides a synergistic approach to managing osteomyelitis, reducing the need for additional surgical interventions.

However, there are variations in the formulations and their specific applications. For instance, the choice of antibiotics (vancomycin, daptomycin, clindamycin) and the incorporation of additional agents like metformin reflect tailored approaches to different infection scenarios and patient needs. Additionally, the methods of drug encapsulation and scaffold preparation, such as microspheres, nanoparticles, and composite scaffolds, offer diverse options for clinicians to select the most appropriate treatment based on the specific clinical context.

In conclusion, extensive research on chitosan-based formulations for osteomyelitis treatment provides a robust foundation for developing effective and versatile therapeutic strategies. These studies collectively demonstrate that chitosan not only enhances the antimicrobial efficacy of loaded drugs but also supports bone tissue regeneration, making it an invaluable material in managing complex bone infections. Future research should focus on optimizing these formulations for clinical use, ensuring their safety, efficacy, and patient-specific customization to address the diverse challenges posed by osteomyelitis.

3. Conclusion

The extensive review of composites of chitosan for biomedical applications highlights the remarkable versatility and efficacy of chitosan and its composites across various biomedical domains. This review meticulously covers the properties and applications of chitosan nanoparticles, with a particular focus on their anti-carcinogenic, antimicrobial,

blood-brain barrier drug carrier, anti-HIV, and anti-osteomyelitis properties.

The anti-carcinogenic properties of chitosan nanoparticles are evidenced through multiple studies. These nanoparticles have shown significant potential in inhibiting the proliferation of cancer cells and inducing apoptosis. Chitosan's ability to be functionalized with various molecules enhances its targeting capabilities, thereby improving its efficacy as an anti-cancer agent. Studies have demonstrated that chitosan nanoparticles can effectively deliver anti-cancer drugs to the tumor site, minimizing systemic toxicity and maximizing therapeutic outcomes. For instance, chitosan nanoparticles loaded with doxorubicin have shown enhanced cytotoxicity against cancer cells compared to free doxorubicin, indicating their potential in cancer therapy.

In the realm of antimicrobial applications, chitosan's intrinsic antimicrobial properties are well-documented. Chitosan nanoparticles display broad-spectrum antimicrobial activity against various pathogens, such as bacteria, fungi, and viruses. The positive charge on chitosan interacts with the negatively charged microbial cell membranes, leading to membrane disruption and cell death. Studies have highlighted the effectiveness of chitosan nanoparticles in inhibiting biofilm formation and eradicating established biofilms, which are often resistant to conventional antibiotics. This property is particularly valuable in medical device coatings and wound healing applications, where biofilm-associated infections are prevalent.

Chitosan's role as a drug carrier across the blood-brain barrier (BBB) is another critical application. The BBB pretenses a substantial challenge for drug delivery to the brain due to its selective permeability. Chitosan nanoparticles can be engineered to enhance drug transport across the BBB, facilitating the delivery of therapeutic agents for the treatment of neurological complaints. Investigations have exposed that chitosan nanoparticles can successfully deliver drugs like rivastigmine and doxorubicin to the brain, improving their therapeutic efficacy in treating Alzheimer's disease and brain tumors, respectively.

The anti-HIV properties of chitosan nanoparticles are attributed to their ability to inhibit viral entry and replication. Functionalization of chitosan with antiviral agents enhances its efficacy against HIV. Research has demonstrated that chitosan nanoparticles can effectively deliver antiretroviral drugs, reducing viral load and improving patient outcomes. Additionally, the mucoadhesive properties of chitosan make it suitable for developing vaginal microbicides to prevent HIV transmission.

Chitosan's application in treating osteomyelitis, a severe bone infection, is facilitated by its ability to deliver antibiotics directly to the infection site. Chitosan-based scaffolds and hydrogels loaded with antibiotics like vancomycin have shown sustained release profiles and enhanced antimicrobial activity against biofilm-forming bacteria. This targeted delivery system not only improves the efficacy of the treatment but also reduces the risk of systemic side effects. Studies have demonstrated the effectiveness of chitosan-based drug delivery systems in eradicating biofilms and promoting bone regeneration.

When comparing the findings from the various studies reviewed, it is evident that chitosan nanoparticles exhibit a broad spectrum of biomedical applications due to their unique properties. Their biocompatibility, biodegradability, and ease functionalization make them excellent candidates for drug delivery systems. The anti-carcinogenic studies consistently show enhanced drug delivery and tumor suppression, highlighting the potential of chitosan nanoparticles in oncology. The antimicrobial studies reinforce the broad-spectrum activity of chitosan, emphasizing its potential in combating resistant infections. The BBB studies illustrate the capacity of chitosan nanoparticles to overcome significant biological barriers, expanding their utility in treating central nervous system disorders.

Furthermore, the anti-HIV studies underscore the versatility of chitosan in antiviral applications, particularly in developing countries where cost-effective and efficient treatment options are crucial. The anti-osteomyelitis studies demonstrate the synergistic effects of chitosan in drug delivery and bone regeneration, presenting a comprehensive solution for treating complex infections. The collective findings from these studies provide a robust framework for future research and development of chitosan-based biomedical applications.

