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REVIEW

Research and Application of Chitosan Nanoparticles in Orthopedic Infections

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Abstract: Orthopedic infection is one of the most intractable orthopedic problems. Bacteria resistant to antibiotics also develop gradually. Chitosan is widely used in the Biomedical field because of its high biocompatibility, biodegradability, and antibacterial activity. Chitosan-based drug delivery systems are frequently utilized to produce controlled medication release. When combined with antibiotics, synergistic antibacterial effects can be achieved. Chitosan-based nanoparticles are one of the most widely used applications in drug delivery systems. The focus of this review is to provide information on new methods being developed for chitosan-based nanoparticles in the field of bone infection treatment, including chitosan nanoparticles for antibacterial purposes, Ch-loaded with antibiotics, Ch-loaded with metal, and used as immune adjuvants. It may Provide ideas for the fundamental research and the prospects of future clinical applications of orthopedic infections.

Keywords: chitosan nanoparticles, orthopedic infection, chitosan-based nanoparticles, staphylococcus aureus

Introduction

Orthopedic infection is one of the most intractable orthopedic problems, and various orthopedic infections were reported, including osteomyelitis, septic arthritis, bursitis, and infections associated with orthopedic devices (Figure 1).[1] Doctors can classify bone infections into acute and chronic categories in clinical practice. Chronic infection is complicated to cure and easily relapses, causing considerable psychological and economic burden to patients.[2] Orthopedic implant-associated infections (OIAIs) are one of the most devastating introduction problems in orthopedic surgery.

With the aging population in developed countries, an increasing number of older adults come with a demand to maintain their quality of life and progress in the surgical technologies and materials used for prostheses. Thus, it is foreseeable that the number of joint replacements will increase continuously over the next few decades.[3] The growing number of joint replacement surgeries is leading to a rise in complications.[4] Among these many complications, Periprosthetic infection is a common complication after implantation.[5]

Periprosthetic joint infection, a complication following joint replacement surgery, occurs at a rate of approximately 1% to 2% in primary total knee arthroplasty(TKA) and up to 20% in the case of prosthesis revisions.[6] The incidence of periprosthetic joint infection (PJI) after total hip arthroplasty (THA) is lower, but it is also a serious and damaging complication.[7] The consequences of periprosthetic infections are severe, often requiring the removal of the prosthesis, increasing the patient's economic costs and suffering, and posing legal risks to doctors.

The patients with joint replacement are usually older. These patients are often combined with some risk factors, which increase the infection rate, including hypertension (HTN), diabetes, hyperlipidemia, rheumatoid arthritis, kidney disease, anemia, and depression.[8] Costs associated with revisions of orthopedic implants and infection management are expected to rise substantially in the coming decades.[9]

Antibiotics have been proven effective in treating infections. However, Systemic antibiotics are not often absorbed; bone tissues are moderately perfused, and blood flow is usually reduced in infected bone tissue. Local Application of antibiotics is also a commonly used treatment method; however, as the number of people using antibiotics to treat bone infections in clinical practice continues to increase, bacterial resistance to antibiotics is also gradually developing.[10]

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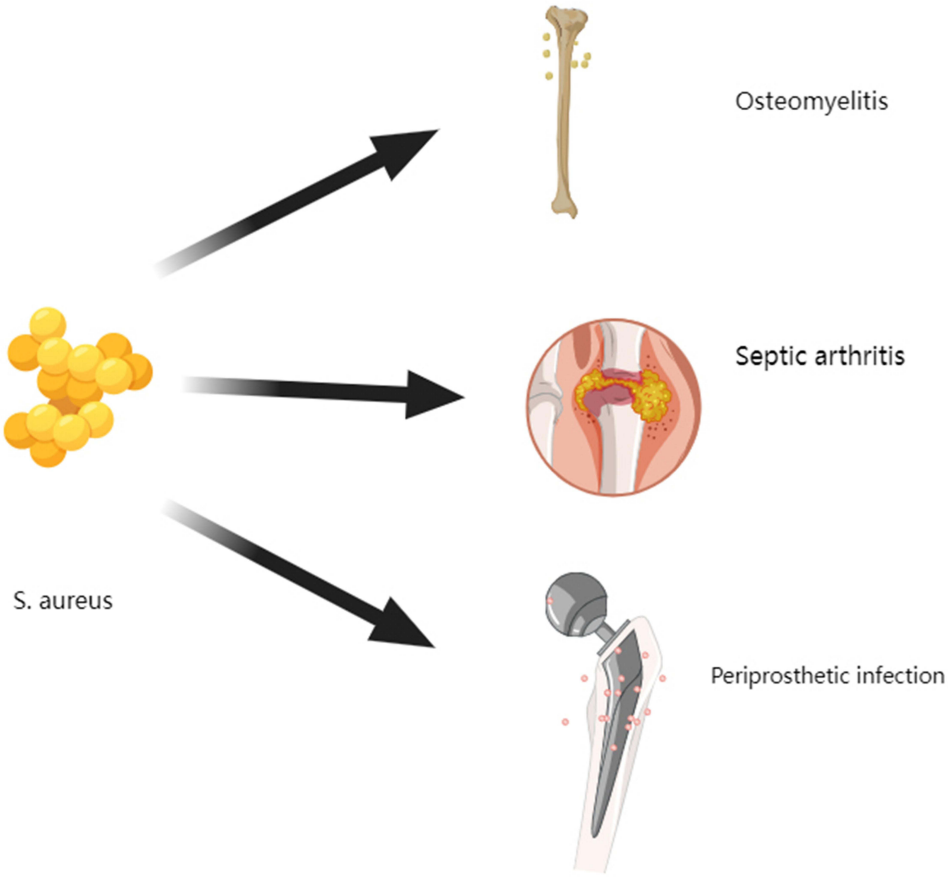

Figure 1 Common infectious diseases in orthopedics, including osteomyelitis, septic arthritis, and implant-associated infections such as periprosthetic infection. Staphylococcus aureus (S. aureus) is the most common bacterium causing orthopedic infections.

Deacetylated derivatives of chitin, chitosan is a hydrophilic cationic polysaccharide found in crustacean shells.[11] It is widely used in the Biomedical field because of its high biocompatibility, biodegradability, and antibacterial activity.[12] Chitosan-based drug delivery systems are frequently utilized to produce controlled medication release.[13] The unique antibacterial ability of Chitosan can disrupt the typical physiological structure of bacteria and achieve rapid bactericidal effects.[13] When combined with antibiotics, synergistic antibacterial effects can be achieved.[14]

Since the term "nanosystems" became known to the scientific community in the 1970s, nanomedicine delivery systems have aroused great scientific interest and have always been a research hotspot in interdisciplinary fields. The size of the system ranges from 10 to 1000 nm and can be composed of different biocompatible materials.[15] Due to its Small volume, large specific surface area, high encapsulation ability of hydrophilic and lipophilic drugs, and suitability for multiple delivery pathways, it can Change drug distribution and improve drug bioavailability. Various nano-delivery systems have been developed and prepared for practical applications.[16] Chitosan-based nanoparticles are among the most widely used applications.[17]

This review provides information on new methods being developed for chitosan-based nanoparticles in bone infection treatment, including chitosan nanoparticles for antibacterial purposes, Ch-loaded with antibiotics, Ch-loaded with metal, and used as immune adjuvants. Finally, it may Provide ideas for fundamental research and the prospects of future clinical applications of orthopedic infections.

The Causes of Difficulty in Treating Bone Infections

The body's initial immunological response to an infection is to alert the infected site to immune cell concentration and aggregation. First, the body's natural defenses against infection—including neutrophils, macrophages, and other

phagocytic cells—and the immune system play a part.[18] Next, T and B cell-specific immune systems generate the required antibodies. These immune cells' concentration, together with immune components (such as histamine), might set off an immunological reaction that results in localized discomfort, swelling, fever, and eventually loss of function, even though this is typically seen as a helpful immune response, a severe or persistent infection may subsequently cause cell and tissue necrosis, as well as the clinical signs and symptoms of osteomyelitis.[19]

The most concerned bacteria in orthopedic infection are Staphylococcus aureus and Staphylococcus epidermidis.[19] From the cultivation of surgical specimens, it can be seen that Staphylococcus aureus is the primary pathogen of chronic bacterial osteomyelitis, accounting for about two-thirds of the isolates.[20]

The antibiotics currently used in the clinical treatment of bone and joint infections include gentamicin, levofloxacin, clindamycin, vancomycin, cephalosporins, amoxicillin, and erythromycin.[21] However, some bacteria, including S. aureus, may develop resistance to all these antibiotics.[22] S. aureus have developed many survival strategies that interact with host cells, including biofilm formation,[23] intracellular infection, small colony variation (SCV), and toxin secretion.[24]

