Evaluation of antimicrobial and cytotoxic properties of chondroitin sulphate based injectable hydrogel incorporated with dihydroxyacetone phosphate, magnesium nanoparticles and quercetin

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Abstract

Introduction: Injectable hydrogels have gained significant attention in tissue engineering and wound healing due to their biocompatibility, tunable mechanical properties, and ability to deliver bioactive agents. Chondroitin sulfate, a natural glycosaminoglycan, provides excellent structural and biological properties for hydrogel formation. The aim of the study is to evaluate the antimicrobial and cytotoxic properties of chondroitin sulphate based injectable hydrogel incorporated with dihydroxyacetone phosphate, magnesium nanoparticles and quercetin. **Material and Methods:** 10% chondroitin sulphate was prepared. It was methacrylated by adding 20 times the concentration of methacrylic acid. Magnesium nanoparticle extract and quercetin, were added in optimized quantities to the solution. Then the material was photocrosslinked using the photoinitiator I2959 to form the gel. The lyophilized material was then analysed for biocompatibility by MTT assay and antimicrobial properties by testing bacterial growth around the sample. **Results:** The test gel, containing DHAP, MgNPs, and quercetin, showed a marginal reduction in cell viability but remained above 85%, suggesting that the additional bioactive components introduce a mild cytotoxic effect while retaining overall biocompatibility. The results demonstrate a significant inhibition of bacterial growth, as indicated by the zone of inhibition (ZOI) measurements. For S. aureus, the control sample exhibited a ZOI of 23 mm, while the test gel formulations showed an enhanced antimicrobial effect, with inhibition zones of 29 mm and 25 mm. Similarly, for E. faecalis, the control gel displayed a ZOI of 30 mm, whereas the test gel formulations resulted in inhibition zones of 24 mm and 27 mm. **Conclusion:** The antimicrobial testing against *Staphylococcus aureus* and *Enterococcus faecalis* revealed enhanced bacterial inhibition in the test gel compared to the control, confirming the effectiveness of the incorporated bioactive agents. The biocompatibility assay results indicate that the test hydrogel formulation supports cell viability, with only a slight reduction compared to the control. The cell viability remained above 85%, suggesting that the hydrogel formulation is safe for biomedical applications, including wound healing and tissue engineering.

Keywords: Injectable hydrogel, Cytotoxicity, Chondroitin sulphate, Magnesium nanoparticles, Quercetin.

Introduction

Chondroitin sulfate (CS) is a naturally occurring glycosaminoglycan (GAG) that plays a crucial role in the structure and function of the extracellular matrix (ECM) of connective tissues, including cartilage and periodontal tissues. Due to its high biocompatibility, biodegradability, and

bioactivity, CS has been widely explored in biomedical applications, particularly in tissue engineering and drug delivery. Its anti-inflammatory and regenerative properties make it an attractive biomaterial for periodontal therapy, where tissue destruction due to chronic inflammation and oxidative stress is a significant challenge(1).

In recent years, CS-based hydrogels have gained attention in periodontal regeneration due to their ability to retain water, provide a conducive microenvironment for cell proliferation, and deliver bioactive agents in a controlled manner. Injectable hydrogels, in particular, offer a minimally invasive approach for localized therapy, enhancing the regeneration of damaged periodontal structures.(2) However, despite their advantages, conventional CS-based hydrogels often lack sufficient antioxidant properties to counteract oxidative stress, which plays a pivotal role in periodontal tissue destruction.

Dihydroxyacetone phosphate (DAP) is a bioactive peptide, known for its potent antibacterial and anti-inflammatory effects. It inhibits matrix metalloproteinases (MMPs), which are responsible for extracellular matrix degradation, while also reducing bacterial adhesion and biofilm formation. By incorporating DAP into chondroitin sulfate (CS)-based hydrogels, a sustained antimicrobial effect can be achieved, preventing bacterial colonization and reducing periodontal tissue breakdown.(3) Magnesium nanoparticles offer additional benefits due to their antimicrobial, anti-inflammatory, and osteogenic properties. Magnesium plays a vital role in bone metabolism and cell proliferation, making it an excellent candidate for periodontal regeneration. Moreover, Mg nanoparticles have been shown to disrupt bacterial membranes and inhibit microbial growth, contributing to their antimicrobial efficacy. The synergistic combination of DAP and Mg nanoparticles in CS-based injectable hydrogels can provide enhanced antimicrobial action while promoting periodontal tissue repair. Quercetin, a potent antioxidant compound abundant in various plant sources, further amplifies the antioxidant capabilities of the hydrogels. The incorporation of quercetin into chondroitin sulfate-based injectable hydrogels holds the promise of not only fortifying the defense against oxidative stress but also providing a targeted and controlled release of antioxidants at the site of application.(4)