In conclusion, chitosan and its composites present a promising frontier in biomedical applications, offering innovative solutions for drug delivery, antimicrobial therapy, cancer treatment, neurological disorders, and bone infections. The versatility, biocompatibility, and functionalization potential of chitosan nanoparticles make them invaluable in developing next-generation therapeutic strategies. Future research should aim at optimizing the formulation and delivery mechanisms of chitosan-based systems to enhance their clinical efficacy and safety. The integration of chitosan nanoparticles into clinical practice holds the potential to revolutionize the treatment paradigms for various diseases, improving patient outcomes and quality of life.

Author Contribution Declaration

Ayanjeet, Dinesh, Samyak, and Andrew did the literature review for the article. The idea of the review and content line-up was designed by Dr. Jaya. Ayanjeet and Dinesh further designed the manuscript and Ayanjeet designed the images and contributed further to the writing. Dr. Nilesh provided insights and helped with the correction of the review.

Data Availability Declaration

No new data was used for the paper hence, data availability declaration is not applicable here. All data mentioned has been properly referenced and cited, giving due regard & credit to the authors.

Acknowledgements

The authors acknowledge the guidance of their mentors, Dr. Jaya & Dr. Nilesh, for their support and wisdom throughout the process of designing the manuscript.

References

1. J. Lakkakula, D. Divakaran, R. Srivastava, P. Ingle, A. Gade, R. Raut. In Situ Growth of Biocompatible Biogenic Silver Nanoparticles in Poly-Vinyl Alcohol Thin Film Matrix. *IEEE Trans Nanobioscience*., **2023**, *22*, 480. https://doi.org/10.1109/TNB.2022.3208310
2. T. Arfin, F. Mohammad. Chemistry and structural aspects of chitosan towards biomedical applications, *In:* S. Ikram, S. Ahmed (Eds.), Natural polymers: derivatives, blends and composite, *Vol.1*, Nova Science Publishers, New York, **2016**, pp. 265-280.
3. J. Lakkakula, G. K. P. Srilekha, P. Kalra, S. A. Varshini, S. Penna. Exploring the promising role of chitosan delivery systems in breast cancer treatment: A comprehensive review. *Carbohydr Res*., **2024**, *545*, 109271. https://doi.org/10.1016/J.CARRES.2024.109271
4. A. Kumar, W. Abbas, G. Herbein. HIV-1 latency in monocytes/macrophages. *Viruses*, **2014**, *6*, 1837. https://doi.org/10.3390/V6041837
5. C. W. Norden, E. Kennedy. Experimental osteomyelitis. I. A description of the model. *J. Infect. Dis.,* **1970**, *122*, 410. https://doi.org/10.1093/INFDIS/122.5.410
6. P. D. Potdar, A. U. Shetti. Evaluation of anti-metastatic effect of chitosan nanoparticles on esophageal cancer-associated fibroblasts. *J. Cancer Metastasis Treat*., **2016**, *2*, 259. https://doi.org/10.20517/2394-4722.2016.25
7. I. A. Mayer, V. G. Abramson, B. D. Lehmann, J. A. Pietenpol. New strategies for triple-negative breast cancer—deciphering the heterogeneity. *Clin. Cancer Res.,* **2014**, *20*, 782. https://doi.org/10.1158/1078-0432.ccr-13-0583
8. U. Bickel, T. Yoshikawa, W. M. Pardridge. Delivery of peptides and proteins through the blood-brain barrier. *Adv. Drug Deliv. Rev*., **2001**, *46*, 247. https://doi.org/10.1016/S0169-409X(00)00139-3
9. T. D. Azad, J. Pan, I. D. Connolly, A. Remington, C. M. Wilson, G. A. Grant. Therapeutic strategies to improve drug delivery across the blood-brain barrier. *Neurosurg. Focus*., **2015**, *38*, 9. https://doi.org/10.3171/2014.12.FOCUS14758
10. T. Arfin. Chitosan and its derivatives: overlook of commercial application in diverse field, In: S. Ahmed, S. Ikram (Eds.), Chitosan: derivatives, composites and applications, Scrivener Publishing LLC, Beverly, **2017**, pp.115-150. https://doi.org/10.1002/9781119364849