Bacteria Biofilms,

Biofilms are microbial communities embedded within polymeric extracellular matrixes that can attach to biotic and abiotic surfaces (Figure 2).[25] Upon implant placement, bacteria can attach to the implant surface and form biofilms at the implant site, resulting in local microbial infections.[26]

Biofilm prevents the attack of antibiotics and immune cells in the environment but also blocks the supply of nutrients and oxygen. Therefore, these conditions cause bacteria to have reduced metabolic activity, a Low-energy state characterized by antibiotic resistance and persistent infectivity. The bacterial cells in the biofilm are resistant to antibiotics that are effective against dividing cells because they have low metabolic activity.[27] In addition, antibiotics are difficult to penetrate the extracellular matrix and effectively interact with bacteria embedded in biofilm.[28] Bacteria within the biofilm have significantly improved resistance to antibiotic treatment compared with planktonic bacteria with the same genes, which can be increased by 10 to 1000 times.[29] However, the diffusion barrier function of the biofilm cannot alone explain the observed sharp decrease in antibiotic sensitivity, as the bacteria inside the biofilm are in a state of low metabolism.[30] On the other hand, unlike planktonic bacteria, bacteria that colonize the host in biofilms have the advantage of being more resistant to the host's immune system.[31] Previous research has revealed that the capacity of S. aureus to produce biofilms accounts for the persistence of many infections.[32]

Intracellular Infection

It has been reported that S. aureus, internalized by cultured osteoblasts, can survive within the cells and significantly reduce osteoblast proliferation (Figure 3).[33]

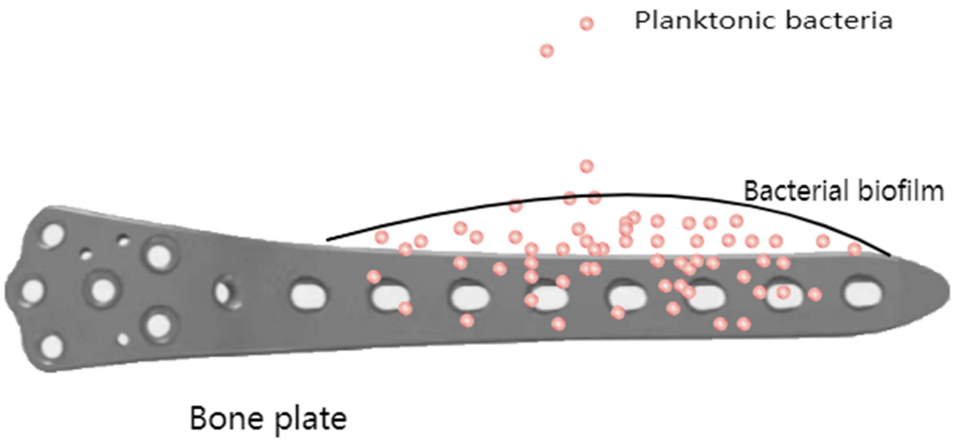

Figure 2 Bacterial biofilm adheres to the bone plate and continuously releases planktonic bacteria, leading to infections that cannot be completely cured.

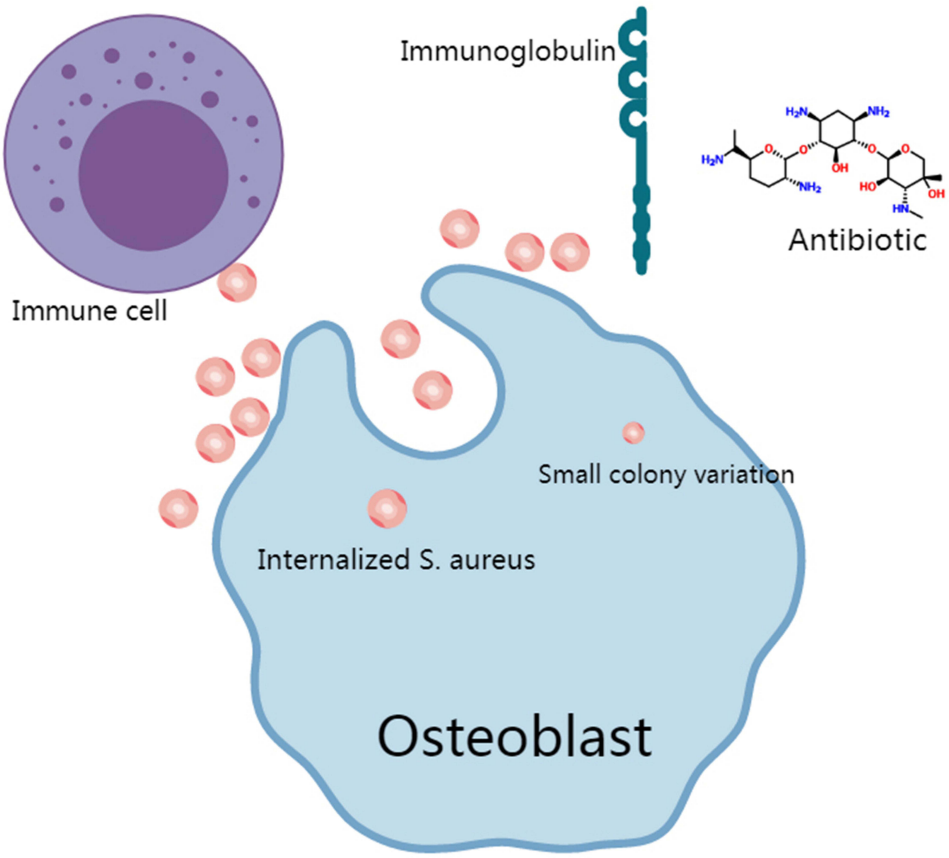

Figure 3 Staphylococcus aureus (S. aureus) is internalized into osteoblast to evade the effects of human immunity and antibiotics. The S. aureus entering the cell is in a low metabolic state and undergoes small colony variation.

In addition to in vitro studies, the infection of human osteoblasts with S. aureus has also been confirmed in vivo. In a human tissue sample from a case of chronic osteomyelitis, Walter Nike employed ultrastructural transmission electron microscopy analysis for the first time to confirm the internalization of Staphylococcus aureus into osteoblasts.[34]

It has been demonstrated that the metabolic rate of bacteria is one of the best predictors of antibiotic susceptibility. Intracellular forms of S. aureus have been shown to have a low metabolic state and are less responsive to antibiotic action, suggesting a switch to a persister phenotype.[35] Osteoblasts have also been implicated in the progression of these infections; Krauss Jennifer elucidates a unique role for osteoclasts to harbor bacteria during infection, providing a possible mechanism by which bacteria could evade destruction by the immune system,[36] S. aureus's intracellular activity plays a role in the development of chronic infection. Creating SCV within the cells may encourage the infection's persistence.[37]

Small Colony Variants (SCVs)

SCVs have been isolated from chronic infection in osteomyelitis cases and are considered a cause of persistence and recurrence.[38]

Research reports showed that small-colony variants (SCVs), a subpopulation of S. aureus related to chronic and relapsing infections, are partially responsible for these infections. SCVs have a size tenth of typical colonies and exhibit several distinctive features, such as reduced or absent pigmentation, decreased hemolytic activity, increased biofilm formation, and enhanced resistance to antimicrobials.[39] Other characteristics of SCVs include their small colony size, slow growth, downregulation of virulence genes, and upregulated genes of adhesiveness.[40] SCVs allow S. aureus to adapt to the environment, reversible phenotypic variations resulting in a quasi-dormant state of infection,[41] and can transform into planktonic bacteria under suitable conditions.[42]

Antibacterial Effects of Chitosan and Chitosan Nanoparticles

Chitosan and Its Antibacterial Effects

Chitosan is widely present in the shells of crustaceans, insects, mollusks, and fungal cell walls and is highly abundant on Earth.[43] As a natural cationic compound, it has many advantages, such as non-toxicity, biodegradability, broad-spectrum antibacterial properties, adsorption, and good biocompatibility.[44] Chitosan itself is not very soluble in water. Due to its cationic polyelectrolyte properties have become a natural cationic flocculant that can be modified through acylation, hydroxylation, grafting, and crosslinking reactions.[45] Modified polymers have broad practical value in fields such as medicine.[46] In recent years, due to the unique antibacterial properties of chitosan, it has attracted more and more attention.[47] Many researchers believe chitosan has unique properties compared to general antibacterial agents, such as broad-spectrum, high sterilization rate, and efficient inhibition of bacterial and fungal growth and reproduction.[48] Many factors affect the antibacterial effect of chitosan, such as molecular weight, concentration, deacetylation degree, environmental pH value, etc.[49,50] Due to the high number of acetylation repeating units, natural chitosan limits the exposure of free amino groups. And because of the close correlation between positively charged free amino groups and antibacterial effects, less exposure of free amino groups exhibits limited antibacterial efficacy. In addition, due to the strong hydrogen bonds within and between molecules, it shows high hydrophobicity.[51] The presence of higher free amino groups in CS is usually increased through the deacetylation process. These amino groups can interact with the surface of microbial cells through electrostatic attraction.[52] In addition, the high solubility of CS in acidic water makes it form cationic polyelectrolytes, which are prone to interact with negatively charged bacterial cells, leading to cell lysis and death. The antibacterial activity of high molecular weight CS mainly exists outside the cell wall of bacteria, as it cannot penetrate the cell wall. It can adhere to the cell wall, decreasing nutrient uptake from the extracellular environment. It also has a chelating effect on metals, reducing bacterial uptake of metal particles. In addition to the extracellular impacts, low molecular weight chitosan can also enter bacteria, affecting mitochondrial function and RNA and protein synthesis.[53]

