Biomedical Sciences and Clinical Medicine 2026;65(1):32-42.
doi: 10.12982/BSCM.2026.03
https://he01.tci-thaijo.org/index.php/CMMJ-MedCMJ/index

BSCM
Biomedical Sciences and Clinical Medicine

Original Article

Open Access

Development of Zinc Oxide Nanoparticle–Infused Carrageenan Membranes with Enhanced Structural and Antimicrobial Properties for Guided Tissue Regeneration

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Received: April 24, 2024;
Revised: July 18, 2025;
Accepted: August 7, 2025

ABSTRACT

OBJECTIVE The fundamental aim of periodontal therapy is to promote the regeneration of tissues affected by the disease. In recent times, there has been a rising trend in using guided tissue regeneration (GTR) membranes. These membranes are increasingly relied upon to direct the growth of gingival tissue away from the root surface. Both resorbable and non-resorbable membranes presently in use serve as physical barriers, hindering the infiltration of connective and epithelial tissues into the defect area, thus fostering periodontal tissue regeneration. This study aims to develop a polymeric membrane reinforced with carrageenan and zinc oxide nanoparticles and assess its potential for periodontal regeneration.

METHODS 3g of dried Kappaphycus was mixed with 100 mL of distilled water in a container and autoclaved for 15 minutes at 121 degrees Celsius. After cooling, the mixture was blended thoroughly for carrageenan extraction. Then, 1.5 g of PEG 6000 was added as a plasticizer. Zinc oxide nanoparticles (ZnO) were added and homogenized into the carrageenan extract. The mixture was microwave-boiled for 2 minutes, poured onto a petri dish, and air-dried for 24 hours. Further drying at 50 degrees Celsius for 2 hours ensured complete moisture removal. Finally, the membrane was carefully peeled from the petri dish for testing and use. The membrane underwent SEM, tensile strength, Fourier transform infrared, and antimicrobial activity analysis.

RESULTS Scanning electron microscopy analysis revealed a densely packed and moderately rough surface in the ZnO-incorporated membrane, with a broader particle size distribution (1.28 ± 3.67 μm) compared to the smoother carrageenan-only membrane (1.15 ± 1.68 μm). Tensile testing showed improved mechanical strength in the ZnO composite (6.89 MPa) relative to the plain carrageenan membrane (5.39 MPa). FTIR spectra confirmed successful integration of ZnO nanoparticles through characteristic Zn–O peaks, along with preserved polysaccharide functional groups. Antimicrobial activity, evaluated via OD_{600} measurements over 4 hours, demonstrated time- and size-dependent bacterial inhibition against S. *mutans*, with the 4 cm^2 membrane showing superior performance, comparable or superior to chloramphenicol at later time points.

CONCLUSIONS The developed membrane reinforced with carrageenan and zinc oxide nanoparticles exhibited adequate tensile strength and sufficient antimicrobial properties, suggesting its suitability for utilization in periodontal therapy as an effective regenerative material.

KEYWORDS biomaterials, carrageenan, guided tissue regeneration, nanoparticles

INTRODUCTION

The human periodontium comprises several components, including the alveolar mucosa, gingiva, periodontal ligament, root covering cementum, and alveolar bone. Periodontitis, a prevalent pathological condition, results from persistent bacterial-induced inflammation of the gingival tissue, leading to progressive and irreversible damage to the periodontium (1). Left untreated, it can lead to soft tissue and hard tissue damage, ultimately tooth loss. Hence, there's a pressing need for restorative procedures to ensure complete and functional regeneration of periodontal tissues.

Guided tissue regeneration (GTR) techniques are employed for bone regeneration in periodontal cases. GTR aims to create a mechanical barrier around the affected periodontal area to facilitate new bone formation (2). The barrier membranes used in GTR must meet specific criteria, including mechanical obstruction, biocompatibility, biological functionality, resistance to exposure, natural degradation, and adequate porosity for new blood vessel formation (3). These membranes are classified into non-resorbable and resorbable types. While non-resorbable membranes like expanded polytetrafluoroethylene (e-PTFE) are common, they require removal post-healing. Resorbable membranes address this issue, but often lack sufficient mechanical properties for soft tissue recovery (4).

Growing apprehensions about the ecological consequences of synthetic materials and the exhaustion of fossil fuels utilized in their manufacturing processes are becoming more apparent. This highlights the significance of advancing eco-friendly materials, such as biomaterials sourced from natural reservoirs, including polysaccharides, proteins, and lipids. To address these challenges, our study focuses on developing a novel polymeric membrane reinforced with carrageenan and zinc oxide nanoparticles (ZnO).