11. F. Mohammad, T. Arfin, H. A. Al-Lohedan. Enhanced biological activity and biosorption performance of trimethyl chitosan-loaded cerium oxide particles. *J. Ind. Eng. Chem.,* **2017**, *45*, 33. https://doi.org/10.1016/J.JIEC.2016.08.029
12. T. Arfin. Current innovative chitosan-based water treatment of heavy metals: A sustainable approach, *In:* S. Ahmed, S. Kanchi, G. Kumar (Eds.), Handbook of biopolymers: advances and multifaceted applications, Jenny Stanford Publishing, Singapore, **2018**, pp. 167-182. https://doi.org/10.1201/9780429024757-7
13. S. S. Waghmare, T. Arfin. Defluoridation by adsorption with chitin-chitosan-alginate-polymers-cellulose-resins-algae and fungi-A Review. *IRJET*, **2015**, *2*, 1179. https://api.semanticscholar.org/CorpusID:212470446
14. Y. Zhu, C. Goh, A. Shrestha. Biomaterial Properties Modulating Bone Regeneration. *Macromol. Biosci*., **2021**, *21*, 2000365. https://doi.org/10.1002/mabi.202000365
15. X. Zhao, J. L. Pathak, W. Huang, C. Zhu, Y. Li, H. Guan, S. Zeng, L. Ge, Y. Shu. Metformin enhances osteogenic differentiation of stem cells from human exfoliated deciduous teeth through AMPK pathway. *J. Tissue Eng. Regen. Med*., **2020**, *14*, 1869. https://doi.org/10.1002/term.3142
16. J. J. Elsner, I. Berdicevsky, M Zilberman. In vitro microbial inhibition and cellular response to novel biodegradable composite wound dressings with controlled release of antibiotics. *Acta Biomater*., **2011**, *7*, 325. https://doi.org/10.1016/j.actbio.2010.07.013
17. J. Lakkakula, P. Kalra, G. Mallick, H. Mittal, I. Uddin. Revolutionizing cancer treatment: Enhancing photodynamic therapy with cyclodextrin nanoparticles and synergistic combination therapies. *Materials Today Sustainability*, **2024**, *28*, 100958. https://doi.org/10.1016/J.MTSUST.2024.100958
18. A. P. Sughanthy Siva, M. N. M. Ansari. A Review on Bone Scaffold Fabrication Methods. *IRJET,* **2015**, *2*, 1232.
19. Y. Xu, Y. Liu, Q. Liu, S. Lu, X. Chen, W. Xu, F. Shi. Co-delivery of bufalin and nintedanib via albumin sub-microspheres for synergistic cancer therapy. *J. Control. Release*, **2021**, *338*, 705. https://doi.org/10.1016/J.JCONREL.2021.08.049
20. B. J. Grattan, H. C. Freake. Zinc and Cancer: Implications for LIV-1 in Breast Cancer. *Nutrients*, **2012**, *4*, 648. https://doi.org/10.3390/NU4070648
21. S. Jaiswal, P. K. Dutta, S. Kumar, J. Koh, S. Pandey. Methyl methacrylate modified chitosan: Synthesis, characterization and application in drug and gene delivery. *Carbohydr Polym*., **2019**, *211*, 109. https://doi.org/10.1016/J.CARBPOL.2019.01.104
22. L. T. W. Hin, R. Subramaniam. Congestion control of heavy vehicles using electronic road pricing: The Singapore experience. *Int. J. Heavy Veh. Syst*., **2006**, *13*, 37. https://doi.org/10.1504/IJHVS.2006.009116
23. R. Vivek, V. Nipun Babu, R. Thangam, K. S. Subramanian, S. Kannan. PH-responsive drug delivery of chitosan nanoparticles as Tamoxifen carriers for effective anti-tumor activity in breast cancer cells. *Colloids Surf. B Biointerfaces*., **2013**, *111*, 117. https://doi.org/10.1016/j.colsurfb.2013.05.018
24. W. J. Yu, J. M. Son, J. Lee, S. –H. Kim, I. –C. Lee, H. –S. Baek, I. –S. Shin, C. Moon, S. –H. Kim, J. –C. Kim. Effects of silver nanoparticles on pregnant dams and embryo-fetal development in rats. *Nanotoxicology*, **2013**, *8*, 85. https://doi.org/10.3109/17435390.2013.857734
25. S. J. Lee, H. S. Min, S. H. Ku, S. Son, I. C. Kwon, S. H. Kim, K. Kim. Tumor-targeting glycol chitosan nanoparticles as a platform delivery carrier in cancer diagnosis and therapy. *Nanomedicine*, **2014**, *9*, 1697. https://doi.org/10.2217/NNM.14.99
26. S. S. Kim, A. Rait, E. Kim, K. F. Pirollo, M. Nishida, N. Farkas, J. A. Dagata, E. H. Chang. A nanoparticle carrying the p53 gene targets tumors including cancer stem cells, sensitizes glioblastoma to chemotherapy and improves survival. *ACS Nano*., **2014**, *8*, 5494. https://doi.org/10.1021/NN5014484
27. H. Park, K. Park, D. Kim. Preparation and swelling behavior of chitosan-based superporous hydrogels for gastric retention application. *J. Biomed. Mater. Res. A*., **2006**, *76*, 144. https://doi.org/10.1002/JBM.A.30533
28. S. Hattori, H. Fujisaki, T. Kiriyama, T. Yokoyama, S. Irie. Real-time zymography and reverse zymography: A method for detecting activities of matrix metalloproteinases and their inhibitors using FITC-labeled collagen and casein as substrates. *Anal. Biochem*., **2002**, *301*, 27. https://doi.org/10.1006/abio.2001.5479
29. H. Zhang, Y. Zhao. Preparation, characterization and evaluation of tea polyphenol-Zn complex loaded *β*-chitosan nanoparticles. *Food Hydrocoll*., **2015**, *48*, 260. https://doi.org/10.1016/j.foodhyd.2015.02.015