Benjamin H Beck studied the antimicrobial activity of chitosan against Streptococcus. He found that after adsorbing bacteria and interacting with their cell surface, chitosan mediates the efflux of intracellular ATP, indicating that chitosan can damage bacterial cells, leading to leakage of cytoplasmic contents and, ultimately, cell death.[54] A recent study established the relationship curve between antibacterial rate and reaction time with the aid of the neutralizing agent MgCl2. Regarding Staphylococcus aureus and Escherichia coli, chitosan's MBC and MIC are both 30 μ g/mL. However, chitosan acetate may attain a 100% antibacterial rate in just three minutes at a concentration of 100 ug/mL.[55] Chitosan chemically modified showed more potent antibacterial activity in the studies. S-nitroso CS has higher antibacterial activity than CS, with a decrease in MIC and MBC values. According to the time-killing curve results, the cell viability of Escherichia coli and Streptococcus mutans decreased 5-fold and 2-fold, respectively, after 0.5 hours of s-nitroso cs treatment.[56] Cristina Ardean et al functionalized chitosan using impregnation technology. And enhanced antibacterial activity in all chitosan derivatives after impregnation Compared with the properties of natural chitosan.[47] Wang employed Quaternary ammonium functionalized chitosan derivatives. Salt and antibacterial results showed good anti-bacterial activity against all microorganisms in the experiment except for Pseudomonas aeruginosa. The materials exhibit high electrochemical activity, and the antibacterial activity against Staphylococcus aureus is higher than that against Escherichia coli.[57]

Chitosan Nanoparticles and Their Antibacterial Effects

Preparation of Chitosan Nanoparticles

CS-based NPs are defined as chitosan nanoparticles with a particle size of less than 1000nm. They are used in various biomedical fields, such as drug delivery, gene delivery, immune induction, etc.[58] There are several methods for preparing chitosan nanoparticles, including ionic gelation, Polyelectrolyte complex, Microemulsion, Coprecipitation, and Emulsification solvent diffusion.[59] The simplicity and efficiency of ionic gelation make it highly popular, as it can be easily performed (Figure 4).[58]

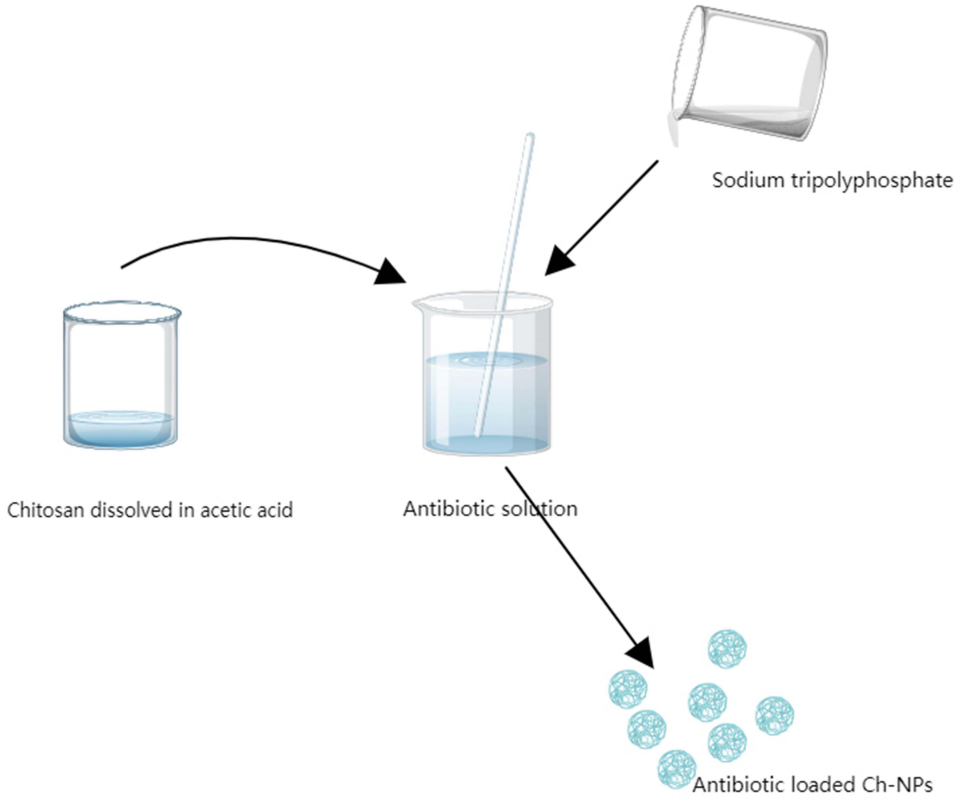

Figure 4 Prepare drug-loaded chitosan nanoparticles using the ionic gelation method.

The Antibacterial Effect of Chitosan Nanoparticles

Many studies have demonstrated that chitosan nanoparticles have good antibacterial effects without drug loading.[59] Compared to chitosan, chitosan nanoparticles have relatively higher antibacterial activity.[60] Azam Aliasghari evaluated the antimicrobial effect of chitosan and nano-chitosan against four bacterial species: Streptococcus mutans, Streptococcus sobrinus, Streptococcus sanguis, and Streptococcus salivarius. As a result, it was found that the mic of chitosan against Streptococcus mutans, Streptococcus sanguineum, Streptococcus salivarius, and Streptococcus sorghum were 1.25, 1.25, 0.625, and 0.625 mg/mL, respectively. Compared with chitosan, chitosan nanoparticles showed significantly enhanced antibacterial activity. The MIC of chitosan nanoparticles against Streptococcus mutans, Streptococcus salivarius, and Streptococcus sorghum was 0.625 mg/mL, and the MIC against Streptococcus sanguineus was 0.312 mg/mL. In addition, chitosan and chitosan nanoparticles can reduce the biofilm formation rate of Streptococcus mutans by 92.5% and 93.4% at a concentration of 5 mg/mL, respectively.[18]

In another study, suspended chitosan nanoparticles were found to effectively inhibit the bacterial attachment of S. aureus as a surgical rinse and were more effective in eradicating bacteria in orthopedic hardware than saline. Thus, they can potentially prevent and treat musculoskeletal infections.[61] In a study, modified chitosan nitrogen-phosphorus co-doped carbonized chitosan nanoparticles (NPCNs) were prepared to clear intracellular S. aureus infection. The results indicate that NPCNs can be used as a fluorescent probe for bacteria imaging and kill extracellular and intracellular bacteria with low cytotoxicity.[62] Aida Haji Hossein Tabrizi prepared chitosan nanoparticles with an average diameter of 246 nm. Using the ion gelation method and isolated 50 clinical uropathogenic Escherichia coli (UPEC) resistant to ciprofloxacin. Antibacterial experiments have shown that minimum inhibitory concentration (MIC) values were 375–750 (μg/mL). The anti-biofilm effect of CNs was evaluated, and 15 (30%) of clinical isolates produced weak biofilm CN in a concentration-dependent manner. However, in combination with CNs, antibiotics showed an additive effect. And decreased the number of viable bacteria in the biofilms.[63] Rivera prepared chitosan nanoparticles with tripolyphosphate (TPP) by using ionotropic gelation. CHNPs were used to perform antibacterial and anti-biofilm experiments on Pseudomonas strains isolated from milk samples of cows diagnosed with BM. The results showed that the nanoparticles inhibited biofilm and could eradicate pre-existing mature biofilms.[64] Costa

found Chitosan nanoparticles can effectively inhibit the biofilm growth of all test microorganisms, including Vancomycin-resistant S. aureus (VRSA), vancomycin-resistant, Enterococcus faecalis (VREF) and Pseudomonas aeruginosa (P. aeruginosa R) and have vigorous anti-quorum sensing activity.[65] R. Ikono investigated nano chitosan inhibition capacity against biofilms of S. mutans. NPs inhibition capacity was observed through biofilm mass quantity and bacterial cell viability. The result showed that the concentration of nano chitosan increased. The cell viability of microorganisms significantly decreased, and biofilm inhibition was observed at 18 h incubation.[66] According to reports, the antibacterial activity of chitosan can be further improved through chemical modification methods, including increasing surface charge concentration, enhancing hydrophobicity, and introducing other antibacterial groups.[67]

