Alginate Membranes with Magnesium Nanoparticles Drive Improved Soft Tissue Regeneration in Zebrafish Models

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**Abstract:** Biopolymers with tunable properties have shown promise for soft tissue regeneration but often fall short in restoring optimal structural and functional integrity. This study investigates the potential of alginate membranes incorporated with magnesium nanoparticles (MgNPs) as scaffolds to enhance mechanical properties, cellular interaction, and tissue regeneration, using zebrafish as an in vivo model. Alginate membranes were synthesized by incorporating magnesium nanoparticles. Zebrafish were used to evaluate the regenerative capacity of these membranes. Histological analysis, including hematoxylin and eosin (H&E) staining, was performed on the 14th day to assess tissue organization and extracellular matrix (ECM) deposition. Antibacterial, antioxidant, and cytotoxic properties of MgNPs were analyzed statistically, with significance determined at a p-value ≤ 0.05.The histological analysis revealed marked tissue regeneration, with densely packed nuclei indicating robust cell proliferation and increased vascularization highlighted by blood-filled vessels. Organized connective tissue was observed, with reduced fibrosis and inflammation. ECM deposition was evident, and the absence of necrotic or apoptotic cells confirmed effective healing. The membranes demonstrated improved mechanical stability, biocompatibility, and regenerative outcomes. Alginate membranes infused with magnesium nanoparticles exhibit significant potential as scaffolds for soft tissue regeneration. They promote cell proliferation, vascularization, and organized ECM deposition while minimizing fibrosis and inflammation, addressing key challenges in tissue repair. These findings suggest strong applicability in clinical settings for wound healing and regenerative therapies.

**Keywords:** Alginate Membranes, Magnesium Nanoparticles, Soft Tissue Repair, Tissue Regeneration, Extracellular Matrix, Zebrafish Model

# Introduction

Soft tissue injuries present a significant global health challenge, affecting millions annually and resulting in substantial medical expenses due to extended treatment durations and complications. Inadequate healing often leads to functional impairments, reduced quality of life, and a substantial economic burden on healthcare systems worldwide[(Chan et al., 2022)](https://paperpile.com/c/2eNg4X/cpsW). Effective solutions to restore soft tissue functionality remain critical, particularly in cases where conventional therapies fail to achieve complete regeneration[(Tang, 2021)](https://paperpile.com/c/2eNg4X/ZOCa);[(Aparna et al., 2021; Poornima et al., 2021; Verma & Muthuswamy Pandian, 2021)](https://paperpile.com/c/2eNg4X/5eqo+OW5o+8Gi7).

Biopolymers, including alginate, have gained international recognition for their potential in tissue engineering due to their biocompatibility, biodegradability, and ability to replicate the natural extracellular matrix (ECM)[(Sun & Tan, 2013)](https://paperpile.com/c/2eNg4X/SL1L); [(Merchant et al., 2022; Pandiyan et al., 2022)](https://paperpile.com/c/2eNg4X/Wbq2+YpD1). The global biomaterials market, valued at billions of dollars, highlights the increasing demand for innovative scaffolding solutions to address soft tissue injuries. However, the widespread use of alginate is hindered by its inherent limitations, such as low mechanical strength and limited bioactivity, which impede its effectiveness in complex tissue repair[(Hadisaputra, 2007)](https://paperpile.com/c/2eNg4X/pMOR)[Merchant et al., 2022; Pandiyan et al., 2022)](https://paperpile.com/c/2eNg4X/Wbq2+YpD1+UIdiy)[(Laghari et al., 2023; Ramakrishnan et al., 2023a)](https://paperpile.com/c/2eNg4X/jLx1H+qWxY)[(Muthuswamy Pandian et al., 2022; Ramakrishnan et al., 2023b)](https://paperpile.com/c/2eNg4X/YcaX+SUqtV).

To overcome these challenges, this study investigates the integration of magnesium nanoparticles (MgNPs) into alginate membranes to enhance their mechanical properties and bioactivity[(Pingale et al., 2024)](https://paperpile.com/c/2eNg4X/gVgi). Magnesium is recognized for its critical role in cellular processes such as proliferation, angiogenesis, and ECM remodeling, which are essential for tissue repair[(Shastri et al., 2010)](https://paperpile.com/c/2eNg4X/Oakq). Furthermore, MgNPs offer antibacterial and antioxidant properties, reducing the risks of infection and oxidative damage during the healing process[(Sankara Narayanan et al., 2015)](https://paperpile.com/c/2eNg4X/yD2K); [(Chokkattu et al., 2022; Ramamurthy et al., 2022)](https://paperpile.com/c/2eNg4X/wQnL+oA9bb); [(Wadhwani et al., 2022)](https://paperpile.com/c/2eNg4X/XSXK); [(Sreevarun et al., 2023)](https://paperpile.com/c/2eNg4X/7V3TL); [(Harikrishnan & Subramanian, 2023)](https://paperpile.com/c/2eNg4X/7Fkk).

