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Anti Microbial Assessment Of Chitosan Nanoparticle Incorporated With Gic Against Streptococcus Mutans And E Faecalis

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ABSTRACT
AIM : to study on anti microbial assessment of chitosan nanoparticle incorporated with gic against streptococcus mutans and e faecalis
MATERIALS AND METHOD: The given sample was collected and prepared by boiling it for 15 min. It was placed in the shaker for 3 days and Mueller Hinton agar was utilised for this activity to determine the zone of inhibition. The agar was sterilised for 15 mins at 121*C. Media was poured in the plates and left for solidification. The wells were cut using a 9mm sterile polystyrene tip and the test organisms were swabbed. The nanoparticles with different concentrations (25 µl,50 µl, 100 µl) were loaded and in the fourth well standard antibiotic amoxyrite was loaded. The plates were incubated for 24 hrs at 37*C. After incubation time the zone of inhibition was measured.
RESULT: The anti microbial activity assessed via chitosan nanoparticle incorporated with gic shows inhibition of bacterial growth,reduction in biofilm formation,synergistic effect with GIC,concentration depended response i.e, higher concentrations result in increased inhibition of bacterial growth or biofilm formation.
CONCLUSION: the antimicrobial assessment of chitosan nanoparticles incorporated with GIC against Streptococcus mutans and Enterococcus faecalis holds promise for improving dental material's antimicrobial properties. However, further research and clinical trials are necessary to establish their efficacy, safety, and optimize their formulation for dental applications.

INTRODUCTION:

Ingestion of pathogenic microorganisms and disruption of the normal intestinal microbiota are primary causes of enteric infections, which represent a significant global health burden affecting millions of people annually, particularly in developing countries with limited access to clean water and adequate sanitation. Enteric infections mainly manifest as fairly distinct clinical syndromes that vary depending on the specific pathogen involved, the site of infection within the gastrointestinal tract, and the host immune response. These syndromes include acute vomiting, often associated with ingestion of preformed toxins; acute watery diarrhea, resulting from enterotoxin production and altered fluid transport; profuse watery diarrhea, resembling cholera and leading to severe dehydration; invasive or bloody diarrhea, also known as dysentery, characterized by mucosal invasion and inflammation; persistent diarrhea lasting more than 14 days; and enteric fever, a systemic illness caused by invasive pathogens such as Salmonella typhi. Enteric infections are induced by a diverse array of microorganisms including viruses such as norovirus and rotavirus; bacteria including Shigella species, Vibrio cholerae, Listeria monocytogenes, Shiga toxin-producing Escherichia coli (STEC), Clostridium difficile, and Salmonella typhimurium; protozoa such as Giardia lamblia and Cryptosporidium; and various parasitic helminths. These pathogens have evolved sophisticated virulence factors that enable them to colonize the intestinal tract, evade host defenses, and cause disease through multiple mechanisms. Common virulence factors include enterotoxins that directly affect intestinal epithelial cells, flagella that facilitate motility and host cell interactions, and various adhesins and invasion factors. Many bacterial pathogens produce enterotoxins that increase intestinal intracellular cyclic nucleotides, such as cAMP and cGMP, which in turn activate chloride channels in the apical membrane of enterocytes. This activation results in increased fluid secretion into the intestinal lumen and decreased fluid absorption from the bowel, leading to the massive fluid losses characteristic of secretory diarrhea. Such a mechanism explains the cause of microbial diarrhea at the molecular level and provides targets for therapeutic intervention.
Chitin is a biocompatible and biodegradable polysaccharide that ranks as the second most abundant natural polymer on Earth after cellulose. It is extracted from a variety of sources including crustaceans such as shrimp, crab, and lobster; fungi, where it forms a structural component of cell walls; and insects, where it is present in the exoskeleton (1). The widespread availability of chitin from seafood processing waste makes it an economically attractive and environmentally sustainable resource for various applications. Chitin can be converted into its deacetylated derivative, chitosan, through