Chitosan Nanoparticles Loaded with Antibiotics

According to previous studies, chitosan nanoparticles loaded with antibiotics exhibited synergistic antibacterial effects.[68] In research, Scolari prepared rifampicin (RIF) and ascorbic acid (ASC) co-loaded into alginate (ALG)/chitosan (CS) nanoparticles (RIF/ASC NPs) and tested for their antibacterial activity against Staphylococcus aureus. The result showed a significant biocide activity against the S. aureus strains with MIC between 2- and 8-fold lower than the free antibiotic RIF. Therefore, he believes that the mechanism of enhancing the antibacterial effect of RIF/ASC NP is the result of the synergistic effect between NP and RIF/ASC antibiotic combination.[69]

Baicalein (BA) has been confirmed as an inhibitor of bacterial biofilm (Figure 5). Zhang uses β-cyclodextrin-grafted chitosan (CD-CS) as a drug carrier to improve its drug efficiency. The results showed that the drug-loaded nanoparticles exhibited a better elimination effect on S. aureus biofilm both in vivo and in vitro. They speculate that NPs may permeate into the biofilm more efficiently and improve the biofilm elimination effect of BA.[70] Pseudomonas aeruginosa (P. aeruginosa) is a common infectious pathogen in orthopedic infections. Zheng engineered self-assembling chitosan-ceftazidime nanoparticles (CSCE) with the capability of penetrating biofilms and eradicating P. aeruginosa. These nanoparticles exhibited significant inhibition of P. aeruginosa growth, reduced pyocyanin production, and decreased biofilm formation, with a maximum inhibition rate of 22.44%.[71] Maya S. created tetracycline-encapsulated O-carboxymethyl chitosan nanoparticles (Tet-O-CMC Nps) to deliver the tetracycline to Infected cells by S. aureus. As a result, Tet-O-CMC Nps demonstrated a six-fold increase in intracellular S. aureus infection-killing efficacy compared to Tet alone, indicating its effectiveness as a nanomedicine (Figure 6).[72] To combat diseases that are resistant to many drugs, Jamil Bushra studies and creates chitosan nanoparticles (CSNPs) coated with cefazolin. The outcome

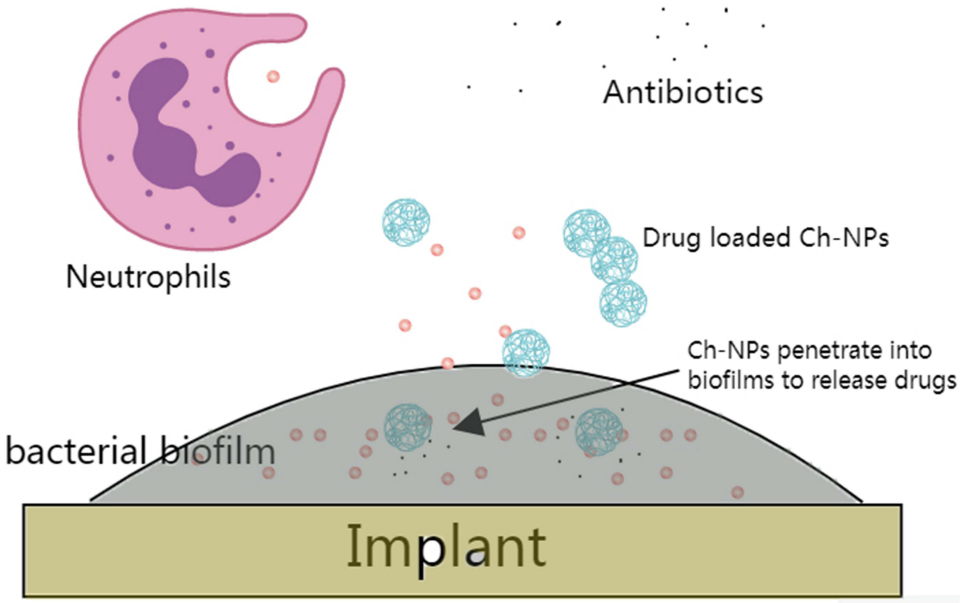

Figure 5 Drug-loaded chitosan nanoparticles (Ch-NPs) can penetrate into bacterial biofilms and release antibiotics, thereby increasing the local antibiotic concentration in the biofilm.

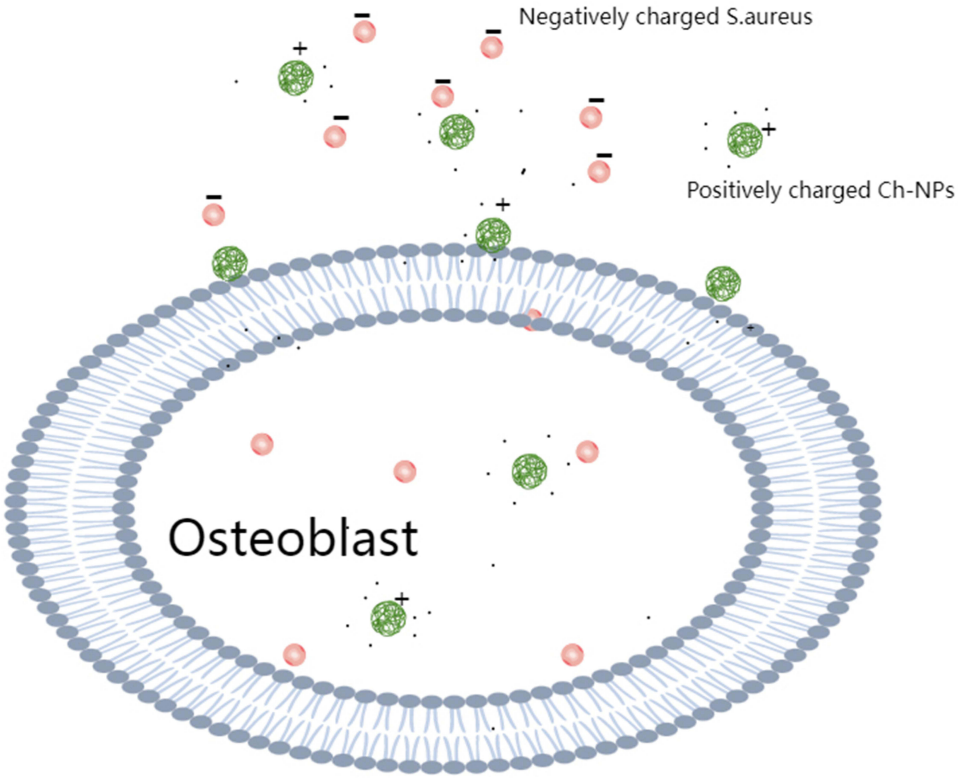

Figure 6 Chitosan nanoparticles (Ch-NPs) with positive charges can effectively kill negatively charged planktonic bacteria. Furthermore, nanoparticles can enter the cell, increase the concentration of antibiotics inside the cell, and effectively kill intracellular bacteria.

showed that cefazolin-loaded CSNPs had outstanding antibacterial potential against Escherichia coli, Pseudomonas aeruginosa, and multidrug-resistant Klebsiella pneumoniae.[73]

Metal-loaded chitosan nanoparticles

In recent years, antibacterial research on metal ions has been widely reported, including silver, gold, iron, and copper.[74–79] Chitosan nanoparticles that chelate with metals also exhibit good antibacterial effects. In a study, by altering the amount of chitosan supplied throughout the synthesis process, four distinct forms of chitosan/Ag nanoparticles were created. Increasing the chitosan content resulted in a more pronounced antibacterial influence, which was especially noticeable in its interaction with the peptidoglycan layer on the bacterial surface, according to the antibacterial results of the nanoparticles. The study also demonstrated the potent antibacterial activity of CS-AgNPs against Staphylococcus aureus and Escherichia coli by causing membrane damage and blocking bacterial growth[80](Figure 7). Shehabeldine Amr M synthesizes Chitosan-stabilized Ag nanoparticles (Chi/Ag-NPs) and evaluates their antibacterial and cytotoxic properties. Using a resazurin-mediated microtiter plate test, the in vitro antibacterial activity was assessed against Pseudomonas aeruginosa and Staphylococcus aureus. At 12.5 μg/mL for both bacterial strains, the lowest concentration of Chi/Ag-NPs was shown to have antibacterial activity.[81]

Hanan M[82] fabricated and characterized Chitosan/Carboxymethyl cellulose Nanocomposites doped with silver nanoparticles. It was discovered that higher Ag NP concentrations improve the electrical characteristics. Increased Ag NP content led to a commensurate improvement in antibacterial effectiveness against Escherichia coli and Staphylococcus aureus. Erisna Mirda[83] synthesized composite particles of silver nanoparticles and chitosan, forming spherical structures. The screening of microbial activity revealed that the AgNP-chi-Spheres with the greatest concentration of NaOH exhibited the largest inhibition zone diameters against S. aureus, E. coli, and C. albicans. The inhibition zone diameters were measured at 19.5 nm, 18.56 nm, and 12.25 nm, respectively. Niloufar[84] reported the synergistic biosynthesized silver nanoparticles (AgNPs) produced by a green synthetic method using chitosan and polyphenols generated from seaweed. Unlike bare chemical silver nanoparticles, the green Ag nano samples exhibited a clear and