Carrageenans (CG) are naturally occurring sulfated polysaccharides extracted from red algae. Under specific conditions, they form a helical structure similar to collagen (5).

CG demonstrate high solubility in water and are frequently employed as gelling agents within the food industry (5). Nonetheless, there is limited documentation regarding their utilization in membrane preparation. There are three main types of carrageenan, each with distinct properties (6). κ-CG, the most abundant type, forms strong gels in the presence of potassium salts, while λ-CG acts solely as a thickening agent (7). The primary limitations of these polymeric membranes include their inadequate mechanical characteristics and their propensity for high solubility in aqueous environments. Consequently, it is imperative to incorporate nanoparticles into the polymer matrix to enhance mechanical properties and mitigate solubility (8).

ZnO exhibit significant antimicrobial activity and can interact with bacterial surfaces, entering the cell and exhibiting distinct bactericidal mechanisms (9). They are effective against various microorganisms like *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumonia*, *Pseudomonas vulgaris*, *Candida albicans*, and *Aspergillus niger* (10) and also possess anti-inflammatory properties (11).

Taking advantage of the individual benefits of these materials, our study aims to fabricate a CG and ZnO reinforced polymeric membrane and evaluate its potential for periodontal regeneration. Assessing various properties of this formulation can enhance its suitability for clinical use in periodontal therapy.

METHODS

3 grams of dried Kappaphycus (Natural Remedies Pvt. Ltd., Bangalore, India) were combined with 100 milliliters of distilled water in a container (Figure 1). This mixture was then transferred to an autoclave-safe container and autoclaved at 121 degrees Celsius for 15 minutes before being allowed to cool to room temperature. Once cooled, the mixture was thoroughly blended to aid in breaking down the Kappaphycus material for carrageenan extraction. After extraction, 1.5 grams of polyethylene glycol (PEG 6000) were added to the extract to act as a plasticizer, enhancing the flexibility and handling properties of the final membrane.

Subsequently, the required quantity of ZnO nanoparticles was introduced into the carrageenan extract mixture and thoroughly homogenized to achieve even dispersion (Figure 2). The mixture was subjected to microwave boiling in a container safe for microwave use for 2 minutes to effectively blend the components.

Figure 1. Kappaphycus seaweed in a borosilicate glass beaker

Figure 2. Carrageenan incorporated with zinc oxide nanoparticles

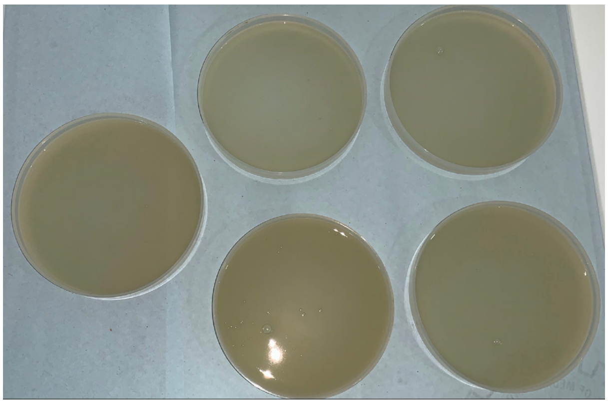

Figure 3. Film cast method of the carrageenan and zinc oxide nanoparticle membrane

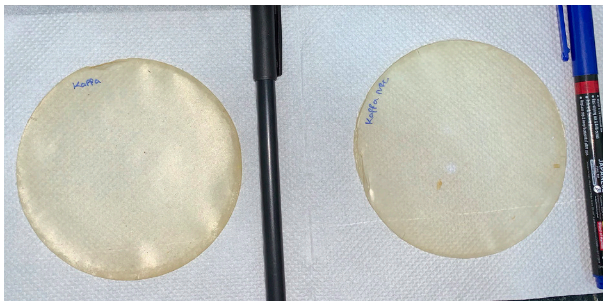

Figure 4. The dried carrageenan and zinc oxide nanoparticle membrane

Following this, the liquid mixture was poured onto a petri dish using the film casting technique and spread evenly to create a thin, uniform film (Figure 3).

Afterward, the film was left to air dry for 24 hours to remove excess moisture and solidify into a membrane. Following air drying, the membrane underwent additional drying at 50 degrees Celsius for 2 hours to ensure thorough moisture removal. Once dried, the membrane was delicately peeled off the surface of the petri dish, making it ready for testing and subsequent utilization (Figure 4).

Morphology of the membrane

For Scanning Electron Microscopy [SEM analysis], the samples were examined utilizing the FEI Quanta FEG 650 SEM [JSM IT-800, JEOL Ltd., Akishima, Tokyo, Japan]. Both the surface and cross-sections of the membrane were photographed at magnifications of 500X.