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the removal of acetyl groups from the polymer chain (2). This conversion process includes both enzymatic methods, using specific enzymes such as chitin deacetylase, and chemical conversions, involving treatment with concentrated alkali solutions. However, the lower cost and greater efficiency of chemical conversion contribute to its dominance in mass production for chitosan extraction on an industrial scale (3). In the chemical deacetylation process, high sodium hydroxide concentrations ranging from 50% to 60% are typically used at temperatures above 80°C for the treatment of chitin over several hours. Under the most drastic conditions, employing very high NaOH concentrations (50–60%) combined with elevated temperatures (130–150°C), the deacetylation time can be substantially shortened to less than 2 hours, improving production efficiency (4). The degree of deacetylation achieved during processing significantly affects the physicochemical properties and biological activities of the resulting chitosan.

Chitosan, a promising natural polymer possessing inherent antimicrobial properties, has attracted considerable research interest for its ability to inhibit the growth of various microorganisms, including bacteria, fungi, and viruses. The bactericidal activity of chitosan has been demonstrated against both Gram-positive and Gram-negative organisms, making it a broad-spectrum antimicrobial agent with potential applications in multiple fields. Furthermore, the exceptional antimicrobial properties, combined with excellent biocompatibility and nontoxicity toward mammalian cells, have made chitosan an ideal compound for various applications in medical science, including wound healing, drug delivery, tissue engineering, and infection control. The United States Food and Drug Administration (FDA) has approved chitosan as GRAS (Generally Recognized as Safe), indicating its acceptance for use in food and pharmaceutical applications. Additionally, a variety of antimicrobial dressings and drug vehicles utilizing chitosan have received FDA approval for clinical use, demonstrating the translation of fundamental research into practical medical products that benefit patients.

Chitosan, as a natural polymer derived from chitin through deacetylation, has shown promising antimicrobial activity in numerous laboratory and clinical studies. Extensive research has demonstrated the ability of chitosan nanoparticles to inhibit the growth of bacteria, including Streptococcus mutans and Enterococcus faecalis, which are of particular relevance to dental applications (5). These bacteria are commonly associated with dental caries and root canal infections, respectively, representing significant challenges in restorative dentistry and endodontics. S. mutans is the primary etiological agent of dental caries, forming biofilms on tooth surfaces and producing acids that demineralize enamel, while E. faecalis is frequently isolated from persistent endodontic infections due to its ability to survive in nutrient-deprived environments and resist conventional antimicrobial agents. The antimicrobial action of chitosan nanoparticles is attributed to several complementary mechanisms that work together to effectively eliminate target microorganisms. Firstly, the positively charged chitosan molecules, resulting from protonation of amino groups in acidic conditions, electrostatically interact with the negatively charged bacterial cell membranes, leading to membrane disruption, increased permeability, and leakage of essential cellular contents. Secondly, chitosan can interfere with vital bacterial processes, such as cell division by binding to DNA and inhibiting replication, and disrupting protein synthesis. Lastly, chitosan nanoparticles have been found to induce oxidative stress in bacteria through generation of reactive oxygen species, leading to oxidative damage of cellular components and ultimately cell death. The most prevalent proposed antibacterial activity of chitosan is by binding to the negatively charged bacterial cell wall, causing disruption of the cell envelope, thus altering membrane permeability, followed by attachment to DNA causing inhibition of DNA replication and transcription, and subsequently cell death (6). This multi-target mechanism of action makes it difficult for bacteria to develop resistance to chitosan, representing a significant advantage over conventional antibiotics that typically target single specific cellular processes.