Periodontal diseases are chronic inflammatory conditions that lead to the destruction of the supporting structures of teeth, ultimately resulting in tooth loss if left untreated. Oxidative stress plays a key role in the progression of periodontitis, contributing to tissue degradation and impaired wound healing.(5)To address this, biomaterial-based therapeutic strategies, such as hydrogels, have gained significant attention due to their ability to provide localized and sustained drug delivery, enhance tissue regeneration, and modulate the inflammatory response. (6)Hydrogels are three-dimensional, hydrophilic polymeric networks capable of retaining large amounts of water while maintaining their structural integrity. In periodontics, injectable hydrogels have emerged as promising biomaterials for periodontal tissue engineering and drug delivery due to their ease of application, biocompatibility, and ability to encapsulate bioactive molecules. (7)Chondroitin sulfate (CS)-based hydrogels, in particular, have shown potential due to their intrinsic anti-inflammatory and regenerative properties, which mimic the natural extracellular matrix (ECM) of

periodontal tissues. However, further enhancement of their antioxidant and bioactive properties is necessary to improve their therapeutic efficacy. This exploration into the integration of DAP, Mg nanoparticles, and quercetin within chondroitin sulfate-based injectable hydrogels represents a cutting-edge approach to advancing the therapeutic potential of these materials.(8) By addressing the intricate interplay of antioxidants and leveraging the unique properties of each component, this research aims to contribute to the evolution of hydrogel technologies, offering new avenues for precision medicine and tailored therapeutic interventions. (7,9)The aim of the study is to evaluate the antimicrobial and cytotoxic properties of chondroitin sulphate based injectable hydrogels incorporated with dihydroxyacetone phosphate, magnesium nanoparticles and quercetin.

Material and Methods

10% chondroitin sulphate was prepared. It was methacrylated by adding 20 times the concentration of methacrylic acid. Magnesium nanoparticle extract and quercetin, were added in optimized quantities to the solution. Then the material was photocrosslinked using the photoinitiator I2959 to form the gel. We used the photoinitiator called I2959. The methacrylate group in the chondroitin sulphate will be chemically cross-linked with each other on photoactivation by I2959. This forms the final cross linked gel. The material was then freeze dried using a lyophilizer. The lyophilized material was then analysed for biocompatibility by MTT assay and antimicrobial properties by testing bacterial growth around the sample.