The research employs zebrafish as an in vivo model, widely used for regenerative studies due to their rapid healing capacity and transparent tissues, allowing detailed histological evaluation[(Cartner et al., 2019; Kalueff & Stewart, 2012)](https://paperpile.com/c/2eNg4X/wDLn+l1Tz)2; [(Jain & Verma, 2022; Marya et al., 2022)](https://paperpile.com/c/2eNg4X/3BLoF+ylKpD). By combining MgNPs with alginate membranes, this study aims to address the existing gap in effective scaffolding materials for soft tissue regeneration.

The objectives of this study are to develop and characterize alginate membranes incorporated with magnesium nanoparticles (MgNPs) and evaluate their regenerative potential using zebrafish models. The research aims to analyze histological changes, focusing on extracellular matrix (ECM) remodeling, collagen synthesis, and vascularization during early and mid-phase healing. Additionally, the study seeks to assess the antibacterial and antioxidant properties of MgNPs and their contributions to enhancing tissue repair outcomes, providing a comprehensive understanding of the membranes’ effectiveness in promoting soft tissue regeneration.

This research proposes an innovative solution to improve soft tissue regeneration by leveraging the synergistic properties of alginate and magnesium nanoparticles, offering a cost-effective and efficient alternative for clinical applications.

# Materials and methods

## Tissue Regeneration in Zebrafish

An artificial injury was induced in the caudal region of zebrafish, and the healing area was exposed to three different concentrations of the compound (20 μl, 40 μl, and 80 μl) during the regeneration process. Tissue sections from the injured area were stained with hematoxylin and eosin (H&E) and examined under a light microscope. The tissues were fixed, dehydrated, and embedded in paraffin before thin sections were cut using a Semi-Automated Rotary Microtome M-240 (Myr, Spain) and mounted on glass slides. H&E staining involved the use of hematoxylin to highlight acidic structures such as nuclei in blue or purple, and eosin to stain the cytoplasm and basic elements in pink or red. Stained sections were then dehydrated, cleared, and coverslipped for microscopic evaluation. Features such as inflammation, cell proliferation, and tissue remodeling were analyzed to assess wound healing and regeneration, with images captured for documentation and analysis.

## Antioxidant Activity

Magnesium nanoparticles (MgNPs) were synthesized following established protocols and characterized using scanning electron microscopy (SEM). The antioxidant activity of MgNPs was assessed using the DPPH assay. A 0.1 mM DPPH solution was prepared in ethanol, and MgNPs at varying concentrations (5, 10, 15, 20, and 25 μg/ml) were diluted in ethanol. In a 96-well plate, 100 μl of each MgNP concentration was mixed with 100 μl of the DPPH solution and incubated at room temperature for 30 minutes. The absorbance was measured at 570 nm using a microplate reader. Statistical analysis of the antioxidant activity was conducted using IBM SPSS Statistics for Windows, Version 23.0, and the results were compared to a standard antioxidant using an unpaired t-test, with a significance threshold of p ≤ 0.05.

## Antimicrobial Activity

The antimicrobial activity of MgNPs was tested using the well diffusion method against clinical pathogenic bacteria, including *Enterococcus faecalis*, *Streptococcus mutans*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. Pure cultures of these bacteria were cultivated on nutrient agar media. Three concentrations of MgNPs (25, 50, and 100 μg/ml) were added to wells in petri dishes inoculated with bacterial isolates. Ampicillin (1 μg/ml) was used as a positive control. Plates were incubated at 37°C for 12–24 hours, and the zones of inhibition were measured manually to evaluate antibacterial efficacy.

## H&E Staining Protocol

Tissue sections from zebrafish were deparaffinized in xylene for 20 minutes and rehydrated through a graded series of ethanol solutions for 10 minutes each. Sections were rinsed under running tap water for 3–5 minutes before nuclear staining with Harris’s hematoxylin for 5 minutes. After a tap water rinse, differentiation was performed using a single dip in acid alcohol, followed by neutralization in ammonia water and further rinsing to achieve bluing. Eosin staining was performed with a single dip, followed by dehydration through ascending alcohol concentrations, clearing in xylene, and mounting with DPX. The stained sections were evaluated microscopically for tissue morphology and regeneration.