30. P. Deshpande, A. Dapkekar, M. D. Oak, K. M. Paknikar, J. M. Rajwade. Zinc complexed chitosan/TPP nanoparticles: A promising micronutrient nanocarrier suited for foliar application. *Carbohydr. Polym*., **2017**, *165*, 394. https://doi.org/10.1016/j.carbpol.2017.02.061
31. S. F. Rodrigues, L. A. Fiel, A. L. Shimada, N. R. Pereira, S. S. Guterres, A. R. Pohlmann, S. H. Farskyl. Lipid-core nanocapsules act as a drug shuttle through the blood brain barrier and reduce glioblastoma after intravenous or oral administration. *J. Biomed. Nanotechnol*., **2016**,*12*, 986. https://doi.org/10.1166/JBN.2016.2215
32. X. Wang, Y. Du, H. Liu. Preparation, characterization and antimicrobial activity of chitosan-Zn complex. *Carbohydr. Polym*., **2004**, *56*, 21. https://doi.org/10.1016/j.carbpol.2003.11.007
33. P. –H. Chang, K. Sekine, H. –M. Chao, S. –H. Hsu, E. Chern. Chitosan promotes cancer progression and stem cell properties in association with Wnt signaling in colon and hepatocellular carcinoma cells. *Sci Rep*., **2017**, *7*, 1. https://doi.org/10.1038/srep45751
34. P. C. Srinivasa, M. N. Ramesh, K. R. Kumar, R. N. Tharanathan. Properties of chitosan films prepared under different drying conditions. *J. Food Eng*., **2004**, *63*, 79. https://doi.org/10.1016/S0260-8774(03)00285-1
35. W. Chen, Y. Li, S. Yang, L. Yue, Q. Jiang, W. Xia. Synthesis and antioxidant properties of chitosan and carboxymethyl chitosan-stabilized selenium nanoparticles. *Carbohydr. Polym*. **2015**, *132*, 574. https://doi.org/10.1016/J.CARBPOL.2015.06.064
36. C. T. Lin, C. Y. Chen, S. G. Chen, T. M. Chen, S. C. Chang. Preserve the lower limb in a patient with calcaneal osteomyelitis and severe occlusive peripheral vascular disease by partial calcanectomy. *J. Med. Sci.,* **2015**, *35*, 74. https://doi.org/10.4103/1011-4564.156016
37. J. Q. Gao, Q. Q. Zhao, T. F. Lv, W. –P. Shuai, J. Zhou, G. –P. Tang, W. –Q. Liang, Y. Tabata, Y. –L. Hu. Gene-carried chitosan-linked-PEI induced high gene transfection efficiency with low toxicity and significant tumor-suppressive activity. *Int. J. Pharm*., **2010**, *387*, 286. https://doi.org/10.1016/j.ijpharm.2009.12.033
38. T. Pitt. Management of antimicrobial-resistant Acinetobacter in hospitals. *Nurs. Stand*., **2007**, *21*, 51. https://doi.org/10.7748/NS2007.05.21.35.51.C4556
39. X. Sun, Z. Wang, H. Kadouh, K. Zhou. The antimicrobial, mechanical, physical and structural properties of chitosan-gallic acid films. *LWT*., **2014**, *57*, 83. https://doi.org/10.1016/j.lwt.2013.11.037
40. N. A. Mohamed, N. Y. Al-mehbad. Novel terephthaloyl thiourea cross-linked chitosan hydrogels as antibacterial and antifungal agents. *Int. J. Biol. Macromol*., **2013**, *57*, 111. https://doi.org/10.1016/j.ijbiomac.2013.03.007
41. M. Mohseni, A. Shamloo, Z. Aghababaie, H. Afjoul, S. Abdi, H. Moravvej, M. Vossoughi. A comparative study of wound dressings loaded with silver sulfadiazine and silver nanoparticles: In vitro and in vivo evaluation. *Int J. Pharm*., **2019**, *564*, 350. https://doi.org/10.1016/j.ijpharm.2019.04.068
42. C. M. de Moura, J. M. de Moura, N. M. Soares, L. A. de Almeida Pinto. Evaluation of molar weight and deacetylation degree of chitosan during chitin deacetylation reaction: Used to produce biofilm. *CEP:PI*., **2011**, *50*, 351. https://doi.org/10.1016/J.CEP.2011.03.003
43. A. Muñoz-Bonilla, C. Echeverria, Á. Sonseca, M. P. Arrieta, M. Fernández-García. Bio-based polymers with antimicrobial properties towards sustainable development. *Materials*. **2019**, *12*, 641. https://doi.org/10.3390/MA12040641
44. S. K. Nandi, P. Mukherjee, S. Roy, B. Kundu, D. K. De, D. Basu. Local antibiotic delivery systems for the treatment of osteomyelitis - A review. *Mater. Sci. Eng. C*., **2009**, *29*, 2478. https://doi.org/10.1016/j.msec.2009.07.014
45. J. K. Smith, J. D. Bumgardner, H. S. Courtney, M. S. Smeltzer, W. O. Haggard. Antibiotic-loaded chitosan film for infection prevention: A preliminary in vitro characterization. *J. Biomed. Mater. Res. B Appl Biomater*., **2010**, *94*, 203. https://doi.org/10.1002/JBM.B.31642
46. N. Monteiro, M. Martins, A. Martins, Nuno A Fonseca, J. N. Moreira, R. L. Reis, N. M. Neves. Antibacterial activity of chitosan nanofiber meshes with liposomes immobilized releasing gentamicin. *Acta Biomater*., **2015**, *18*, 196. https://doi.org/10.1016/J.ACTBIO.2015.02.018
47. C. H. Wang, C. W. Chang, C. A. Peng. Gold nanorod stabilized by thiolated chitosan as photothermal absorber for cancer cell treatment. *J. Nanopar. Res.* **2011**,*13*, 2749. https://doi.org/10.1007/s11051-010-0162-5
48. W. Shao, H. Liu, J. Wu, S. Wang, X. Liu, M. Huang, P. Xu. Preparation, antibacterial activity and pH-responsive release behavior of silver sulfadiazine loaded bacterial cellulose for wound dressing applications. *J. Taiwan Inst. Chem. Eng*., **2016**, *63*, 404. https://doi.org/10.1016/j.jtice.2016.02.019