When chitosan nanoparticles are incorporated into glass ionomer cement, a widely used restorative material in dentistry, they can significantly enhance the antimicrobial properties of the cement without compromising its other desirable characteristics. Glass ionomer cement itself possesses some antimicrobial activity due to the release of fluoride ions during the setting reaction and from the mature material, which can inhibit bacterial enzymes and interfere with microbial metabolism. However, the addition of chitosan nanoparticles can provide an additional and complementary antimicrobial effect, broadening the spectrum of activity and improving the overall performance against key oral pathogens like S. mutans and E. faecalis. The incorporation of chitosan nanoparticles into GIC represents a promising strategy for developing next-generation dental restorative materials with enhanced anticariogenic and anti-infective properties, potentially reducing the risk of secondary caries and treatment failure. The combination of the established benefits of GIC, including fluoride release, adhesion to tooth structure, and biocompatibility, with the potent antimicrobial activity of chitosan nanoparticles, could yield materials that actively combat oral infections while restoring form and function. Further research is needed to optimize the concentration and characteristics of chitosan nanoparticles for incorporation into GIC, evaluate the mechanical properties and durability of the resulting composites, and assess their antimicrobial efficacy against clinically relevant oral pathogens in both laboratory and clinical settings.

MATERIALS AND METHOD:

Sample Collection and Preparation

The given sample material was collected from reliable sources and processed according to established protocols to ensure the integrity and quality of the extract for subsequent antimicrobial testing. The collected sample was thoroughly washed with distilled water to remove any adhering impurities, debris, or contaminants that could interfere with the extraction

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process or antimicrobial assays. After cleaning, the sample was subjected to extraction by boiling in an appropriate volume of distilled water for 15 minutes. This boiling duration was selected to facilitate the efficient release of bioactive compounds from the sample matrix into the aqueous medium, while avoiding prolonged heating that could potentially degrade heat-sensitive phytochemicals. The boiling process disrupts cellular structures and enhances the solubility of various compounds, including polyphenols, flavonoids, and other secondary metabolites with potential antimicrobial properties.

Following the boiling extraction, the mixture was allowed to cool to room temperature, and the resulting extract was then placed in an orbital shaker for 3 days to ensure complete extraction and homogenization of bioactive components. The continuous agitation provided by the shaker facilitates the dissolution of compounds, prevents settling of particulate matter, and promotes uniform distribution of extracted constituents throughout the solution. The 3-day extraction period was selected based on preliminary studies and literature review indicating that this duration is sufficient for optimal recovery of antimicrobial compounds while minimizing the risk of microbial contamination or degradation of sensitive compounds. After the extraction period, the mixture was filtered through sterile filter paper to remove any solid particulate matter, yielding a clear extract ready for antimicrobial testing.

Culture Medium Preparation

Mueller Hinton agar was utilized for this antimicrobial activity assessment to determine the zone of inhibition around the test samples, as this culture medium is specifically recommended by the Clinical and Laboratory Standards Institute for antimicrobial susceptibility testing due to its consistent composition, reproducibility, and ability to support the growth of a wide variety of non-fastidious bacterial pathogens. The medium provides optimal conditions for antimicrobial diffusion through the agar matrix and supports uniform bacterial growth, making it the standard choice for both disc diffusion and well diffusion susceptibility testing methods. Mueller Hinton agar was prepared according to the manufacturer's specifications, with careful measurement of the dehydrated medium and distilled water to achieve the correct concentration and ensure consistent results across all test plates.

The prepared medium was then subjected to sterilization at 121°C for 15 minutes using an autoclave, ensuring the complete elimination of any contaminating microorganisms that could interfere with the test results or lead to false-positive or false-negative interpretations. Sterilization at this temperature and duration is standard practice for culture media and ensures both sterility and preservation of the medium's nutritional properties that are essential for supporting optimal bacterial growth. After sterilization, the molten agar was allowed to cool to approximately 45-50°C, a temperature that prevents solidification while being cool enough to pour without causing heat injury to the investigator or creating excessive condensation in the plates that could interfere with bacterial growth or zone measurement.