## Statistical Analysis

Data were collected, tabulated, and analyzed using IBM SPSS Statistics for Windows, Version 23.0 (Released 2015; IBM Corp., Armonk, NY, USA). A one-way ANOVA was used to assess the antibacterial activity of MgNPs in comparison to the antibiotic standard, with a significance level of p ≤ 0.05. An unpaired t-test was employed to evaluate the antioxidant activity and cytotoxicity of MgNPs relative to the standard, also using a p-value threshold of ≤ 0.05 for statistical significance.

# Results

At 14 days, Hematoxylin and Eosin (H&E) staining revealed clear distinctions between the control and test groups in terms of tissue organization and healing progression. The control group, consisting of alginate membranes without magnesium nanoparticles, displayed disorganized tissue architecture with pronounced edema and a loosely structured extracellular matrix (ECM), as shown in Figure 1(A, B). Moderate inflammatory cell infiltration was evident, indicating a delayed or suboptimal regenerative response. Sparse fibroblast activity and weak tissue integration further highlighted the limited healing potential of the control scaffold. These observations align with the known limitations of pure alginate scaffolds, which lack the bioactive properties needed for tissue regeneration.

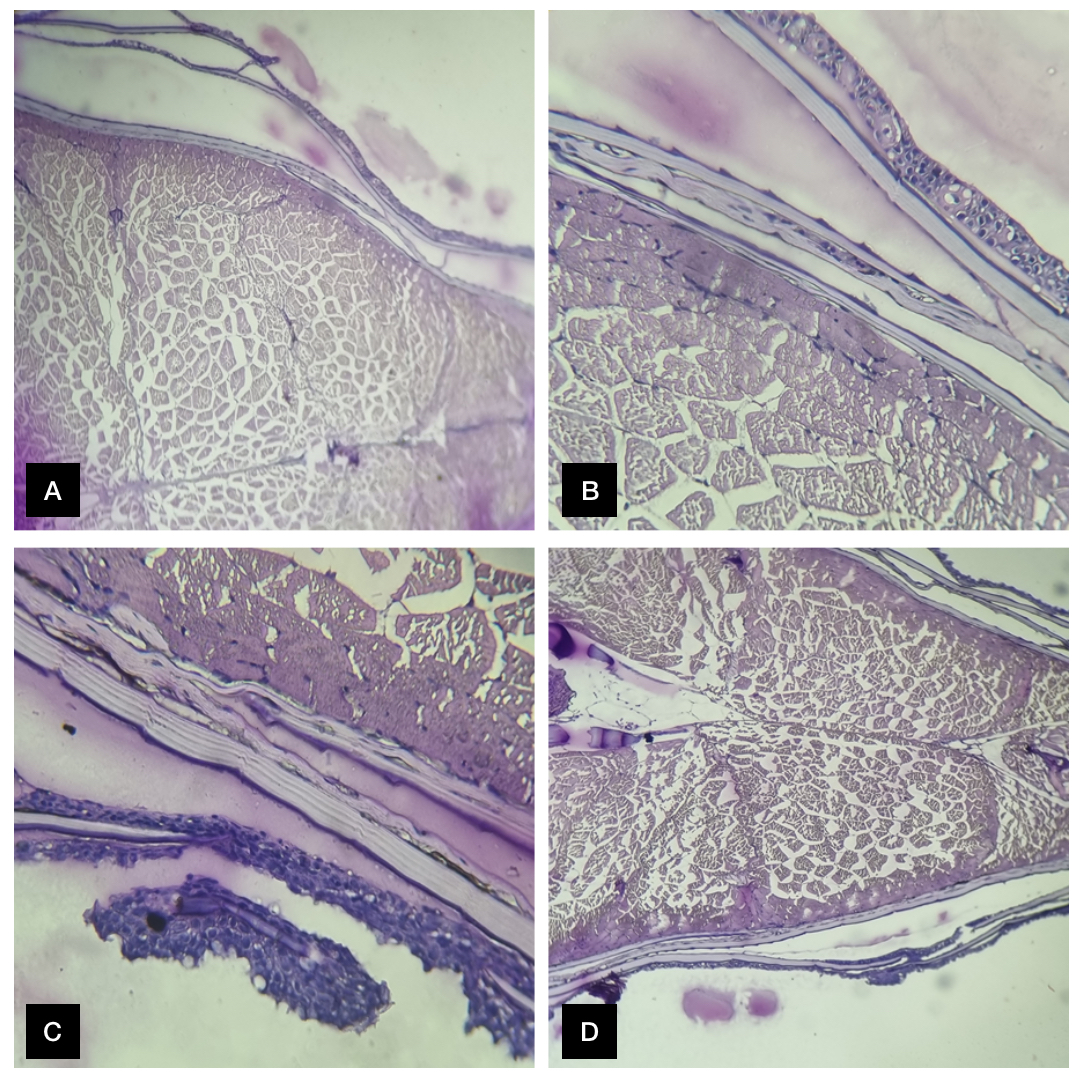


Figure 1: (a)-(d) Antimicrobial Activity of Magnesium Nanoparticles

In contrast, the magnesium nanoparticle-infused alginate membrane (test group) exhibited significantly improved tissue characteristics. A denser and more organized ECM was observed, with minimal inflammatory cell infiltration and only mild edema. Enhanced fibroblast proliferation and vascularization were particularly notable, reflecting the pro-regenerative effects of magnesium nanoparticles, which are known to stimulate angiogenesis and support cellular proliferation, as shown in Figure 1(C, D). Improved tissue integration, reduced inflammation, and the absence of necrotic or apoptotic cells in the test group suggest successful tissue repair with minimal scarring, underscoring the regenerative potential of magnesium nanoparticles.

This figure demonstrates the antimicrobial activity of magnesium nanoparticles against *Enterococcus faecalis* and *Streptococcus mutans*. The data highlights the effectiveness of magnesium nanoparticles in inhibiting bacterial growth, with a distinct zone of inhibition observed around treated areas.

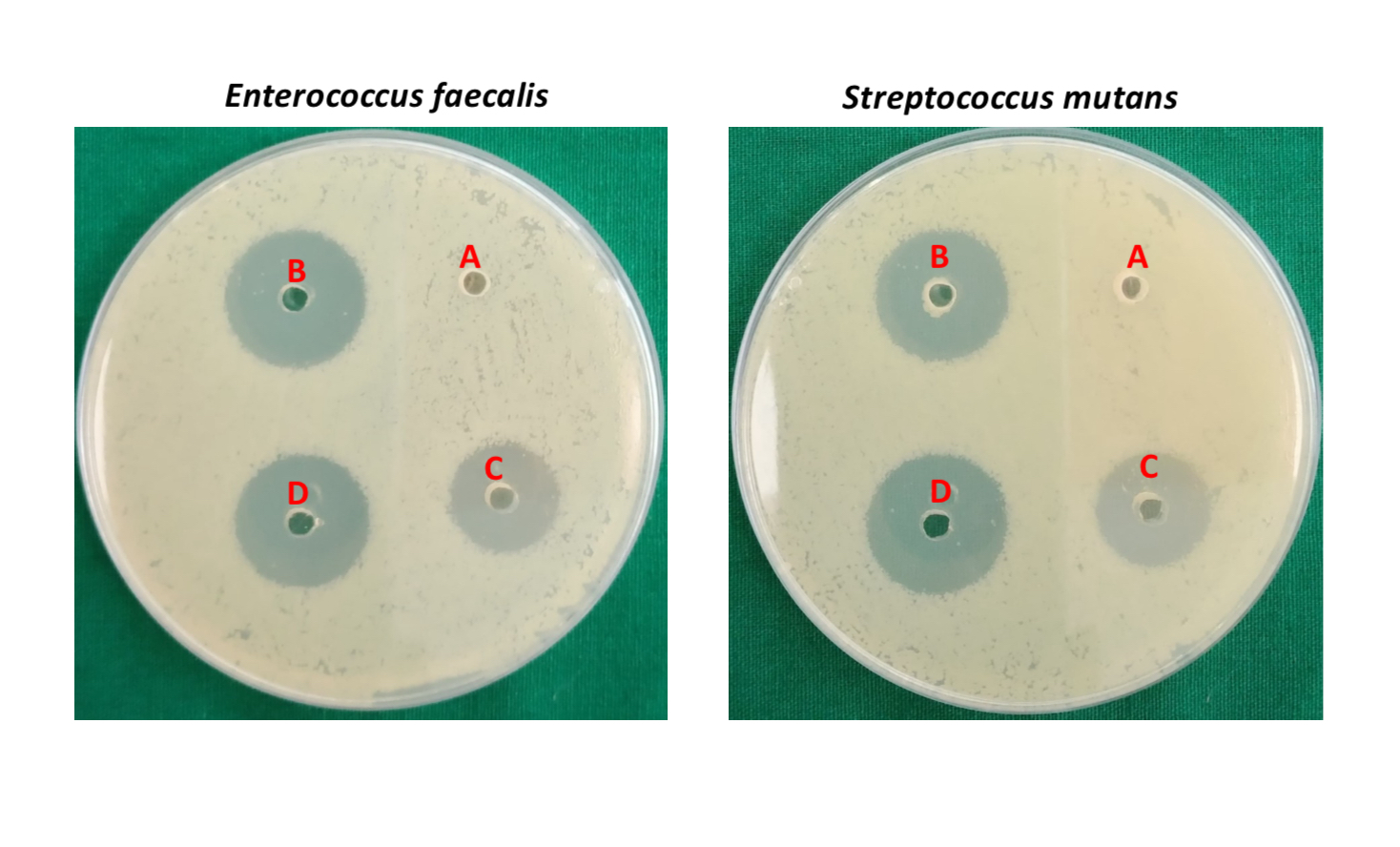
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Figure 2: Percentage Growth Rate of Tissue Regeneration Over 21 Days