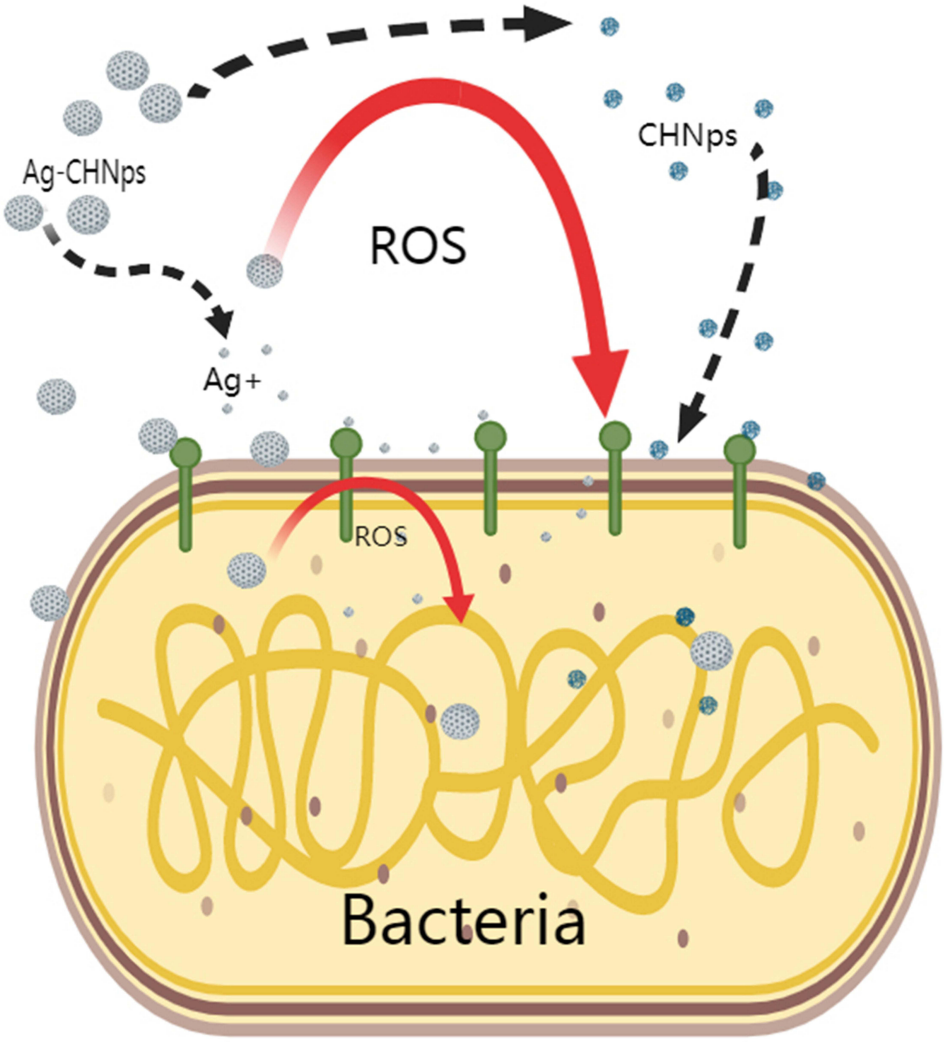

Figure 7 Silve/Chitosan nanoparticles(Ag-CHNps) can be decomposed into silver nanoparticles(AgNps) and chitosan nanoparticles (CHNps), which have antibacterial activity respectively. 1, Antibacterial mechanism of AgNps: a), Attach to the cell membrane, membrane proteins, and DNA bases, causing the disturbance of regular function of bacteria; b), Emit silver ions (Ag+), which impact the membrane, DNA, and proteins; c), Produce reactive oxidative species (ROS), which can also impact DNA, cell membrane, and membrane proteins. 2, Antibacterial mechanism of Chitosan nanoparticles (CHNps): bind to negatively charged cell membranes, causing leakage of bacterial cells.

powerful antibacterial effect in laboratory tests against all chosen human pathogens. The nano samples have a high concentration of biomolecules on their surface, contributing to their effectiveness. The findings demonstrate that combining chitosan and polyphenol increases the bactericidal activities of biogenic AgNPs.

Hashem describes producing, characterizing, and assessing gold nanoparticles modified with chitosan (Ch/AuNPs) with effective antibacterial activities. The results indicated that S. aureus had the least inhibitory effect, with a zone of inhibition diameter of 16 ± 2.1 mm at the highest dose tested, while P. aeruginosa showed the most inhibitory impact at 500 µg/mL, with a zone of inhibition diameter of 26 ± 1.8 mm.[85] The authors of another work describe gold nanoparticles modified with chitosan with effective antibacterial effects. The antibacterial mechanism of Ch/AuNPs is the contact of nanoparticles with bacterial cell membranes, which causes cellular disruption during the antibacterial process.[86]

Studies were also conducted on chitosan nanoparticles coated with Fe2+ or Fe3+ to enhance the antibacterial properties of chitosan. The antibacterial properties of the nanoparticles were assessed in vitro against Staphylococcus aureus and Escherichia coli at varying concentrations. The Fe/chitosan NPs' zeta potentials were found to be +28.82 and

+28.26 mV, respectively, according to the results. Compared to chitosan, their antibacterial activity was significantly more potent at lower doses.[87]

Chitosan NPs Used as Vaccine Adjuvants for Infection Prevention

Although research is ongoing, vaccination against S. aureus infection is currently used in veterinary medicine (eg, to prevent mastitis among dairy calves). However, despite numerous attempts, human testing has not yielded positive results with this vaccine to date.[88]

Acevedo Villanueva conducted tests to assess the broilers' immune response after being challenged with Salmonella and given the Salmonella chitosan-nanoparticle (CNP) vaccination. When CNP-vaccinated birds were compared to the control, the amount of Salmonella Enteritidis loads in their cecal content two days after the challenge was reduced by 65.9%. Moreover, the chitosan-nano vaccine had no adverse effects on the bird's production performance.[89] When employed as an immunomodulator, research revealed that nanoparticles made from chitosan derivatives exhibited more cellular immunological activity. Xu administered the chitosan nanoparticle vaccine to mice and then examined the tissues and tissue sections to determine the expression levels of immunological components, immune genes, and immunoglobulins. The findings demonstrated that the immune factors IL-6, TNF, and IL-1β can be significantly improved by using C236-HACC-OVA (C2,3,6-chitosan sulfate-chitosan quaternary ammonium salt-ovalbumin) and NO-HACC-OVA (NO-carboxymethyl chitosan-chitosan quaternary ammonium salt-ovalbumin) nanoparticles. After giving both nanoparticles, there was a considerable increase in the amount of IgG1.[89]

Staphylococcal enterotoxin B (SEB), a potent superantigen, is responsible for many disorders caused by Staphylococcus aureus. In research, SEB protein was entrapped into chitosan nanoparticles, and nano-formulation immunogenicity was investigated. According to the study, the immunogenicity of recombinant SEB was evaluated following nasal administration to mice. Serum and stool IgG and IgA antibodies showed that SEB protein-loaded NPs could evoke the mice's immune responses.[90] The immune triggering mechanism of chitosan NPs is based on enhancing antigen uptake and inducing macrophages to secrete inflammatory factors regulating Th1/Th2 balance, tailoring a specific immune response.[91] Several studies have demonstrated the potential use of chitosan NPs and derivatives as promising vehicles for vaccine delivery.[92]

Future Perspectives and Challenges

Orthopedic infections are progressively rising as more orthopedic implant procedures are performed. Antibiotics have historically been used to treat these medical conditions. Still, overuse and ineffectiveness have created bacteria that are resistant to antibiotics, necessitating the use of a more extensive variety of antibiotics.[93] The current clinical method for treating bone and joint infections is extensive surgical debridement to remove bacteria and necrotic tissue. Antibiotics are essential,[94] but infections are difficult to completely cure due to the limitations of antibiotics and bacterial immune evasion.[94] Therefore, chitosan nanoparticle technology provides a new option for the treatment of infections, especially in terms of antibiotic delivery. Due to the degradable properties of chitosan, in addition to systematic administration, chitosan-loaded nanoparticles can also be embedded in bone cement, artificial bone, bone defect scaffold materials, etc., achieving the goal of local treatment.[95]