Tensile strength measurement

To assess tensile strength, the membrane was divided into smaller sections and affixed to the testing equipment with the cut ends. Tensile load, measured in MPa units, was incrementally applied to the membrane at a predefined rate until it fractured. Recorded data encompassed the tensile load observed at the moment of fracture. This examination was carried out utilizing a universal testing machine (Figure 5).

Fourier transform infrared (FTIR)

FTIR analysis was carried out using a BRUKER FTIR spectrometer. Attenuated total reflectance fourier transform infrared (ATR-FTIR) spectra were obtained from dry films to explore chemical interactions among carrageenan, ZnO, and other components involved in the manufacturing process.

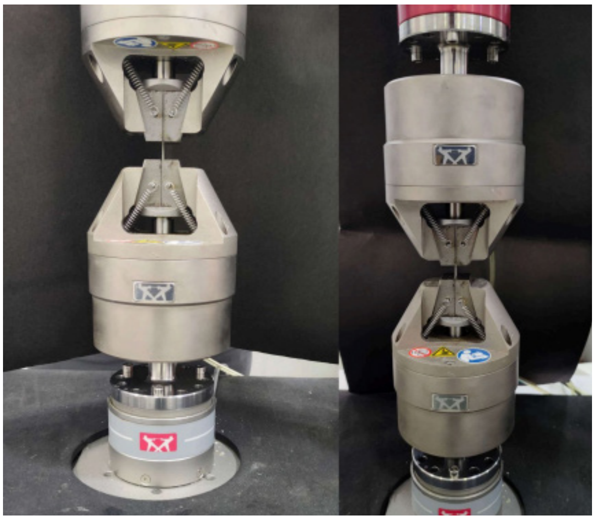

Figure 5. Universal testing machine, Model- Instron Expert Electropulse 3000

Antimicrobial activity of carrageenan and zinc oxide nanoparticle membrane

The antimicrobial activity of the carrageenan and zinc oxide nanoparticle-based membrane was evaluated against *Streptococcus mutans* (*S. mutans*) using a broth turbidity assay. Membrane discs of four different surface areas (1 cm^2, 2 cm^2, 3 cm^2, and 4 cm^2) were aseptically introduced into individual test tubes containing 5 mL of Mueller-Hinton broth inoculated with a standardized suspension of *S. mutans*. Chloramphenicol at a concentration of 5 mg/mL served as the positive control. All samples were incubated at 37 °C, and bacterial growth was monitored by measuring optical density (OD) at 600 nm using a UV-Vis spectrophotometer at regular intervals of 1, 2, 3, and 4 hours. A reduction in OD values indicated bacterial growth inhibition by the test membrane. Each condition was tested in a single replicate due to material constraints.

Statistical analysis

Normality of the particle size data for the SEM analysis in each group was assessed using the Shapiro–Wilk test. Depending on the distribution, an independent samples t-test was applied to compare average particle sizes. Additionally, the F-test for equality of variances was used to evaluate differences in variance between groups.

RESULTS

The membranes were cut into 1 × 1 cm pieces and subjected to sterilization. Following sterilization, various evaluations were conducted, including SEM analysis, tensile strength measurement, FTIR analysis, and antimicrobial activity assay.

Scanning electron microscopy (SEM) analysis

SEM allows for direct observation of the surface of solid objects, providing detailed insights into the morphological structure of the membrane. In this study, SEM analysis was conducted at 300× magnification to evaluate the surface characteristics of the fabricated membranes. The membrane composed solely of carrageenan exhibited a relatively smooth and uniform morphology (Figure 6). In contrast, the membrane incorporating ZnO displayed a densely packed surface with moderate roughness, characterized by micro-scale protrusions and depressions, along with a relatively narrow pore size distribution (Figure 7).

Particle size analysis was performed on the SEM images using image analysis software calibrated to a 10 μm scale bar. For the pure carrageenan membrane, 88 particles were measured, revealing an average particle size of 1.15 ± 1.68 μm (mean ± SD), with a distribution range from 0.17 to 12.26 μm. The ZnO-incorporated membrane showed a more heterogeneous morphology; 36 particles were analyzed with an average size of 1.28 ± 3.67 μm and a distribution range from 0.17 to 22.65 μm. The higher standard deviation and broader size range in the ZnO-containing membrane suggest particle aggregation and structural modifications induced by the incorporation of ZnO into the carrageenan matrix.