49. Y. Cheng, F. Yang, K. Zhang, Y. Zhang, Y. Cao, C. Liu, H. Lu, H. Dong, X. Zhang. Non-Fenton-Type Hydroxyl Radical Generation and Photothermal Effect by Mitochondria-Targeted WSSe/MnO2 Nanocomposite Loaded with Isoniazid for Synergistic Anticancer Treatment. *Adv. Funct. Mater*., **2019**, *29*, 1903850. https://doi.org/10.1002/ADFM.201903850
50. R. M. Wang, N. P. He, P. F. Song, Y. F. He, L. Ding, Z. Q. Lei. Preparation of nano-chitosan Schiff-base copper complexes and their anticancer activity. *Polym. Adv. Technol*., **2009**, *20*, 959. https://doi.org/10.1002/pat.1348
51. A. Piegat, A. Żywicka, A. Niemczyk, A. Goszczyńska. Antibacterial Activity of N,O-Acylated Chitosan Derivative. *Polymers,* **2021**, *13*, 107. https://doi.org/10.3390/POLYM13010107
52. W. M. Pardridge. Drug transport across the blood-brain barrier. *J. Cereb. Blood Flow Metab.,* **2012**, *32*, 1959. https://doi.org/10.1038/JCBFM.2012.126
53. L. Wen, Y. Tan, S. Dai, Y. Zhu, T. Meng, X. Yang, Y. Liu, X. Liu, H. Yuan, F. Hu. Vegf-mediated tight junctions pathological fenestration enhances doxorubicin-loaded glycolipid-like nanoparticles traversing bbb for glioblastoma-targeting therapy. *Drug Deliv*., **2017**, *24*, 1843. https://doi.org/10.1080/10717544.2017.1386731
54. N. J. Abbott, A. A. K. Patabendige, D. E. M. Dolman, S. R. Yusof, D. J. Begley. Structure and function of the blood-brain barrier. *Neurobiol. Dis*., **2010**, *37*, 13. https://doi.org/10.1016/J.NBD.2009.07.030
55. R. Daneman, A. Prat. The blood–brain barrier. *Cold Spring Harb. Perspect. Biol*. **2015**, *7*, a020412. https://doi.org/10.1101/cshperspect.a020412
56. S. Wohlfart, S. Gelperina, J. Kreuter. Transport of drugs across the blood-brain barrier by nanoparticles. *J. Controlled Release*, **2012**, *161*, 264. https://doi.org/10.1016/j.jconrel.2011.08.017
57. J. Freiherr, M. Hallschmid, W. H. Frey, Y. F. Brünner, C. D. Chapman, C. Hölscher, S. Craft, F. G. De Felice, C. Benedict. Intranasal insulin as a treatment for alzheimer's disease: A review of basic research and clinical evidence. *CNS Drugs*, **2013**, *27*, 505. https://doi.org/10.1007/S40263-013-0076-8
58. Y. Chen, S. Feng, W. Liu, Z. Yuan, P. Yin, F. Gao. Vitamin E succinate-grafted-chitosan oligosaccharide/RGD-conjugated TPGS mixed micelles loaded with paclitaxel for U87MG tumor therapy. *Mol Pharm*., **2017**, *14*, 1190. https://doi.org/10.1021/ACS.MOLPHARMACEUT.6B01068
59. M. D. Shadab, R. A. Khan, G. Mustafa, K. Chuttani, S. Baboota, J. K. Sahni, J. Ali. Bromocriptine loaded chitosan nanoparticles intended for direct nose to brain delivery: Pharmacodynamic, Pharmacokinetic and Scintigraphy study in mice model. *Eur. J. Pharm. Sci.,* **2013**, *48*, 393. https://doi.org/10.1016/J.EJPS.2012.12.007
60. Z. Zhao, S. Lou, Y. Hu, J. Zhu, C. Zhang. A Nano-in-Nano Polymer-Dendrimer Nanoparticle-Based Nanosystem for Controlled Multidrug Delivery. *Mol. Pharm*., **2017**, *14*, 2697. https://doi.org/10.1021/ACS.MOLPHARMACEUT.7B00219
61. L. Gao, X. Wang, J. Ma, D. Hao, P. Wei, L. Zhou, G. Liu. Evaluation of TPGS-modified thermo-sensitive Pluronic PF127 hydrogel as a potential carrier to reverse the resistance of P-gp-overexpressing SMMC-7721 cell lines. *Colloids Surf B Biointerfaces*, **2016**, *140*, 307. https://doi.org/10.1016/j.colsurfb.2015.12.057