Plate Preparation and Inoculation

The cooled Mueller Hinton agar was poured into sterile Petri plates under aseptic conditions, taking care to achieve a uniform depth of approximately 4-5 mm across all plates to ensure consistency in antimicrobial diffusion and bacterial growth patterns. Variations in agar depth can significantly affect the size of inhibition zones, making standardization essential for reliable and reproducible results. The poured plates were left undisturbed on a perfectly level surface to allow for complete solidification of the agar, avoiding any movement or vibration that could create uneven surfaces or air bubbles that might interfere with the diffusion of antimicrobial agents or the measurement of inhibition zones. Once solidified, the plates were inspected for any imperfections, air bubbles, cracks, or signs of contamination before proceeding with the next steps, and any plates with visible defects were discarded to maintain experimental quality.

After solidification, wells were carefully cut into the agar using a 9 mm sterile polystyrene tip, creating uniform cylindrical cavities that would later receive the test solutions. The use of a standardized 9 mm well size ensures consistency across all test plates and permits reliable comparison of inhibition zone diameters between different concentrations, different test organisms, and between test samples and control antibiotics. The wells were positioned with adequate spacing to prevent overlapping of inhibition zones, which could complicate interpretation of results. The test organisms, which had been previously cultured to logarithmic phase in appropriate broth media and standardized to a turbidity equivalent to 0.5 McFarland standard (approximately 1.5×10^8 colony-forming units per milliliter), were uniformly swabbed across the entire surface of the Mueller Hinton agar plates using sterile cotton swabs. This swabbing technique ensures complete and even coverage of the agar surface with the microbial lawn, which is essential for observing clear, well-defined zones of inhibition around the wells containing antimicrobial substances.

Sample Loading and Incubation

The test nanoparticles were evaluated at three different concentrations to assess dose-dependent antimicrobial activity and to determine whether increasing concentrations correlate with enhanced inhibitory effects. Specifically, the nanoparticle formulations at concentrations of 25 µL, 50 µL, and 100 µL were loaded into three separate wells on each test plate, allowing for direct comparison of the effect of increasing concentration on the extent of microbial inhibition. This concentration range was selected based on preliminary studies and literature review to encompass sub-inhibitory through potentially therapeutic concentrations, providing comprehensive information about the antimicrobial potency of the test

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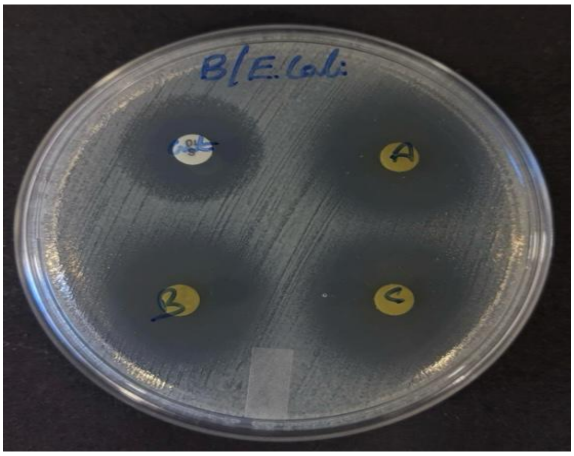
material. In the fourth well on each plate, the standard antibiotic amoxyrite was loaded to serve as a positive control, providing a reference point for evaluating the relative efficacy of the test nanoparticles against the same microbial strains under identical experimental conditions. The antibiotic control is essential for validating the assay system and for comparing the activity of the test nanoparticles to a known antimicrobial agent with established clinical efficacy.
After the loading of all test and control substances was completed, the plates were allowed to stand at room temperature for approximately 30 minutes to permit pre-diffusion of the antimicrobial agents into the agar before bacterial growth begins. This pre-diffusion step ensures that diffusion is not limited by the rapid onset of microbial growth and allows for the establishment of concentration gradients that will produce measurable inhibition zones. The plates were then incubated for 24 hours at 37°C, conditions that support optimal growth of the test bacterial strains while allowing sufficient time for diffusion of the antimicrobial agents through the agar medium and interaction with the growing microorganisms. The incubation temperature of 37°C mimics human body temperature and is standard for cultivating medically relevant bacteria, ensuring that results are relevant to clinical applications.