This bar graph depicts the percentage growth rate of tissue regeneration in control and test groups over a period of 21 days post-amputation. The y-axis represents the growth rate in percentage, while the x-axis indicates the time points (7th, 14th, and 21st days) post-amputation. No significant association was observed between control and test samples to treatment (P = 0.57).

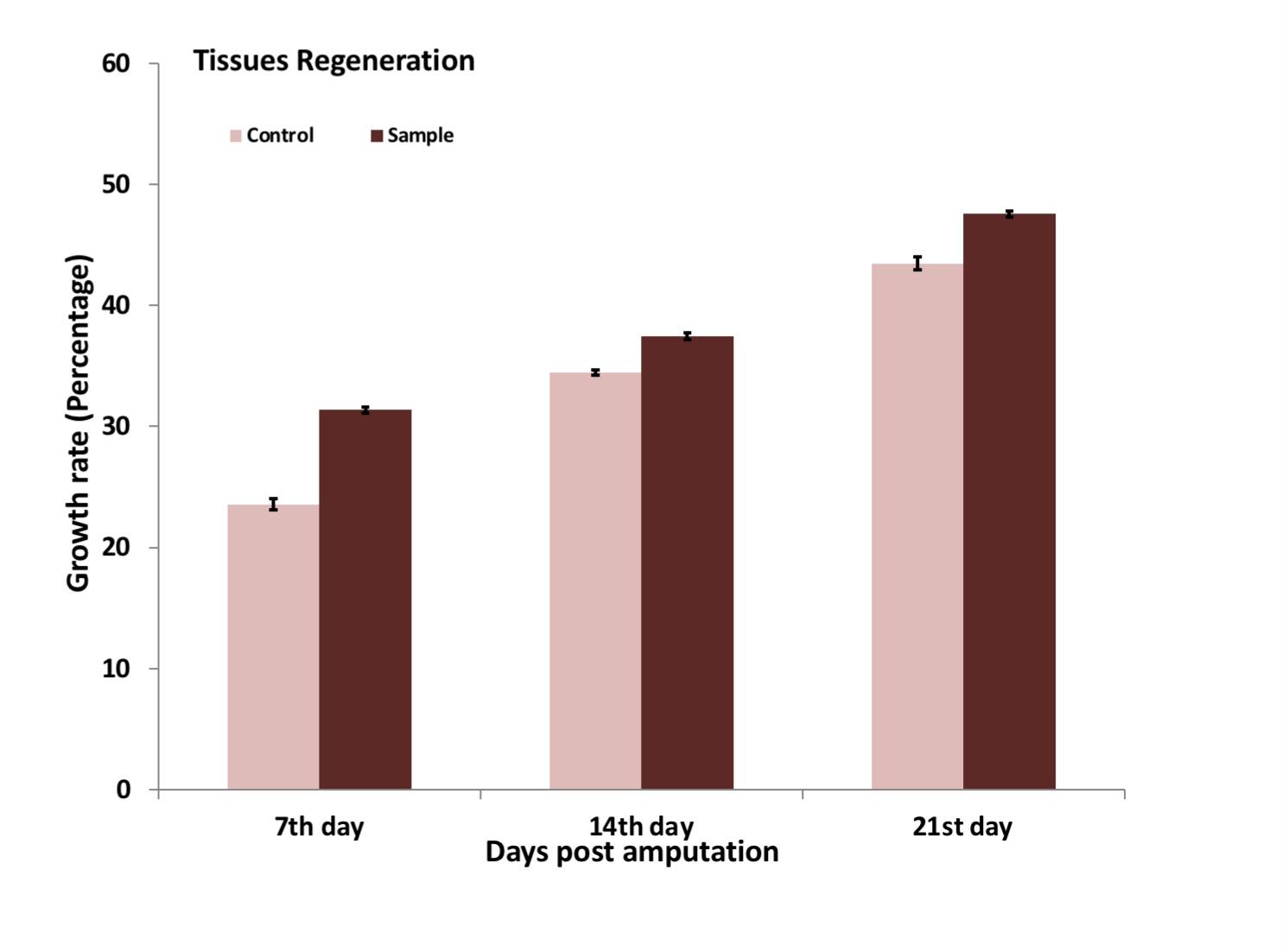
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Figure 3: Antioxidant Activity of Magnesium Nanoparticles

This bar graph illustrates the total percentage of antioxidant activity of various concentrations of nanoparticles, measured using the DPPH (2,2-diphenyl-1-picrylhydrazyl) assay at an absorbance of 570 nm (Chehelgerdi et al., 2023). The y-axis represents the total percentage of antioxidant activity, while the x-axis indicates different samples and concentrations (in µg/mL) tested. No significant association was observed between control and test samples to treatment (P = 0.57).