To achieve synergistic antibacterial effects, ChNP is used to carry various antibacterial agents, including many types of antibacterial metals.[96,97] Due to the unique advantages of chitosan in terms of physical and chemical properties, as well as its non-toxic and biodegradable properties, chitosan nanoparticles are foreseeable as a sufficiently broad application in basic medical research and clinical applications for treating infections.[98]

As the positive charge on the surface of chitosan, chitosan nanoparticles have a broad-spectrum antibacterial effect, and it is difficult to develop drug resistance because they interact with bacterial cell membranes, resulting in cytoplasmic exposure.[99] Due to the excellent permeability of chitosan nanoparticles, they can enter bacterial biofilms and hinder biofilm formation. Chitosan loaded with antibiotics or metal nanoparticles has unique advantages in killing intracellular bacteria and biofilm bacteria, as many antibiotics make it difficult to enter the interior of cells and biofilms.[100] When chitosan is used as a drug carrier, it can form a synergistic antibacterial effect and more effectively kill bacteria that evade the immune system.[101]

Conclusions

This article reviews the clinical status and difficult-to-cure situations of orthopedic infections, as well as the common pathogenic bacteria Staphylococcus aureus; the mechanisms that make infection difficult to cure include biofilm formation, intracellular infection, and small colony variation. The application of chitosan and the construction of nanoparticles are discussed. We review the antibacterial effects of chitosan and nanoparticles, as well as the in vitro study of chitosan-based nanoparticles in the antibacterial properties of antibiotics and metal-loaded materials. At the same time, the possible application prospects of chitosan nanoparticles in vaccine immunity against bacterial infections are also introduced.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors declare that they have no conflicts of interest in this work.

References

1. Masters EA, Ricciardi BF, Bentley KL, et al. Skeletal infections: microbial pathogenesis, immunity, and clinical management. *Nat Rev Microbiol*. 2022;20(7):385–400. doi:10.1038/s41579-022-00686-0
2. Pincher B, Fenton C, Jeyapalan R, et al. A systematic review of the single-stage treatment of chronic osteomyelitis. *J Orthop Surg Res*. 2019;14(1):393. doi:10.1186/s13018-019-1388-2
3. Hoskins W, Rainbird S, Peng Y, et al. Incidence, Risk Factors, and Outcome of Ceramic-On-Ceramic Bearing Breakage in Total Hip Arthroplasty. *J Arthroplasty*. 2021;36(8):2992–2997. doi:10.1016/j.arth.2021.03.021
4. D'Amore T, Goh GS, Courtney PM, et al. Hospital Charges Are Not Associated With Episode-of-Care Costs or Complications After Total Joint Arthroplasty. *J Arthroplasty*. 2022;37(8S):S727–S731. doi:10.1016/j.arth.2022.01.018
5. Patel R. Periprosthetic Joint Infection. *N Engl J Med*. 2023;388(3):251–262. doi:10.1056/NEJMra2203477
6. Li S, Meng Y, Pan J, et al. Novel, static, permanent spacers to treat chronic knee periprosthetic joint infections. *Int Orthop*. 2023;47(10):2585–2589. doi:10.1007/s00264-023-05884-w
7. Senneville E, Dinh A, Ferry T, et al. Tolerance of Prolonged Oral Tedizolid for Prosthetic Joint Infections: results of a Multicentre Prospective Study. *Antibiotics*. 2020;10(1):4. doi:10.3390/antibiotics10010004
8. Basile G, Gallina M, Passeri A, et al. Prosthetic joint infections and legal disputes: a threat to the future of prosthetic orthopedics. *J Orthop Traumatol*. 2021;22(1):44. doi:10.1186/s10195-021-00607-6
9. Roof MA, Aggarwal VK, Schwarzkopf R. The Economics of Revision Arthroplasty for Periprosthetic Joint Infection. *Arthroplast Today*. 2023;23:101213. doi:10.1016/j.artd.2023.101213
10. Negut I, Ristoscu C, Tozar T, et al. Implant Surfaces Containing Bioglasses and Ciprofloxacin as Platforms for Bone Repair and Improved Resistance to Microbial Colonization. *Pharmaceutics*. 2022;14(6):1175. doi:10.3390/pharmaceutics14061175
11. Ferreira PG, Ferreira VF, da Silva FDC, et al. Chitosans and Nanochitosans: recent Advances in Skin Protection, Regeneration, and Repair. *Pharmaceutics*. 2022;14(6):1307. doi:10.3390/pharmaceutics14061307
12. Kankariya Y, Chatterjee B. Biomedical Application of Chitosan and Chitosan Derivatives: a Comprehensive Review. *Curr Pharm Des*. 2023;29(17):1311–1325. doi:10.2174/1381612829666230524153002
13. Lee JH, Tachibana T, Wadamori H, et al. Drug-Loaded Biocompatible Chitosan Polymeric Films with Both Stretchability and Controlled Release for Drug Delivery. *ACS Omega*. 2023;8(1):1282–1290. doi:10.1021/acsomega.2c06719
14. Mu H, et al. Chitosan conjugation enables intracellular bacteria susceptible to aminoglycoside antibiotic. *Glycobiology*. 2016;26(11):1190–1197.
15. Seaberg J, Montazerian H, Hossen MN, et al. Hybrid Nanosystems for Biomedical Applications. *ACS Nano*. 2021;15(2):2099–2142. doi:10.1021/acsnano.0c09382
16. Fornaguera C, Garcia-Celma MJ. Personalized Nanomedicine: a Revolution at the Nanoscale. *J Pers Med*. 2017;7(4):12. doi:10.3390/jpm7040012