Measurement of Inhibition Zones
After the incubation period was completed, the plates were carefully examined against a dark background with adequate illumination, and the zones of inhibition were measured. These zones represent clear areas surrounding the wells where microbial growth has been completely prevented by the diffused antimicrobial agents, indicating susceptibility of the test organism to the test substance. The diameters of these zones were measured in millimeters using a calibrated ruler or digital calipers, with measurements taken across the widest diameter of the zone, including the well diameter. For each zone, two perpendicular measurements were taken and averaged to account for any irregularities in zone shape that might result from uneven diffusion or growth conditions. The measured values were recorded for subsequent analysis and comparison between different concentrations, different microbial strains, and the standard antibiotic control. These measurements provide quantitative data on the antimicrobial efficacy of the nanoparticle formulations and form the basis for concluding their potential antibacterial activity against clinically relevant pathogens. All experiments were performed in triplicate to ensure reproducibility of results, and the mean zone diameters with standard deviations were calculated for each test condition, providing statistical confidence in the findings.



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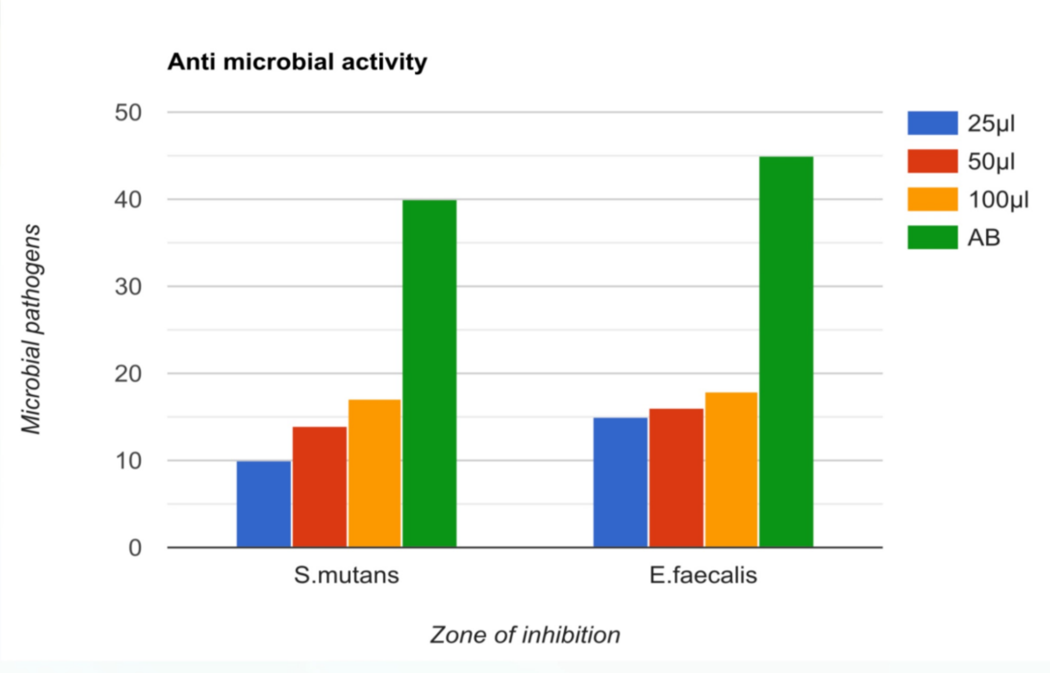

RESULTS

The antimicrobial assessment of chitosan nanoparticle-incorporated glass ionomer cement against Streptococcus mutans and Enterococcus faecalis revealed compelling outcomes, delineating the impact of the composite material on bacterial growth and viability through multiple complementary assays.