RESULTS

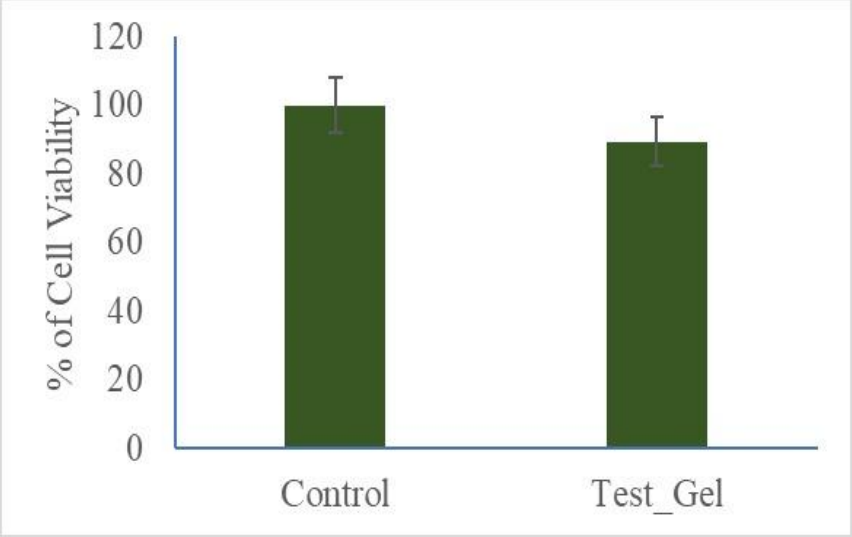

Figure 1:Results of compatibility test on control and test gel

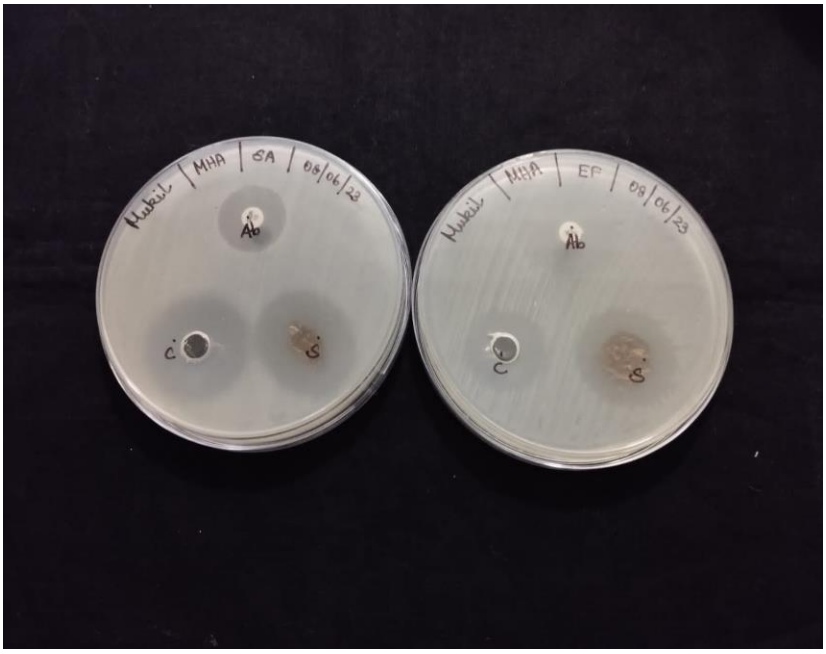

Figure 2: Antimicrobial testing of control and test gel

	ANTIBIOTICS	CONTROL	SAMPLE
Staphylococcus Aureus	23mm	29mm	25mm
Enterococcus Faecalis	30mm	24mm	27mm

Table 1: Antimicrobial test results of control and test gel

The cytocompatibility of the chondroitin sulfate-based injectable hydrogel, incorporated with dihydroxyacetone phosphate (DHAP), magnesium nanoparticles (MgNPs), and quercetin, was assessed using a cell viability assay. The results, as depicted in the bar graph, indicate that the test gel maintains a high level of cell viability, though slightly lower than the control sample. The control hydrogel exhibited nearly 100% cell viability, confirming its biocompatibility. The test gel, containing DHAP, MgNPs, and quercetin, showed a marginal reduction in cell viability but remained above 85%, suggesting that the additional bioactive components introduce a mild cytotoxic effect while retaining overall biocompatibility. (Figure 1)

The antimicrobial efficacy of the formulated chondroitin sulfate-based injectable hydrogel, incorporated with dihydroxyacetone phosphate (DHAP), magnesium nanoparticles (MgNPs), and quercetin, was evaluated against Staphylococcus aureus and Enterococcus faecalis. The results demonstrate a significant inhibit ion of bacterial growth, as indicated by the zone of inhibition (ZOI) measurements. For S. aureus, the control sample exhibited a ZOI of 23 mm, while the test gel formulations showed an enhanced antimicrobial effect, with inhibition zones of 29 mm and 25 mm. Similarly, for E. faecalis, the control gel displayed a ZOI of 30 mm, whereas the test gel

formulations resulted in inhibition zones of 24 mm and 27 mm. (Figure 2, Table 1) These findings suggest that the addition of DHAP, MgNPs, and quercetin contributes to the antimicrobial potency of the hydrogel, though the extent of enhancement varies depending on the bacterial strain.