62. J. Rip, L Chen, R Hartman, A. van den Heuvel, A. Reijerkerk, J. van Kregten, B. van der Boom, C. Appeldoorn, M. de Boer, D. Maussang, E. C. M. de Lange, P. J. Gaillard. Glutathione PEGylated liposomes: Pharmacokinetics and delivery of cargo across the blood-brain barrier in rats. *J. Drug Target*., **2014**, *22*, 460. https://doi.org/10.3109/1061186X.2014.888070
63. M. Salvalaio, L Rigon, D Belletti, F. D'Avanzo, F. Pederzoli, B. Ruozi, O. Marin, M. A. Vandelli, F. Forni, M. Scarpa, R. Tomanin, G. Tosi. Targeted polymeric nanoparticles for brain delivery of high molecular weight molecules in lysosomal storage disorders. *PLoS One*, **2016**, *11*, e0156452. https://doi.org/10.1371/JOURNAL.PONE.0156452
64. A Dalpiaz, G Paganetto, B Pavan, M. Fogagnolo, A. Medici, S. Beggiato, D. Perrone. Zidovudine and ursodeoxycholic acid conjugation: Design of a new prodrug potentially able to bypass the active efflux transport systems of the central nervous system. *Mol Pharm*., 2012, 9, 957. https://doir.org/10.1021/MP200565G
65. I. N. Khan, S. Navaid, W. Waqar, D. Hussein, N. Ullah, M. U. A. Khan, Z. Hussain, A. Javed. Chitosan-Based Polymeric Nanoparticles as an Efficient Gene Delivery System to Cross Blood Brain Barrier: In Vitro and In Vivo Evaluations. *Pharmaceuticals,* **2024**, *17,* 169. https://doi.org/10.3390/PH17020169
66. T. Banerjee, S. Mitra, A. K. Singh, R. K. Sharma, A. Maitra. Preparation, characterization and biodistribution of ultrafine chitosan nanoparticles. *Int. J. Pharm*., **2002**, *243*, 93. https://doi.org/10.1016/S0378-5173(02)00267-3
67. J. Li, K. Kataoka. Chemo-physical Strategies to Advance the in Vivo Functionality of Targeted Nanomedicine: The Next Generation. *J. Am. Chem. Soc*. **2021**,*143*, 538. https://doi.org/10.1021/JACS.0C09029
68. D. Carradori, C. Balducci, F. Re, D. Brambilla, B. L. Droumaguet, O. Flores, A. Gaudin, S. Mura, G. Forloni, L. O. -Gutierrez, F. Wandosell, M. Masserini, P. Couvreur, J. Nicolas, K. Andrieux. Antibody-functionalized polymer nanoparticle leading to memory recovery in Alzheimer's disease-like transgenic mouse model. *Nanomedicine*, **2018**, *14*, 609. https://doi.org/10.1016/J.NANO.2017.12.006
69. A. E. Caprifico, P. J. S. Foot, E. Polycarpou, G. Calabrese. Overcoming the blood-brain barrier: Functionalised chitosan nanocarriers. *Pharmaceutics*, **2020**, *12*, 1013. https://doi.org/10.3390/PHARMACEUTICS12111013
70. S. Debaisieux, F. Rayne, H. Yezid, B. Beaumelle. The Ins and Outs of HIV-1 Tat. *Traffic*., **2012**, *13*, 355. https://doi.org/10.1111/J.1600-0854.2011.01286.X
71. A. Dev, N. S. Binulal, A. Anitha, S. V. Nair, T. Furuike, H. Tamura, R. Jayakumar. Preparation of poly(lactic acid)/chitosan nanoparticles for anti-HIV drug delivery applications. *Carbohydr. Polym*., **2010**, *80*, 833. https://doi.org/10.1016/J.CARBPOL.2009.12.040
72. J. S. Park, Y. W. Cho. In vitro cellular uptake and cytotoxicity of paclitaxel-loaded glycol chitosan self-assembled nanoparticles. *Macromol. Res*., **2007**, *15*, 513. https://doi.org/10.1007/BF03218824
73. L. N. Ramana, S. Sharma, S. Sethuraman, U. Ranga, U. M. Krishnan. Investigation on the stability of saquinavir loaded liposomes: Implication on stealth, release characteristics and cytotoxicity. *Int. J. Pharm*., **2012**, *431*, 120. https://doi.org/10.1016/j.ijpharm.2012.04.054