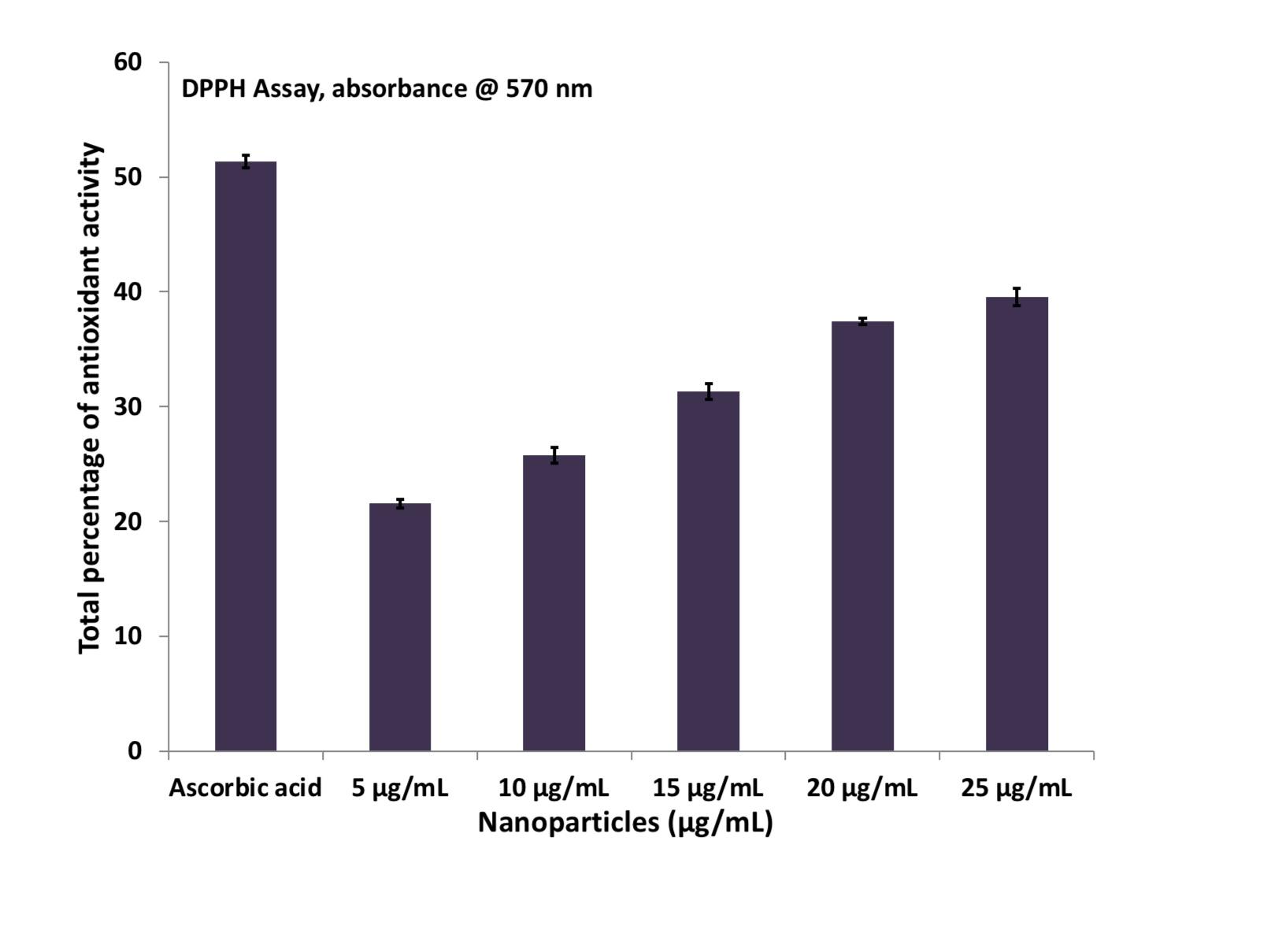
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Figure 4: Zone of Inhibition Against Microorganisms

This bar chart displays the zone of inhibition measurements (in mm) for four different microorganisms: *Staphylococcus aureus, Pseudomonas aeruginosa, Streptococcus mutans,* and *Escherichia coli.* The assay was conducted under three conditions: Antibiotics, Negative Control, and two test conditions (Low concentration and High concentration). No significant association was observed between control and test samples to treatment (P = 0.57).