17. Jha R, Mayanovic RA. A Review of the Preparation, Characterization, and Applications of Chitosan Nanoparticles in Nanomedicine. *Nanomaterials (Basel)*. 2023;13(8):1302. doi:10.3390/nano13081302
18. Chen YT, Ricciardi BF, Bentley KL, et al. Immunometabolic Regulation of Bacterial Infection, Biofilms, and Antibiotic Susceptibility. *J Innate Immun*. 2024;16(1):143–158.
19. Snoddy B, Jayasuriya AC. The use of nanomaterials to treat bone infections. *Mater Sci Eng C Mater Biol Appl*. 2016;67:822–833. doi:10.1016/j.msec.2016.04.062
20. Lu Y, Cai W-J, Ren Z, et al. The Role of Staphylococcal Biofilm on the Surface of Implants in Orthopedic Infection. *Microorganisms*. 2022;10(10):1909. doi:10.3390/microorganisms10101909
21. Anagnostakos K, Fink B. Antibiotics in Orthopedic Infections. *Antibiotics (Basel)*. 2021;10(11).
22. Guo Y, Song G, Sun M, et al. Prevalence and Therapies of Antibiotic-Resistance in Staphylococcus aureus. *Front Cell Infect Microbiol*. 2020;10:107. doi:10.3389/fcimb.2020.00107
23. Nasser A, Azimi T, Ostadmohammadi S, et al. A comprehensive review of bacterial osteomyelitis with emphasis on Staphylococcus aureus. *Microb Pathog*. 2020;148:104431. doi:10.1016/j.micpath.2020.104431
24. Mlynarczyk-Bonikowska B, Kowalewski C, Krolak-Ulinska A, et al. Molecular Mechanisms of Drug Resistance in Staphylococcus aureus. *Int J Mol Sci*. 2022;23(15):8088. doi:10.3390/ijms23158088
25. Mirzaei R, Campoccia D, Ravaioli S, et al. Emerging Issues and Initial Insights into Bacterial Biofilms: from Orthopedic Infection to Metabolomics. *Antibiotics (Basel)*. 2024;13(2):184.
26. Aransiola SA, Selvaraj B, Maddela NR. Bacterial biofilm formation and anti-biofilm strategies. *Res Microbiol*. 2023;104172.
27. Uruen C, et al. Biofilms as Promoters of Bacterial Antibiotic Resistance and Tolerance. *Antibiotics (Basel)*. 2020;10(1).
28. Hathroubi S, Mekni MA, Domenico P, et al. Biofilms: microbial Shelters Against Antibiotics. *Microb Drug Resist*. 2017;23(2):147–156. doi:10.1089/mdr.2016.0087
29. Gebreyohannes G, et al. Challenges of intervention, treatment, and antibiotic resistance of biofilm-forming microorganisms. *Heliyon*. 2019;5(8):e02192.
30. Penesyan A, Nagy SS, Kjelleberg S, et al. Rapid microevolution of biofilm cells in response to antibiotics. *NPJ Biofilms Microbiomes*. 2019;5(1):34. doi:10.1038/s41522-019-0108-3
31. Zhang K, et al. Promising Therapeutic Strategies Against Microbial Biofilm Challenges. *Front Cell Infect Microbiol*. 2020;10:359.
32. Gomes P, Forrester M, Pace M, et al. May the force be with you: the role of hyper-mechanostability of the bone sialoprotein binding protein during early stages of Staphylococci infections. *Front Chem*. 2023;11:1107427. doi:10.3389/fchem.2023.1107427
33. Khalid M, Bora T, Ghaithi AA, et al. Raman Spectroscopy detects changes in Bone Mineral Quality and Collagen Cross-linkage in Staphylococcus Infected Human Bone. *Sci Rep*. 2018;8(1):9417. doi:10.1038/s41598-018-27752-z
34. Walter N, Mendelsohn D, Brochhausen C, et al. Intracellular S. aureus in Osteoblasts in a Clinical Sample from a Patient with Chronic Osteomyelitis-A Case Report. *Pathogens*. 2021;10(8):1064. doi:10.3390/pathogens10081064
35. Peyrusson F, Varet H, Nguyen TK, et al. Intracellular Staphylococcus aureus persisters upon antibiotic exposure. *Nat Commun*. 2020;11(1):2200. doi:10.1038/s41467-020-15966-7
36. Krauss JL, Roper PM, Ballard A, et al. Staphylococcus aureus Infects Osteoclasts and Replicates Intracellularly. *mBio*. 2019;10(5). doi:10.1128/mBio.02447-19
37. Proctor R. Respiration and Small Colony Variants of Staphylococcus aureus. *Microbiol Spectr*. 2019;7(3). doi:10.1128/microbiolspec.GPP3-0069-2019
38. Bogut A, Koper P, Marczak M, et al. The first genomic characterization of a stable, hemin-dependent small colony variant strain of Staphylococcus epidermidis isolated from a prosthetic-joint infection. *Front Microbiol*. 2023;14:1289844. doi:10.3389/fmicb.2023.1289844
39. Zhou S, Rao Y, Li J, et al. Staphylococcus aureus small-colony variants: formation, infection, and treatment. *Microbiol Res*. 2022;260:127040. doi:10.1016/j.micres.2022.127040
40. Sato T, Uno T, Kawamura M, et al. In vitro Tolerability of Biofilm-Forming Trimethoprim-/Sulfamethoxazole-Resistant Small Colony Variants of Staphylococcus aureus Against Various Antimicrobial Agents. *Microb Drug Resist*. 2021;27(9):1282–1289. doi:10.1089/mdr.2020.0379
41. Guo H, et al. Biofilm and Small Colony Variants-An Update on Staphylococcus aureus Strategies toward Drug Resistance. *Int J Mol Sci*. 2022;23(3):567.
42. Kriegeskorte A, Grubmã¼ller S, Huber C, et al. Staphylococcus aureus small colony variants show common metabolic features in central metabolism irrespective of the underlying auxotrophism. *Front Cell Infect Microbiol*. 2014;4:141. doi:10.3389/fcimb.2014.00141
43. Pal K, Bharti D, Sarkar P, et al. Selected Applications of Chitosan Composites. *Int J Mol Sci*. 2021;22(20):10968. doi:10.3390/ijms222010968
44. Shariatinia Z. Carboxymethyl chitosan: properties and biomedical applications. *Int J Biol Macromol*. 2018;120(Pt B):1406–1419. doi:10.1016/j.ijbiomac.2018.09.131
45. Wang W, Xue C, Mao X. Chitosan: structural modification, biological activity and application. *Int J Biol Macromol*. 2020;164:4532–4546. doi:10.1016/j.ijbiomac.2020.09.042
46. Lima R, Fernandes C, Pinto MMM. Molecular modifications, biological activities, and applications of chitosan and derivatives: a recent update. *Chirality*. 2022;34(9):1166–1190. doi:10.1002/chir.23477
47. Ardean C, Davidescu CM, Nemeş NS, et al. Antimicrobial Activities of Chitosan Derivatives. *Pharmaceutics*. 2021;13(10):1639. doi:10.3390/pharmaceutics13101639
48. Ardean C, Davidescu CM, Nemeş NS, et al. Factors Influencing the Antibacterial Activity of Chitosan and Chitosan Modified by Functionalization. *Int J Mol Sci*. 2021;22(14):7449. doi:10.3390/ijms22147449
49. Hosseinnejad M, Jafari SM. Evaluation of different factors affecting antimicrobial properties of chitosan. *Int J Biol Macromol*. 2016;85:467–475. doi:10.1016/j.ijbiomac.2016.01.022
50. Tian Y, Wu D, Wu D, et al. Chitosan-Based Biomaterial Scaffolds for the Repair of Infected Bone Defects. *Front Bioeng Biotechnol*. 2022;10:899760. doi:10.3389/fbioe.2022.899760
51. Webber JL, Namivandi-Zangeneh R, Drozdek S, et al. Incorporation and antimicrobial activity of nisin Z within carrageenan/chitosan multilayers. *Sci Rep*. 2021;11(1):1690. doi:10.1038/s41598-020-79702-3