Tensile strength

The tensile strength of the fabricated membranes was evaluated to qualitatively assess their mechanical integrity. The membrane incorporating ZnO into the carrageenan matrix exhibited a higher maximum tensile stress of approximately 6.89 MPa, in contrast to 5.39 MPa for the membrane composed of carrageenan alone. This increase indicates that the incorporation of ZnO nanoparticles contributed to enhanced structural reinforcement, likely due to improved intermolecular interactions and nanoparticle dispersion within the polymer matrix. However, owing to the preliminary scope of the study and limited availability of materials, tensile testing was performed on only a single membrane sample per formulation.

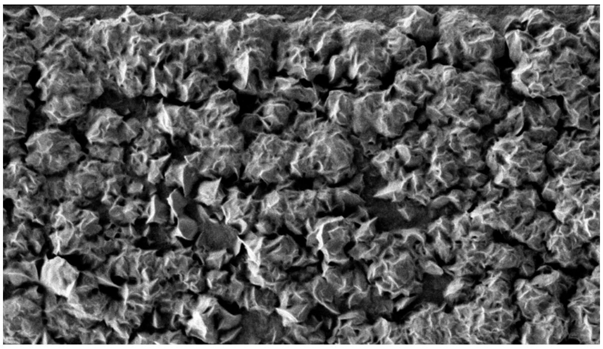

Figure 6. Scanning electron microscopy analysis of the carrageenan membrane

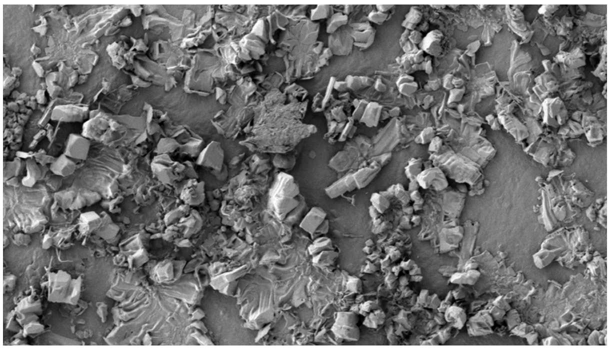

Figure 7. Scanning electron microscopy analysis of the carrageenan and zinc oxide nanoparticle membrane

FTIR analysis of carrageenan membrane

The FTIR spectrum of the pure carrageenan membrane (Figure 8) exhibited characteristic absorption bands corresponding to functional groups typically present in polysaccharides. A broad band at 3,393.12 cm^{-1} was attributed to O–H stretching vibrations, indicating the presence of hydroxyl groups involved in hydrogen bonding. A peak at 2,938.77 cm^{-1} was due to C–H stretching of aliphatic $-CH_2$ groups.

The absorption band at 1,611.03 cm^{-1} likely represented C = O stretching or O–H bending from absorbed moisture. Peaks observed at 1,456.06 cm^{-1} and 1,408.96 cm^{-1} were assigned to CH_2 bending vibrations and symmetric COO^- stretching, respectively, suggesting the presence of carboxylate groups. A strong peak at 1,238.56 cm^{-1} corresponded to S = O stretching vibrations, indicative of sulfate ester groups typical of sulfated polysaccharides like carrageenan.

Further, peaks in the region 1,147.35 to 1,021.24 cm^{-1} were attributed to C–O–C ether linkages and C–O stretching, representing glycosidic bonds in the polymer backbone. The fingerprint region below 930 cm^{-1}, including peaks at 928.31, 845.21, 772.24, and 578.29 cm^{-1}, corresponded to C–O–S stretching, sugar ring skeletal vibrations, and other out-of-plane bending modes. These findings confirmed the presence of key functional groups such as hydroxyl, carboxylate, sulfate, and ether linkages, consistent with the structural features of carrageenan.

FTIR analysis of carrageenan–ZnO nanocomposite membrane

The FTIR spectrum of the carrageenan membrane infused with ZnO (Figure 9) showed absorption bands characteristic of both the polysaccharide backbone and the incorporated inorganic phase. A broad peak at 3,353.32 cm^{-1} was attrib-