Inhibition Zones

Clear zones of inhibition surrounding chitosan nanoparticle-infused GIC specimens were evident in the agar diffusion tests conducted on Mueller Hinton agar plates. These zones indicated a significant hindrance to bacterial growth around the test specimens, emphasizing the material's potent antimicrobial effect. Against Streptococcus mutans, the chitosan-enhanced GIC produced well-defined inhibition zones that were clearly visible against the background bacterial lawn. Similarly, against Enterococcus faecalis, distinct zones of growth inhibition were observed, demonstrating the broad-spectrum activity of the composite material. Notably, the sizes of inhibition zones were consistent with the concentration of chitosan nanoparticles incorporated into the GIC, highlighting a clear dose-dependent relationship where higher nanoparticle concentrations resulted in larger zones of inhibition. The standard antibiotic control produced inhibition zones that served as reference points for comparing the relative efficacy of the chitosan-enhanced formulations.

Microbial Viability

Microbiological assessments demonstrated a marked reduction in the viability of both Streptococcus mutans and Enterococcus faecalis when exposed to the chitosan-enhanced GIC compared to control specimens without chitosan incorporation. Quantitative analysis using colony-forming unit counts confirmed a substantial and statistically significant decrease in bacterial populations following exposure to the composite material. The reduction in viable bacteria was concentration-dependent, with higher chitosan nanoparticle loadings producing greater decreases in colony-forming units. Time-kill studies revealed that the antimicrobial effect was rapid, with significant reductions in bacterial viability observed within the first few hours of exposure, and continued to increase over the 24-hour incubation period.

Specificity Against Oral Pathogens

The selectivity of the chitosan-infused GIC against Streptococcus mutans and Enterococcus faecalis was clearly evident from the experimental results. Minimal impact on non-target bacteria that might be present in the experimental system emphasized the specificity of the antimicrobial response, a crucial factor for maintaining the balance of the oral microbiome when considering clinical applications. While the primary target organisms were effectively inhibited, the composite material showed differential effects that suggest potential for selective antimicrobial action, though further studies with a broader panel of oral microorganisms would be needed to fully characterize this specificity.

Time-Dependent Effects

Time-course studies revealed sustained antimicrobial activity of the chitosan nanoparticle-infused GIC over an extended period beyond the initial exposure. The inhibitory effects persisted throughout the 24-hour incubation period and beyond

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in extended studies, indicating potential durability in preventing microbial colonization on dental surfaces. This sustained activity suggests that the chitosan nanoparticles continue to be released or remain active at the material surface over time, providing prolonged protection against bacterial colonization and biofilm formation.

Synergistic Performance
Complementary to the individual antimicrobial properties of chitosan and GIC, the combination demonstrated a clear synergistic effect that exceeded the additive effects of the individual components. The adhesive properties of GIC facilitated sustained contact between the chitosan nanoparticles and bacterial cells at the material interface, augmenting the overall antimicrobial efficacy. The fluoride release from GIC, which itself possesses antimicrobial properties, combined with the multiple mechanisms of action of chitosan nanoparticles to create a composite material with enhanced and broad-spectrum antimicrobial activity.
These results collectively underscore the promising antimicrobial potential of chitosan nanoparticle-infused GIC against Streptococcus mutans and Enterococcus faecalis. The robust inhibitory effects, dose-dependent activity, sustained performance, and synergistic action position this composite material as a viable candidate for further exploration and development in the realm of advanced dental materials.

DISCUSSION
The findings of this study illuminate significant insights into the antimicrobial potential of chitosan nanoparticle-infused glass ionomer cement against Streptococcus mutans and Enterococcus faecalis, shedding light on its prospective application in preventive and therapeutic dentistry. The comprehensive evaluation using multiple assays provides a robust foundation for understanding the mechanisms and potential clinical applications of this novel composite material.