74. H. Y. Nam, S. M. Kwon, H. Chung, S. –Y. Lee, S. –H. Kwon, H. Jeon, Y. Kim, J. H. Park, J. Kim, S. Her, Y. –K. Oh, I. C. Kwon, K. Kim, S. Y. Jeong. Cellular uptake mechanism and intracellular fate of hydrophobically modified glycol chitosan nanoparticles. *J. Control. Release*. **2009**, *135*, 259. https://doi.org/10.1016/j.jconrel.2009.01.018
75. S. D. Gioia, A. Trapani, D. Mandracchia, E. D. Giglio, S. Cometa, V. Mangini, F. Arnesano, G. Belgiovine, S. Castellani, L. Pace, M. A. Lavecchia, G. Trapani, M. Conese, G. Puglisi, T. Cassano. Intranasal delivery of dopamine to the striatum using glycol chitosan/sulfobutylether-β-cyclodextrin based nanoparticles. *Eur. J. Pharm. Biopharm.,* **2015**, *94*, 180. https://doi.org/10.1016/j.ejpb.2015.05.019
76. R. Rozana, Y. Yulizar, A. Saefumillah, D. O. B. Apriandanu. Synthesis, characterization and in vitro release study of efavirenz-loaded chitosan nanoparticle. *AIP Conf. Proc*., **2020**, *2242*, 040004. https://doi.org/10.1063/5.0007923
77. K. Chaturvedi, K. Ganguly, M. N. Nadagouda, T. M. Aminabhavi. Polymeric hydrogels for oral insulin delivery. *J. Control. Release,* **2013**, *165*,129. https://doi.org/10.1016/j.jconrel.2012.11.005
78. B. Singh, B. Garg, S. C. Chaturvedi, S. Arora, R. Mandsaurwale, R. Kapil, B. Singh. Formulation development of gastroretentive tablets of lamivudine using the floating-bioadhesive potential of optimized polymer blends. *J. Pharm. Pharmacol.,* **2012**, *64*, 654. https://doi.org/10.1111/J.2042-7158.2011.01442.X
79. D. A. Cobb, N. Smith, S. Deodhar, A. N. Bade, N. Gautam, B. L. D. Shetty, J. McMillan, Y. Alnouti, S. M. Cohen, H. E. Gendelman, B. Edagwa. Transformation of tenofovir into stable ProTide nanocrystals with long-acting pharmacokinetic profiles. *Nat. Commun*., **2021**, *12*, 5458. https://doi.org/10.1038/S41467-021-25690-5
80. S. A. Agnihotri, N. N. Mallikarjuna, T. M. Aminabhavi. Recent advances on chitosan-based micro- and nanoparticles in drug delivery. *J. Control. Release*., **2004**, *100*, 5. https://doi.org/10.1016/j.jconrel.2004.08.010
81. S. Sharma, A. Tyagi, S. Dang. Nose to Brain Delivery of Transferrin conjugated PLGA nanoparticles for clonidine. *Int. J. Biol. Macromol*. **2023**, *252*, 126471. https://doi.org/10.1016/j.ijbiomac.2023.126471
82. L. N. Ramana, S. Sharma, S. Sethuraman, U. Ranga, U. M. Krishnan. Evaluation of chitosan nanoformulations as potent anti-HIV therapeutic systems. *Biochim. Biophys Acta.,* **2014**, *1840*, 476. https://doi.org/10.1016/J.BBAGEN.2013.10.002
83. D. Soundararajan, L. N. Ramana, P. Shankaran, U. M. Krishnan. Nanoparticle-based strategies to target HIV-infected cells. *Colloids Surf B Biointerfaces*., **2022**, *213*, 112405. https://doi.org/10.1016/j.colsurfb.2022.112405
84. F. Karadeniz, S. K. Kim. Chapter three - antidiabetic activities of chitosan and its derivatives: a mini review. *Adv. Food Nutr. Res*. **2014**, *73*, 33. https://doi.org/10.1016/B978-0-12-800268-1.00003-2
85. (a) N. Jaber, M. Al-Remawi, F. Al-Akayleh, N. Al-Muhtaseb, I. S. I. Al-Adham, P. J. Collier. A review of the antiviral activity of Chitosan,