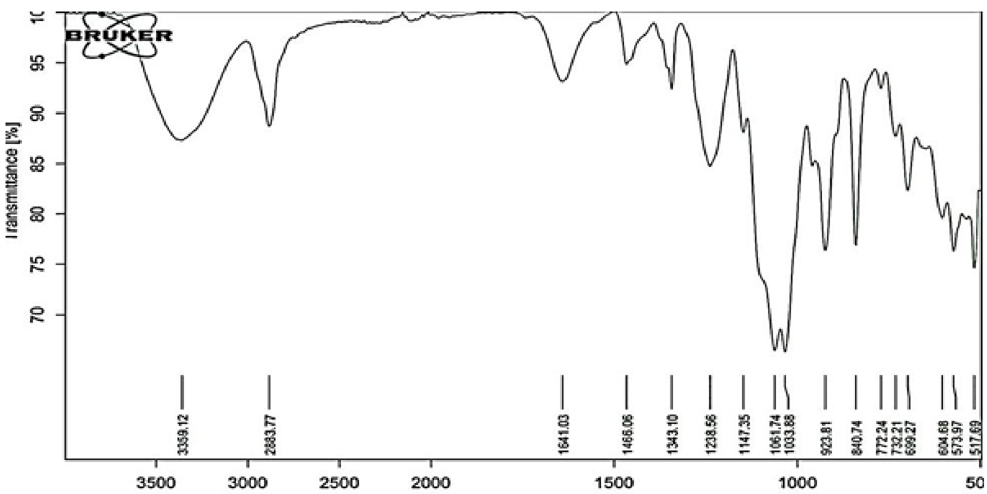

Figure 8. Fourier transform infrared spectra of carrageenan membrane

uted to O–H stretching vibrations, reflecting hydroxyl groups involved in hydrogen bonding within the carrageenan matrix. A peak observed at 2,885.91 cm^{-1} was assigned to C–H stretching from methylene groups.

The band at 1,644.39 cm^{-1} was likely due to C=O stretching or O–H bending from residual water content or carboxylate groups. Peaks at 1,458.30 cm^{-1} and 1,406.93 cm^{-1} corresponded to CH_2 bending and COO^- symmetric stretching, respectively, indicative of aliphatic hydrocarbon chains and carboxylate functionalities. A strong band at 1,236.42 cm^{-1} was assigned to S = O stretching, confirming the presence of sulfate ester groups within the sulfated polysaccharide.

The spectral region between 1,090.42 and 1,006.02 cm^{-1} exhibited strong bands corresponding to C–O–C and C–O stretching vibrations, associated with glycosidic linkages in the carrageenan polymer. Additional sharp peaks at 857.27 cm^{-1}, 701.82 cm^{-1}, and neighboring lower wavenumbers were attributed to Zn–O bond stretching vibrations, providing spectral evidence for the successful incorporation of ZnO into the membrane. These Zn–O peaks were distinct from the typical polysaccharide signals and indicated interaction between the ZnO nanoparticles and functional groups (such as hydroxyl or sulfate) in the carrageenan matrix. Collectively, the presence of characteristic hydroxyl, carboxylate, sulfate, ether, and metal-oxygen (Zn–O) vibrational modes confirmed the formation of a hybrid organic–inorganic membrane and supported the successful integration of ZnO within the carrageenan network.

Antimicrobial activity

The antimicrobial efficacy of the carrageenan–zinc oxide nanoparticle membrane was evaluated over time by measuring the OD at 600 nm. A general decline in OD values indicated effective bacterial inhibition (Figure 10).

At 1 hour, all membrane sizes showed moderate antimicrobial activity, with OD values decreasing progressively from the 1 cm² to 4 cm² membranes, suggesting a dose-dependent effect. The 4 cm² membrane exhibited the lowest OD (~0.14), while the 1 cm² membrane showed higher turbidity (~0.26), indicating lesser inhibition. Chloramphenicol, the positive control, had an OD of ~0.30.

At 2 hours, the 1 cm² membrane showed the highest OD (~0.36), indicating poor inhibition, while the 4 cm² membrane maintained a lower OD (~0.18), comparable to chloramphenicol (~0.22), demonstrating effective bacterial suppression with a larger membrane size.

At 3 hours, all membranes showed a notable decrease in OD values (~0.14–0.18), approaching the efficacy of chloramphenicol (~0.18), indicating time-dependent enhancement of antimicrobial activity.