52. Ke CL, Deng F-S, Chuang C-Y, et al. Antimicrobial Actions and Applications of Chitosan. *Polymers*. 2021;13(6):904. doi:10.3390/polym13060904
53. Perinelli DR, Fagioli L, Campana R, et al. Chitosan-based nanosystems and their exploited antimicrobial activity. *Eur J Pharm Sci*. 2018;117:8–20. doi:10.1016/j.ejps.2018.01.046
54. Beck BH, Yildirim-Aksoy M, Shoemaker CA, et al. Antimicrobial activity of the biopolymer chitosan against Streptococcus iniae. *J Fish Dis*. 2019;42(3):371–377. doi:10.1111/jfd.12938
55. Wang M, Bi S, Qin D, et al. Quantitative evaluation of the antibacterial effectiveness and efficiency of chitosan considering the effect of neutralization. *Carbohydr Polym*. 2021;265:117918. doi:10.1016/j.carbpol.2021.117918
56. Pelegrino MT, Pieretti JC, Nakazato G, et al. Chitosan chemically modified to deliver nitric oxide with high antibacterial activity. *Nitric Oxide*. 2021;106:24–34. doi:10.1016/j.niox.2020.10.003
57. Wang L, Xin M, Li M, et al. Effect of the structure of chitosan quaternary phosphonium salt and chitosan quaternary ammonium salt on the antibacterial and antibiofilm activity. *Int J Biol Macromol*. 2023;242(Pt 2):124877. doi:10.1016/j.ijbiomac.2023.124877
58. Jonassen H, Kjoniksen A-L, Hiorth M. Stability of chitosan nanoparticles cross-linked with tripolyphosphate. *Biomacromolecules*. 2012;13(11):3747–3756. doi:10.1021/bm301207a
59. Chae K-S, Shin C-S, Shin W-S. Characteristics of cricket (Gryllus bimaculatus) chitosan and chitosan-based nanoparticles. *Food Sci Biotechnol*. 2018;27(3):631–639. doi:10.1007/s10068-018-0314-4
60. Ganesan S, Alagarasan JK, Sonaimuthu M, et al. Preparation and Characterization of Salsalate-Loaded Chitosan Nanoparticles: in Vitro Release and Antibacterial and Antibiofilm Activity. *Mar Drugs*. 2022;20(12):733. doi:10.3390/md20120733
61. Holmes MD, Narro AJ, Jones HL, et al. The potential of suspended chitosan nanoparticles as a surgical irrigation fluid. *J Orthop Res*. 2024;42(1):223–229. doi:10.1002/jor.25667
62. Dai X, Liu X, Li Y, et al. Nitrogen-phosphorous co-doped carbonized chitosan nanoparticles for chemotherapy and ROS-mediated immunotherapy of intracellular Staphylococcus aureus infection. *Carbohydr Polym*. 2023;315:121013. doi:10.1016/j.carbpol.2023.121013
63. Haji Hossein Tabrizi A, Habibi M, Foroohi F, et al. Investigation of the effects of antimicrobial and anti-biofilm peptide IDR1018 and chitosan nanoparticles on ciprofloxacin-resistant Escherichia coli. *J Basic Microbiol*. 2022;62(10):1229–1240. doi:10.1002/jobm.202200156
64. Rivera Aguayo P, Bruna Larenas T, Alarcón Godoy C, et al. Antimicrobial and Antibiofilm Capacity of Chitosan Nanoparticles against Wild Type Strain of Pseudomonas sp. Isolated from Milk of Cows Diagnosed with Bovine Mastitis. *Antibiotics*. 2020;9(9):551. doi:10.3390/antibiotics9090551
65. Costa EM, Silva S, Veiga M, et al. Exploring chitosan nanoparticles as effective inhibitors of antibiotic resistant skin microorganisms - From in vitro to ex vitro testing. *Carbohydr Polym*. 2018;201:340–346. doi:10.1016/j.carbpol.2018.08.083
66. Ikono R, Vibriani A, Wibowo I, et al. Nanochitosan antimicrobial activity against Streptococcus mutans and Candida albicans dual-species biofilms. *BMC Res Notes*. 2019;12(1):383. doi:10.1186/s13104-019-4422-x
67. Zhao D, Yu S, Sun B, et al. Biomedical Applications of Chitosan and Its Derivative Nanoparticles. *Polymers (Basel)*. 2018;10(4):462. doi:10.3390/polym10040462
68. Nie B, et al. Bone infection site targeting nanoparticle-antibiotics delivery vehicle to enhance treatment efficacy of orthopedic implant related infection. *Bioact Mater*. 2022;16:134–148.
69. Scolari IR, Páez PL, Musri MM, et al. Rifampicin loaded in alginate/chitosan nanoparticles as a promising pulmonary carrier against Staphylococcus aureus. *Drug Deliv Transl Res*. 2020;10(5):1403–1417. doi:10.1007/s13346-019-00705-3
70. Zhang Z, Chen J, Zou L, et al. Preparation, Characterization, and Staphylococcus aureus Biofilm Elimination Effect of Baicalein-Loaded beta-Cyclodextrin-Grafted Chitosan Nanoparticles. *Int J Nanomed*. 2022;17:5287–5302. doi:10.2147/IJN.S383182
71. Zheng X, Gao M, Wu L, et al. Ceftazidime-assisted synthesis of ultrasmall chitosan nanoparticles for biofilm penetration and eradication of Pseudomonas aeruginosa. *Sci Rep*. 2023;13(1):13481. doi:10.1038/s41598-023-40653-0
72. Maya S, Indulekha S, Sukhithasri V, et al. Efficacy of tetracycline encapsulated O-carboxymethyl chitosan nanoparticles against intracellular infections of Staphylococcus aureus. *Int J Biol Macromol*. 2012;51(4):392–399. doi:10.1016/j.ijbiomac.2012.06.009
73. Jamil B, Habib H, Abbasi S, et al. Cefazolin loaded chitosan nanoparticles to cure multi drug resistant Gram-negative pathogens. *Carbohydr Polym*. 2016;136:682–691. doi:10.1016/j.carbpol.2015.09.078
74. Kovacova M, Daneu N, Tkáčiková Ľ, et al. Sustainable One-Step Solid-State Synthesis of Antibacterially Active Silver Nanoparticles Using Mechanochemistry. *Nanomaterials (Basel)*. 2020;10(11):2119. doi:10.3390/nano10112119
75. Bruna T, Maldonado-Bravo F, Jara P, et al. Silver Nanoparticles and Their Antibacterial Applications. *Int J Mol Sci*. 2021;22(13):7202. doi:10.3390/ijms22137202
76. Fanoro OT, Oluwafemi OS. Bactericidal Antibacterial Mechanism of Plant Synthesized Silver, Gold and Bimetallic Nanoparticles. *Pharmaceutics*. 2020;12(11):1044. doi:10.3390/pharmaceutics12111044
77. Chithambharan A, Pottail L, Mirle RM, et al. Bioinspired Gold Nanoparticle Synthesis Using Terminalia bellerica Fruit Parts and Exploring Their Anti-bacterial Potency In Vitro. *Indian J Microbiol*. 2021;61(3):298–305. doi:10.1007/s12088-021-00937-3
78. Ermini ML, Voliani V. Antimicrobial Nano-Agents: the Copper Age. *ACS Nano*. 2021;15(4):6008–6029. doi:10.1021/acsnano.0c10756
79. Yi G, Teong SP, Liu S, et al. Iron-based nano-structured surfaces with antimicrobial properties. *J Mater Chem B*. 2020;8(44):10146–10153. doi:10.1039/D0TB01941K
80. Ge J, Li M, Fan J, et al. Synthesis, characterization, and antibacterial activity of chitosan-chelated silver nanoparticles. *J Biomater Sci Polym Ed*. 2024;35(1):45–62. doi:10.1080/09205063.2023.2265629
81. Shehabeldine AM, et al. Multifunctional Silver Nanoparticles Based on Chitosan: antibacterial, Antibiofilm, Antifungal, Antioxidant, and Wound-Healing Activities. *J Fungi (Basel)*. 2022;8(6):865.
82. Ragab HM, et al. Fabrication and Characterization of Silver Nanoparticle-Doped Chitosan/Carboxymethyl Cellulose Nanocomposites for Optoelectronic and Biological Applications. *ACS Omega*. 2024;9(20):22112–22122.
83. Mirda E, Idroes R, Khairan K, et al. Synthesis of Chitosan-Silver Nanoparticle Composite Spheres and Their Antimicrobial Activities. *Polymers (Basel)*. 2021;13(22):3990. doi:10.3390/polym13223990
84. Rezazadeh NH, Buazar F, Matroodi S. Synergistic effects of combinatorial chitosan and polyphenol biomolecules on enhanced antibacterial activity of biofunctionalaized silver nanoparticles. *Sci Rep*. 2020;10(1):19615. doi:10.1038/s41598-020-76726-7

85. Hashem AH, Shehabeldine AM, Ali OM, et al. Synthesis of Chitosan-Based Gold Nanoparticles: antimicrobial and Wound-Healing Activities. *Polymers (Basel)*. 2022;14(11):2293. doi:10.3390/polym14112293
86. Fuster MG, Montalbán MG, Carissimi G, et al. Antibacterial Effect of Chitosan-Gold Nanoparticles and Computational Modeling of the Interaction between Chitosan and a Lipid Bilayer Model. *Nanomaterials (Basel)*. 2020;10(12):2340. doi:10.3390/nano10122340
87. Qian J, Pan C, Liang C. Antimicrobial activity of Fe-loaded chitosan nanoparticles. *Eng Life Sci*. 2017;17(6):629–634. doi:10.1002/elsc.201600172
88. Barie PS, Narayan M, Sawyer RG. Immunization Against Staphylococcus aureus Infections. *Surg Infect (Larchmt)*. 2018;19(8):750–756. doi:10.1089/sur.2018.263
89. Xu C, Xing R, Liu S, et al. In vivo immunological activity of chitosan-derived nanoparticles. *Int J Biol Macromol*. 2024;262(Pt 2):130105. doi:10.1016/j.ijbiomac.2024.130105
90. Hosseini SA, et al. Immunogenicity Evaluation of Recombinant Staphylococcus aureus Enterotoxin B (rSEB) and rSEB-loaded Chitosan Nanoparticles Following Nasal Administration. *Iran J Allergy Asthma Immunol*. 2020;19(2):159–171.
91. Mehrabi M, et al. Chitosan-based Nanoparticles in Mucosal Vaccine Delivery. *Arch Razi Inst*. 2018;73(3):165–176.
92. Zhao J, Li J, Jiang Z, et al. Chitosan, N,N,N-trimethyl chitosan (TMC) and 2-hydroxypropyltrimethyl ammonium chloride chitosan (HTCC): the potential immune adjuvants and nano carriers. *Int J Biol Macromol*. 2020;154:339–348. doi:10.1016/j.ijbiomac.2020.03.065
93. Elifranji ZO, Haddad B, Salameh A, et al. Microbiological Profile and Drug Resistance Analysis of Postoperative Infections following Orthopedic Surgery: a 5-Year Retrospective Review. *Adv Orthop*. 2022;2022:7648014. doi:10.1155/2022/7648014
94. Runner RP, Mener A, Roberson JR, et al. Prosthetic Joint Infection Trends at a Dedicated Orthopaedics Specialty Hospital. *Adv Orthop*. 2019;2019:4629503. doi:10.1155/2019/4629503
95. Gaglio SC, et al. Efficiency of Chitosan Nanocarriers in Vaccinology for Mucosal Immunization. *Vaccines (Basel)*. 2023;11(8).
96. Miyazawa N, Hakamada M, Mabuchi M. Antimicrobial mechanisms due to hyperpolarisation induced by nanoporous Au. *Sci Rep*. 2018;8(1):3870. doi:10.1038/s41598-018-22261-5
97. Jedrzejczyk RJ, Turnau K, Jodłowski PJ, et al. Antimicrobial Properties of Silver Cations Substituted to Faujasite Mineral. *Nanomaterials (Basel)*. 2017;7(9):240. doi:10.3390/nano7090240
98. Sonin D, Pochkaeva E, Zhuravskii S, et al. Biological Safety and Biodistribution of Chitosan Nanoparticles. *Nanomaterials*. 2020;10(4):810. doi:10.3390/nano10040810
99. Rathinam S, Solodova S, Kristjánsdóttir I, et al. The antibacterial structure-activity relationship for common chitosan derivatives. *Int J Biol Macromol*. 2020;165(Pt B):1686–1693. doi:10.1016/j.ijbiomac.2020.09.200
100. Xu J, et al. Antibiofilm Effect of Cinnamaldehyde-Chitosan Nanoparticles against the Biofilm of Staphylococcus aureus. *Antibiotics (Basel)*. 2022;11(10).
101. Lin X, Gong X, Ruan Q, et al. Antimicrobial Application of Chitosan Derivatives and their Nanocomposites. *Curr Med Chem*. 2023;30(15):1736–1755. doi:10.2174/0929867329666220803114729