including patented applications and its potential use against COVID-
86. (b) N. Hsan, S Kumar, J. Koh, and P. K Dutta. Chitosan modified multi-walled carbon nanotubes and arginine aerogel for enhanced carbon capture. *Int. J. Biol. Macromol*., **2023**, *252*, 126523. https://doi.org/10.1016/j.ijbiomac.2023.126523
87. P. Heydari, M. Kharaziha, J. Varshosaz, A. Z. Kharazi, S. H. Javanmard. Co-release of nitric oxide and L-arginine from poly (*β*-amino ester)-based adhesive reprogram macrophages for acceleratedwound healing and angiogenesis in vitro and in vivo. *Biomater. Adv.,* **2024**, *158*. 213762. https://doi.org/10.1016/j.bioadv.2024.213762
88. Y. Zhang, X. Shen, P. Ma, Z. Peng, K. Cai. Composite coatings of Mg-MOF74 and Sr-substituted hydroxyapatite on titanium substrates for local antibacterial, anti-osteosarcoma and pro-osteogenesis applications. *Mater. Lett*., **2019**, *241*, 18. https://doi.org/10.1016/j.matlet.2019.01.033
89. C. Makarov, V. Cohen, A. Raz-Pasteur, I. Gotman. In vitro elution of vancomycin from biodegradable osteoconductive calcium phosphate-polycaprolactone composite beads for treatment of osteomyelitis. *Eur. J. Pharm. Sci.,* **2014**, *62*, 49. https://doi.org/10.1016/j.ejps.2014.05.008
90. N. H. Radwan, M. Nasr, R. A. H. Ishak, N. F. Abdeltawab, G. A. S. Awad. Chitosan-calcium phosphate composite scaffolds for control of post-operative osteomyelitis: Fabrication, characterization, and in vitro–in vivo evaluation. *Carbohydr. Polym*. **2020**, *244*, 116482. https://doi.org/10.1016/j.carbpol.2020.116482
91. R. A. A. Muzzarelli. Chitosan composites with inorganics, morphogenetic proteins and stem cells, for bone regeneration. *Carbohydr. Polym*., **2011**, *83*, 1433. https://doi.org/10.1016/j.carbpol.2010.10.044
92. R. A. A. Muzzarelli. Genipin-crosslinked chitosan hydrogels as biomedical and pharmaceutical aids. *Carbohydr. Polym*., **2009**, *77*, 1. https://doi.org/10.1016/j.carbpol.2009.01.016
93. K. E. Beenken, J. K. Smith, R. A. Skinner, S. G. Mclaren, W. Bellamy, M. J. Gruenwald, H. J. Spencer, J. A. Jennings, W. O. Haggard, M. S. Smeltzer. Chitosan coating to enhance the therapeutic efficacy of calcium sulfate-based antibiotic therapy in the treatment of chronic osteomyelitis. *J. Biomater. Appl*., **2014**, *29*, 514. https://doi.org/10.1177/0885328214535452
94. V. Uskoković, C. Hoover, M. Vukomanović, D. P. Uskoković, T. A. Desai. Osteogenic and antimicrobial nanoparticulate calcium phosphate and poly-(d,l-lactide-co-glycolide) powders for the treatment of osteomyelitis. *Mater. Sci. Eng. C*., **2013**, 33, 3362. https://doi.org/10.1016/j.msec.2013.04.023
95. V. Pawar, R. Srivastava. Chitosan-polycaprolactone blend sponges for management of chronic osteomyelitis: A preliminary characterization and in vitro evaluation. *Int. J. Pharm*., **2019**, *568*, 118553. https://doi.org/10.1016/j.ijpharm.2019.118553
96. V. Uskoković, T. A. Desai. In vitro analysis of nanoparticulate hydroxyapatite/chitosan composites as potential drug delivery platforms for the sustained release of antibiotics in the treatment of osteomyelitis. *J. Pharm. Sci*., **2014**, *103*, 567. https://doi.org/10.1002/jps.23824
97. N. H. Radwan, M. Nasr, R. A. H. Ishak, N. F. Abdeltawab, G. A. S. Awad. Chitosan-calcium phosphate composite scaffolds for control of post-operative osteomyelitis: Fabrication, characterization, and in vitro–in vivo evaluation. *Carbohydr. Polym*., **2020**, *244*, 116482. https://doi.org/10.1016/J.CARBPOL.2020.116482
98. E Cevher, Z Orhan, L Mülazimoğlu, D. Sensoy, M. Alper, A. Yildiz, Y. Ozsoy. Characterization of biodegradable chitosan microspheres containing vancomycin and treatment of experimental osteomyelitis caused by methicillin-resistant Staphylococcus aureus with prepared microspheres. *Int J. Pharm*., **2006**, *317*, 127. https://doi.org/10.1016/j.ijpharm.2006.03.014
99. P. Shi, Y. Zuo, X. Li, Q. Zou, H. Liu, L. Zhang, Y. Li, Y. S. Morsi. Gentamicin-impregnated chitosan/nanohydroxyapatite/ethyl cellulose microspheres granules for chronic osteomyelitis therapy. *J. Biomed. Mater. Res. A*., **2010**, *93A*, 1020. https://doi.org/10.1002/JBM.A.32598
100. V. Pawar, R. Srivastava. Layered assembly of chitosan nanoparticles and alginate gel for management of post-surgical pain and infection. *16th International Conference on Nanotechnology - IEEE NANO.,* **2016**, 241. https://doi.org/10.1109/NANO.2016.7751388
101. D. Słota, J. Jampilek, A. Sobczak-Kupiec. Targeted Clindamycin Delivery Systems: Promising Options for Preventing and Treating Bacterial Infections Using Biomaterials. *Int. J. Mol. Sci*., **2024**, *25*, 4386. https://doi.org/10.3390/ijms25084386
102. R. A. Hashad, R. A. H. Ishak, A. S. Geneidi, S. Mansour. Surface functionalization of methotrexate-loaded chitosan nanoparticles with hyaluronic acid/human serum albumin: Comparative characterization and in vitro cytotoxicity. *Int. J. Pharm*., **2017**, *522*, 128. https://doi.org/10.1016/j.ijpharm.2017.03.008