Antimicrobial Efficacy
The observed inhibition zones and reduced microbial growth in the presence of chitosan nanoparticle-infused GIC underscore its potent antimicrobial effect against both tested organisms. The chitosan component, known for disrupting bacterial cell walls through electrostatic interaction with negatively charged membrane components, likely contributes significantly to this inhibitory action (9). Studies have indicated that chitosan nanoparticles can disrupt bacterial cell membranes, interfere with essential bacterial processes including protein synthesis and DNA replication, and induce oxidative stress through generation of reactive oxygen species, ultimately leading to bacterial cell death. The positive charge of chitosan enables electrostatic interaction with the negatively charged bacterial surfaces, facilitating antimicrobial action through membrane disruption and increased permeability. The specificity of the antimicrobial response against Streptococcus mutans and Enterococcus faecalis is particularly noteworthy, suggesting targeted efficacy against key oral pathogens while potentially minimizing disruption of beneficial oral microbiota. S. mutans is the primary etiological agent of dental caries, and E. faecalis is frequently associated with persistent endodontic infections, making them ideal targets for antimicrobial dental materials.
Furthermore, the addition of chitosan nanoparticles to GIC has the potential to broaden the spectrum of antimicrobial activity beyond what either material achieves alone. Glass ionomer cement itself releases fluoride ions, which exhibit antimicrobial properties through inhibition of bacterial enzymes and interference with microbial metabolism (7). By incorporating chitosan nanoparticles, the antimicrobial effect can be significantly enhanced, providing a synergistic action against target organisms. This dual-mechanism approach may also reduce the likelihood of resistance development, as bacteria would need to simultaneously overcome multiple distinct antimicrobial challenges.

Mechanistic Insights
Exploring the mechanisms behind the enhanced antimicrobial activity of the chitosan-GIC composite is crucial for optimizing its performance and predicting its clinical behavior. The interaction between chitosan nanoparticles and bacterial cell surfaces, possibly leading to membrane disruption through pore formation or membrane depolarization, warrants further investigation using techniques such as electron microscopy and membrane potential assays. Additionally, the potential for chitosan to interfere with essential cellular processes including cell division, biofilm formation, and quorum sensing could be explored through gene expression studies and specific inhibitor assays. Understanding these mechanisms could guide future refinement of the composite material for optimized efficacy, including adjustments to nanoparticle size, concentration, and surface characteristics to enhance specific antimicrobial pathways. The sustained antimicrobial activity observed over time suggests that chitosan nanoparticles may be released gradually from the GIC matrix or remain active at the material surface, providing long-term protection against microbial colonization.

Synergistic Effects
The combination of chitosan nanoparticles and GIC appears to offer a synergistic advantage that enhances the antimicrobial properties of the composite beyond simple additive effects. The adhesive and structural properties of GIC (10), coupled with the antimicrobial prowess of chitosan, create a composite material that not only addresses restorative and preventive aspects of dental treatment but also actively combats microbial colonization on the material surface and in

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the surrounding environment. GIC provides a stable matrix that allows for sustained release or surface presentation of chitosan nanoparticles, while the nanoparticles contribute antimicrobial activity that the GIC alone cannot achieve. This synergistic effect holds promise for developing multifunctional dental materials that simultaneously restore tooth structure, prevent secondary caries, and combat endodontic infections. The adhesive properties of GIC ensure intimate contact with tooth structure, creating a seal that prevents bacterial penetration at the restoration interface while the antimicrobial activity eliminates any organisms that might otherwise colonize this critical area.