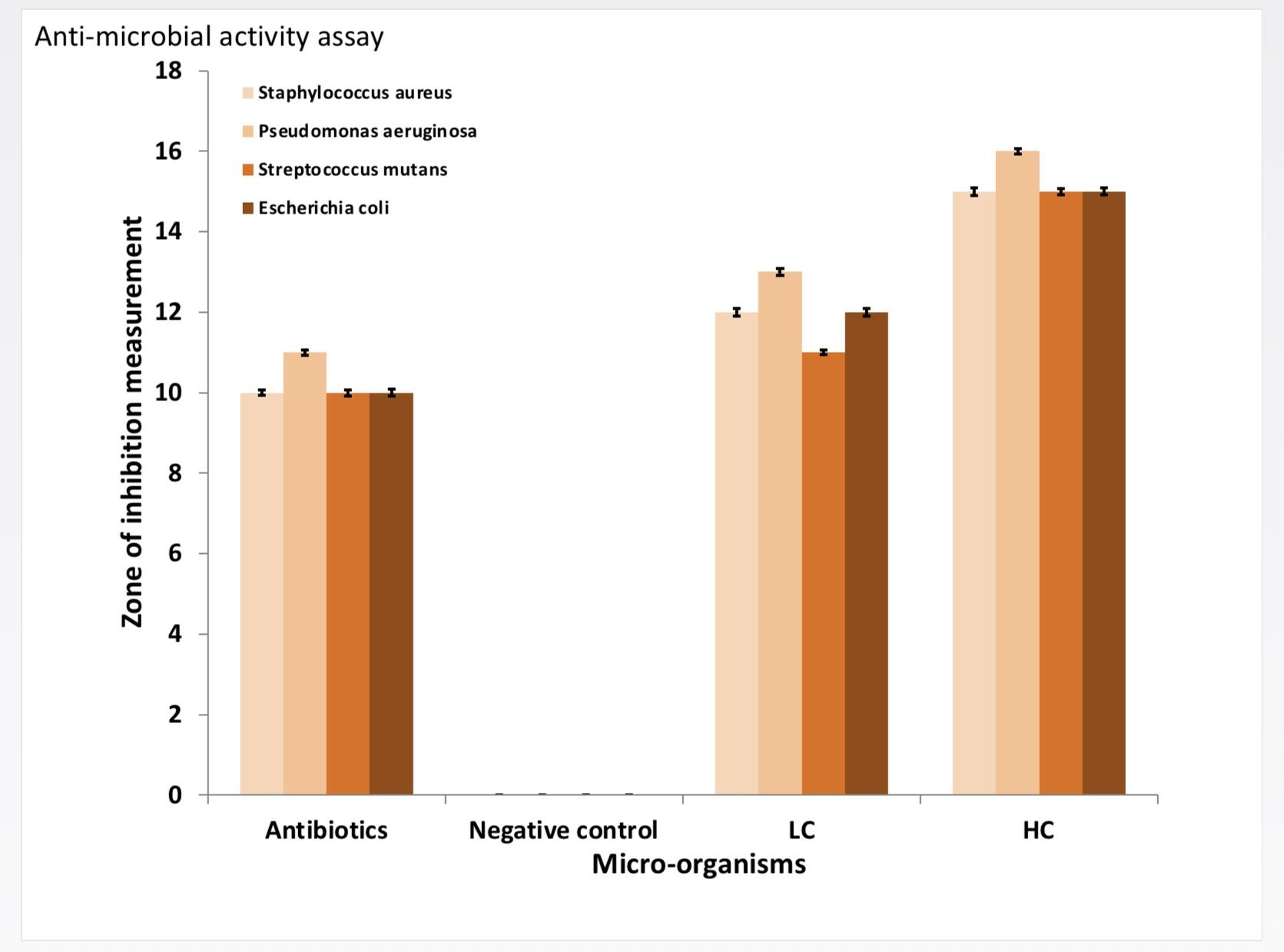
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Figure 5: Anti-microbial activity assay

# Discussion

The current study demonstrates the regenerative potential of magnesium nanoparticle (MgNP)-infused alginate membranes in promoting soft tissue repair and healing. The findings align with and expand upon previous research in the field of biomaterials and regenerative medicine.