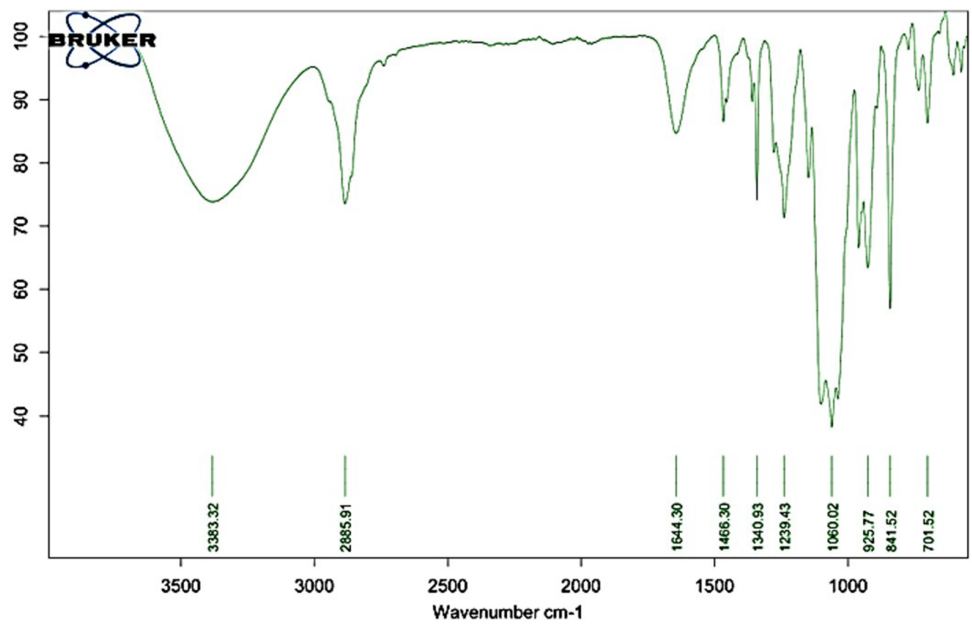

Figure 9. Fourier transform infrared spectra of carrageenan and zinc oxide nanoparticle membrane

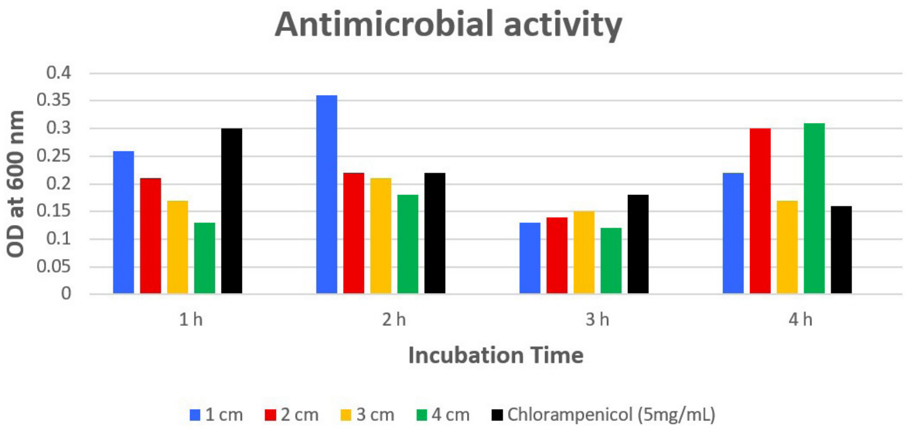

Figure 10. Antimicrobial activity of carrageenan and zinc oxide nanoparticle membrane, with the X-axis representing the incubation time, and the Y-axis representing the optical density

By 4 hours, the 2 cm² and 4 cm² membranes demonstrated the highest bacterial inhibition (OD ~0.30 and ~0.31, respectively), surpassing the chloramphenicol control (OD ~0.16), whereas the 3 cm² membrane showed lower OD (~0.18), indicating better performance.

DISCUSSION

Periodontitis is a chronic inflammatory disease of the supporting structures of the teeth, primarily caused by bacterial biofilm accumulation (12). It leads to progressive destruction of the periodontal ligament and alveolar bone, often resulting in tooth mobility and eventual tooth loss if left untreated. One of the hallmark manifestations of advanced periodontitis is the formation of intrabony defects, vertical bone loss patterns confined within the bone walls (13). These defects present a significant clinical challenge due to their complex anatomy and limited self-regenerative capacity. Effective management of intrabody defects is crucial for restoring periodontal health and function, often requiring regenerative strategies such as bone grafts, barrier membranes, or bioactive materials to promote tissue regeneration and bone fill.

GTR utilizes membranes as mechanical barriers to create a secluded space around periodontal defects, preventing the rapid migration of epithelial and connective tissue cells into the wound area. This selective repopulation encourages the regeneration of the periodontal ligament, cementum, and alveolar bone (14). Although current GTR membranes have demonstrated potential in supporting partial periodontal regeneration, full and predictable regeneration remains elusive. Many available membranes are often associated with biocompatibility concerns, risk of immunogenicity, and high costs.

To overcome these limitations, there is a growing focus on developing naturally derived, biocompatible membranes that can provide both mechanical support and biological activity. In this context, carrageenan, a natural marine-derived polysaccharide, offers an attractive alternative due to its biodegradability, hydrophilicity, and gel-forming properties (15). When combined with zinc oxide nanoparticles (ZnO NPs)—known for their antimicrobial, anti-inflammatory, and osteoinductive properties the resulting composite membrane holds promise for enhancing the regenerative microenvironment. Such carrageen-Zno NP-infused membranes not only act as physical barriers but may also actively promote tissue healing and prevent microbial contamination, making them highly suitable candidates for next-generation GTR applications.