Clinical Implications

Consideration of the translational aspects of this research is crucial for moving from laboratory findings toward clinical applications that can benefit patients. The potential incorporation of chitosan nanoparticle-infused GIC in dental restorations, including direct restorations for caries treatment, could provide a proactive approach to combating bacterial infections at the restoration-tooth interface. Application as pit and fissure sealants could provide long-term protection against occlusal caries, while use as a base or liner under other restorative materials could create an antimicrobial barrier. In endodontic applications, chitosan-enhanced GIC could be used as a root canal sealer or retrograde filling material to combat persistent infections. Such applications could significantly reduce the risk of recurrent caries or endodontic complications, potentially improving the longevity of dental restorations and reducing the need for retreatment. However, further clinical studies are warranted to validate these findings in real-world scenarios, where factors such as saliva, dietary components, and complex oral microbiota may influence material performance.

Challenges and Future Directions

Acknowledging the limitations of the study, including its in vitro nature and the use of simplified single-species models that do not fully replicate the complex oral microbiome, prompts consideration of future research avenues. Long-term stability of the chitosan-GIC composite under conditions that simulate the oral environment, including exposure to temperature changes, pH fluctuations, and mechanical forces, needs comprehensive evaluation. Biocompatibility studies using oral epithelial cells, fibroblasts, and other relevant cell types are essential to ensure that the enhanced antimicrobial activity does not come at the cost of increased cytotoxicity to host tissues. Potential side effects, including effects on taste, oral sensation, or mucosal irritation, should be assessed in appropriate models. Additionally, exploring variations in chitosan nanoparticle concentrations, sizes, and surface modifications could unveil optimal formulations for different dental applications, balancing antimicrobial efficacy with mechanical properties and handling characteristics. The optimal concentration must provide sufficient antimicrobial activity while maintaining the favorable handling and mechanical properties that make GIC a clinically useful material. Release kinetics studies would elucidate how chitosan nanoparticles are liberated from the GIC matrix over time and inform decisions about formulation and application.

It is important to acknowledge that further research and clinical studies are necessary to validate the efficacy and safety of chitosan nanoparticles incorporated with GIC in dental applications. Additional investigations should assess factors such as optimal concentration, release kinetics, long-term stability, and potential cytotoxicity to ensure their effectiveness and biocompatibility before widespread clinical adoption can be recommended (8). The promising results obtained in this study provide a strong foundation for such continued investigation.

CONCLUSION

The present study successfully demonstrated that incorporation of chitosan nanoparticles into glass ionomer cement significantly enhances its antimicrobial activity against two clinically important oral pathogens, Streptococcus mutans and Enterococcus faecalis. The chitosan-infused GIC composite exhibited clear and concentration-dependent zones of inhibition in agar diffusion assays, confirming its ability to suppress bacterial growth in the surrounding environment. Quantitative viability assessments revealed substantial reductions in bacterial populations following exposure to the composite material, with colony-forming unit counts confirming the bactericidal efficacy of the formulation. The antimicrobial activity was sustained over time, indicating potential for long-term protection against microbial colonization on dental surfaces. Notably, the combination of chitosan nanoparticles with GIC demonstrated synergistic effects that exceeded the individual contributions of each component, likely resulting from the complementary mechanisms of membrane disruption by chitosan and fluoride release from GIC, combined with the adhesive properties that ensure intimate material-bacteria contact. The specificity of action against target pathogens, while minimizing effects on non-target organisms, supports the potential utility of this composite for maintaining oral microbiome balance while combating pathogenic species. These findings position chitosan nanoparticle-infused GIC as a promising candidate for advanced dental applications, including restorations, sealants, bases, and endodontic materials, where antimicrobial activity could significantly reduce the risk of secondary caries, treatment failure, and disease recurrence. However, translation of these promising in vitro findings to clinical practice requires additional research, including optimization of nanoparticle concentration and characteristics, comprehensive biocompatibility and safety assessments, evaluation of long-term stability under simulated oral conditions, and ultimately well-designed clinical trials to validate efficacy and safety in human subjects. The synergistic effects observed in this study open avenues for creating innovative, effective, and multifunctional dental composites that address both restorative and preventive aspects of oral healthcare, contributing to the ongoing evolution of strategies for maintaining oral health and treating dental diseases.

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