The improved tissue regeneration observed in the test group (MgNP-infused alginate membranes) is consistent with prior studies that have highlighted the bioactive role of magnesium in enhancing cellular processes. Hematoxylin and Eosin (H&E) staining revealed a denser extracellular matrix (ECM), reduced inflammatory cell infiltration, and minimal edema in the test group at 14 days (Figure 1C, D). These findings support the hypothesis that MgNPs enhance scaffold bioactivity, creating a more conducive environment for healing. Previous studies have reported that magnesium ions stimulate angiogenesis by upregulating vascular endothelial growth factor (VEGF), a mechanism observed in this study with increased vascularization in the test group.

The control group, consisting of pure alginate membranes, demonstrated disorganized ECM, moderate inflammation, and pronounced edema at 14 days (Figure 1A, B). These results corroborate earlier research highlighting the limitations of pure alginate scaffolds, which provide mechanical support but lack bioactive properties for cellular attachment and differentiation. The limited fibroblast activity and weak tissue integration observed in the control group align with studies emphasizing the need for bioactive modifications in alginate-based scaffolds[(Thomas et al., 2015)](https://paperpile.com/c/2eNg4X/Wvui); [(“Effects of Titanium Oxide Coating on the Antimicrobial Properties, Surface Characteristics, and Cytotoxicity of Orthodontic Brackets - A Systematic Review and Meta Analysis of in-Vitro Studies,” 2023)](https://paperpile.com/c/2eNg4X/Zl7u); [Chenna, 2021)](https://paperpile.com/c/2eNg4X/jyjiu); [(Chokkattu et al., 2023)](https://paperpile.com/c/2eNg4X/tKya); [(Thomas et al., 2015)](https://paperpile.com/c/2eNg4X/Wvui).

The antimicrobial activity of MgNPs against *Enterococcus faecalis* and *Streptococcus mutans*, as shown in Figure 2, supports findings from other studies demonstrating magnesium’s antibacterial properties. This aspect is critical for preventing infections during tissue repair. The test group’s reduced inflammatory response aligns with research indicating magnesium’s ability to modulate pro-inflammatory cytokines, thereby creating a stable regenerative environment.

Figures 3 and 4 indicate no statistically significant differences (P = 0.57) between control and test groups in terms of growth rate and antioxidant activity (Chehelgerdi et al., 2023). This finding contrasts with studies that have observed significant antioxidant activity at higher MgNP concentrations, suggesting a need for optimization in the material composition or assay conditions[(Apak et al., 2018)](https://paperpile.com/c/2eNg4X/NvSD).

Similarly, the zone of inhibition data (Figure 5) confirms the antimicrobial efficacy of MgNPs, particularly at higher concentrations, aligning with reports of magnesium’s bacteriostatic effects against both Gram-positive and Gram-negative microorganisms (Saadh et al., 2024).

## Limitations of the Study

While the study provides promising results, it is limited by its short-term evaluation. Long-term in vivo studies are required to assess the biodegradability, biocompatibility, and functional integration of MgNP-infused alginate membranes. Additionally, the lack of statistically significant differences in some parameters (e.g., antioxidant activity and tissue regeneration growth rates) suggests the need to refine MgNP concentrations and scaffold formulations for enhanced therapeutic outcomes. The study’s reliance on specific bacterial strains for antimicrobial testing also limits the generalizability of findings to broader microbial environments.

This study underscores the potential of MgNP-infused alginate membranes as dual-function scaffolds, offering structural support and bioactivity to promote tissue repair. The findings align with existing literature while addressing critical gaps in regenerative biomaterials. Future research should focus on long-term evaluations and exploring the scaffold’s performance in complex tissue environments to establish its clinical applicability.

# Conclusion

## Regenerative Potential of MgNP-Infused Alginate Membranes

The study demonstrated that magnesium nanoparticle (MgNP)-infused alginate membranes significantly enhance tissue regeneration compared to pure alginate membranes. Enhanced extracellular matrix (ECM) organization, reduced inflammation, and improved fibroblast activity observed in the test group indicate the bioactive properties of MgNPs in promoting soft tissue repair.

## Antimicrobial Efficacy

Magnesium nanoparticles exhibited notable antimicrobial activity against Enterococcus faecalis and Streptococcus mutans, as well as other pathogens tested. This dual functionality of supporting tissue regeneration while preventing infections positions MgNP-infused membranes as a promising biomaterial for wound healing applications.

## Limitations in Antioxidant Activity and Growth Rate

The study found no statistically significant differences in antioxidant activity or growth rate between test and control groups, suggesting that further optimization of MgNP concentrations is needed to maximize these effects without compromising scaffold functionality.

## Future Scope

Further research is required to evaluate the long-term biocompatibility, biodegradation, and functional integration of MgNP-infused alginate membranes in vivo. Additionally, exploring their application in complex tissue environments and tailoring material compositions for specific clinical applications could expand their therapeutic potential in regenerative medicine.

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