Several studies have highlighted the individual advantages of carrageenan and ZnO. This study combines these materials to develop a polymeric membrane, leveraging their synergistic effects. CG, chosen for its biocompatibility and hydrophilic nature, is conducive to cell proliferation and migration, essential in regenerative membrane applications like wound healing or tissue engineering mechanisms, and has anti-viral activity (16). ZnO with antimicrobial properties (17) complement carrageenan's characteristics, offering a promising combination for periodontal regeneration.

A study investigating bi- and tri-layer nanofibrous membranes formulated with sulfated polysaccharide carrageenan for periodontal regeneration reported favorable outcomes. The membranes maintained structural stability and demonstrated a sustained release of Ca^2 ions for at least three weeks, indicative of their potential to support osteoblast proliferation and enhance bone regeneration in periodontal applications. (18).

Another study explored an antibiotic-loaded cellulose nanofiber and κ-carrageenan hydrogel which showed strong antibacterial effects against key periodontal pathogens, significantly increasing MDA levels and reducing biofilm formation and bacterial activity, indicating promising potential for periodontitis treatment (19).

A separate study focused on the fabrication and characterization of a stimuli-responsive scaffold/bio-membrane using a novel carrageenan biopolymer for biomedical applications. The bio-membrane exhibited a high strength of 89.21 MPa, surpassing that of commercial membranes. Moreover, its smooth surface favored cell adhesion and proliferation (20).

One study evaluated the preparation of a carrageenan- and fucoidan-silica nanoparticle–based membrane for guided bone regeneration in dental implant sites, demonstrating its potential to promote osteoconduction. Another study investigated the applications of seaweed biopolymers, highlighting their effectiveness in scaffolds, due to their favourable biocompatibility and bioactive properties (21, 22).

ZnO nanoparticles were incorporated into the carrageenan-based membrane to enhance both its antimicrobial efficacy and mechanical performance. ZnO nanoparticles contribute to bacterial inhibition through multiple mechanisms, including the generation of reactive oxygen species (ROS), disruption of bacterial cell membranes, and the release of $Zn^2{}_2$ ions, which are known to interfere with microbial metabolism and replication (23). Simultaneously, the nanoscale size and uniform distribution of ZnO within the carrageenan matrix contribute to improved tensile strength and elasticity of the membrane, as reflected by the measured tensile strength of approximately 6.89 MPa.

Several previous studies have highlighted the potential of combining carrageenan with reinforcing agents like ZnO to develop composite biomaterials with enhanced properties. Based on these insights, the present study aimed to develop a polymeric membrane that harnesses the synergistic benefits of carrageenan's biocompatibility and ZnO's multifunctionality. The resulting membrane demonstrates strong potential for application in GTR, offering both antimicrobial protection and mechanical stability essential for periodontal healing.

In one study, an overview of recent progress in dental applications of ZnO was provided. It stated that ZnO nanoparticles have osteoconductive ability, antibacterial activity, and flexibility suitable for periodontal tissue regeneration (24).

In another investigation, the antimicrobial efficacy of zinc against periodontal pathogens was examined, revealing its ability to hinder the vital functions, growth, and reproduction of various microorganisms and disrupting the integrity of bacterial cell walls (25).

Another study focused on the favorable properties of ZnO nanoparticles in promoting tissue integration of biomaterials. It was concluded that ZnO nanoparticles possess antibacterial properties and can enhance the cellular metabolic activity of fibroblasts and endothelial cells relevant for wound healing (26).

A study investigated the use of green nanomaterials such as zinc oxide and chitosan for their antimicrobial activity against oral pathogens, demonstrating significant inhibitory effects (27). Another study evaluated the antibiofilm efficacy of surface-modified gutta-percha incorporated with ZnO, revealing enhanced resistance to microbial colonization and improved potential for endodontic applications (28).

In our study, the polymeric membrane formulated with carrageenan and ZnO underwent *in-vitro* characterization. SEM analysis revealed that the pure carrageenan membrane had a smooth, uniform surface, ideal for controlled degradation. Incorporation of ZnO nanoparticles introduced micro-scale roughness, enhancing cell adhesion and regenerative interaction. The well-distributed surface features and moderate porosity are beneficial for periodontal applications. Particle size analysis confirmed successful ZnO integration with favorable morphological variation. These characteristics collectively improve the membrane's functional potential in GTR.

These findings align with previous research, which investigated carrageenan-based antimicrobial bionanocomposite films incorporated with ZnO nanoparticles stabilized by melanin, revealing irregularly placed rod-shaped particles with protrusions and depressions (29).

The carrageenan–ZnO membrane showed a tensile strength of ~6.89 MPa, supporting its role in maintaining structural integrity during tissue regeneration. This mechanical property helps preserve space for tissue growth and prevents membrane collapse. Comparable findings were reported in a study showing 5.50 ± 2.62 MPa for κ-carrageenan/ZnO composites, aligning with the present results (30).

In FTIR analysis, the FTIR spectra confirmed the successful incorporation of ZnO into the carrageenan matrix. Characteristic peaks for hydroxyl, carboxylate, sulfate, and ether groups were present in both membranes, validating the structural integrity of carrageenan. In the ZnO-infused membrane, additional peaks corresponding to Zn–O stretching were observed, indicating effective interaction between the nanoparticles and functional groups in the polymer. These spectral features demonstrate the formation of a stable hybrid organic–inorganic membrane, suitable for biomedical applications.

Regarding antimicrobial activity, the results were comparable to those obtained from the control group treated with chloramphenicol alone. Chloramphenicol, a broad-spectrum antibiotic commonly used to treat bacterial infections, served as the standard antimicrobial agent in the study. There was a close resemblance between the antimicrobial effects observed in the tested group and the control group, indicating that the evaluated membrane demonstrated antimicrobial activity comparable to that of chloramphenicol. These results align with a study where zinc oxide nanoparticle and carrageenan-based composite films exhibited high antibacterial properties, particularly against *Escherichia coli* and *Listeria monocytogenes* species (31).

Thus, the formulated membrane, composed of naturally derived ingredients such as ZnO and carrageenan, presents a biocompatible and eco-friendly solution tailored for periodontal applications. This study is among the first to evaluate the synergistic potential of zinc oxide and carrageenan within a single membrane, highlighting the growing relevance of nanotechnology in oral care. Based on the findings, the developed polymeric membrane holds significant promise for regenerative periodontal therapy. Future comparative studies with existing membranes are warranted to further validate their clinical efficacy and real-world applicability.

Limitations

Study limitations include the use of a single sample per membrane type for tensile testing, limiting statistical strength. Additionally, MIC values for carrageenan-ZnO and chloramphenicol were not determined, restricting quantitative antimicrobial comparisons. Although OD data showed time- and dose-dependent inhibition, future studies will address these gaps and include *in-vivo* validation for clinical relevance.

CONCLUSIONS

The membrane developed using carrageenan reinforced with ZnO demonstrated promising characteristics for periodontal regeneration. SEM analysis confirmed a denser, moderately rough surface morphology, favorable for cell attachment. Mechanical testing showed improved tensile strength in the ZnO-reinforced membrane compared to carrageenan alone, indicating enhanced structural integrity. FTIR analysis validated the successful integration of ZnO nanoparticles within the carrageenan matrix through distinct Zn–O vibrational peaks. Moreover, antimicrobial assays revealed that the membrane exhibited time-dependent inhibition of S. *mutans*, with activity comparable to chloramphenicol by 4 hours of incubation. These findings support the membrane's potential as a multifunctional material for GTR in periodontal therapy.

Areas of future research

Future research can focus on enhancing the membrane by incorporating additional antimicrobial agents for synergistic effects, as well as bioactive molecules like growth factors or peptides to boost cell proliferation and tissue regeneration. These strategies could pave the way for more targeted and effective periodontal therapies.

ACKNOWLEDGEMENTS

The authors of this study express their sincere gratitude to the management and the Department of Periodontics at Saveetha Dental College and Hospitals, Chennai, for their invaluable guidance and support in facilitating the successful completion of this research.

FUNDING

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors

CONFLICTS OF INTEREST

The authors have no conflicts of interest to report.

AUTHOR'S CONTRIBUTION

P.T.: data curation, data curation, investigation, project administration, supervision, visualization, writing- original draft, writing- review & editing; A.M.: investigation, formal analysis, methodology, visualization, writing- original draft, writing- review & editing; B.G.S.: formal analysis, methodology, supervision, writing- original draft.

DATA AVAILABILITY STATEMENT

All the necessary data has been provided in the manuscript.

INSTITUTIONAL REVIEW BOARD STATEMENT

The institutional scientific research board [SRB/SDC/UG-2094/23/PERIO/152] was obtained before the start of the study.

INFORMED CONSENT STATEMENT

Not applicable.This study does not involve human participants or human tissue.

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