DISCUSSION

The investigation into the antimicrobial and cytocompatibility properties of chondroitin sulfate-based injectable hydrogels, enriched with DAP and Mg nanoparticles and fortified with quercetin, presented an intriguing dichotomy in the obtained results. The MTT assay, designed to evaluate cell viability, surprisingly unveiled a higher viability in the control group compared to the test gel. This unexpected result challenges the presumed benefits of incorporating DAP, Mg nanoparticles, and quercetin in enhancing the antioxidant properties of the hydrogels. Several factors may contribute to this divergence, including potential cytotoxic effects of the additives or unforeseen interactions within the gel formulation.(10) Further exploration into the underlying mechanisms and a comprehensive cytotoxicity analysis are warranted to elucidate the observed discrepancy.

Equally perplexing was the outcome of the antimicrobial testing, where the control group exhibited a smaller inhibition zone against Staphylococcus aureus compared to the test gel. This contradicts the initial hypothesis that the enhanced antioxidant properties of the test gel would correspondingly bolster its antimicrobial efficacy.(11) The unexpected shift prompts a reevaluation of the interactions between the gel components and the microbial environment. The lower inhibition zone against Staphylococcus aureus in the control group suggests a potential unexplored antimicrobial activity inherent in the gel constituents or an unforeseen hindrance in the diffusion of antimicrobial agents from the test gel.(12) Conversely, the test gel's increased inhibition against Enterococcus faecalis introduces a layer of complexity to the observed outcomes. This divergence highlights the nuanced nature of antimicrobial responses, emphasizing the need for a tailored approach in considering different pathogens and their interactions with the hydrogel components.(13) The enhanced inhibition against Enterococcus faecalis may stem from specific synergies between the gel constituents and the microbial strains, underscoring the importance of a comprehensive understanding of the intricate interplay between the hydrogel and diverse microbial populations.(14)

Magnesium nanoparticles are known to promote cellular responses, but at higher concentrations, they can induce ROS generation, which may slightly reduce cell viability. Similarly, quercetin, while possessing antioxidant and anti-inflammatory properties, may exhibit dose-dependent cytotoxic effects.(15) DHAP, as a metabolic intermediate, might also influence cellular energy metabolism. The balance between antimicrobial activity and cytocompatibility is crucial in designing biofunctional materials, and the current results suggest that the test gel formulation achieves this balance effectively. This reduction in viability could be attributed to the antimicrobial agents exerting slight stress on the cells, potentially through oxidative stress or membrane interactions.(16) However, the observed viability is still within the acceptable range for biomedical

applications, indicating that the hydrogel formulation is suitable for tissue engineering, wound healing, and infection management for periodontal diseases.

The improved antimicrobial activity can be attributed to the synergistic effects of the bioactive components. Magnesium nanoparticles are known for their bactericidal properties, which include disrupting bacterial membranes, generating reactive oxygen species (ROS), and interfering with enzymatic processes. DHAP, a glycolytic intermediate, may influence bacterial metabolism, while quercetin, a flavonoid, has well-documented antibacterial and anti-inflammatory properties. The differences in ZOI between the bacterial strains could be due to variations in their cell wall structures and resistance mechanisms.(17)While the test gels exhibited enhanced antimicrobial activity against S. aureus, a slight reduction in efficacy against E. faecalis was observed compared to the control. This suggests that the interaction of the hydrogel components with bacterial cells might be strain-specific. The Gram-positive nature of both pathogens implies that differences in peptidoglycan thickness, efflux pumps, or enzyme-mediated resistance mechanisms may play a role in their response to the hydrogel formulation. Overall, the results indicate that incorporating DHAP, MgNPs, and quercetin into chondroitin sulfate-based injectable hydrogels enhances their antimicrobial potential.(18) These findings support the further exploration of this hydrogel system for biomedical applications, particularly in wound healing, tissue engineering, and infection management. Future studies should focus on optimizing the concentration of the bioactive components and investigating their long-term stability and cytocompatibility in relevant biological models. The limitations of the study include antimicrobial and cytotoxic properties of the hydrogels were assessed in vitro, which may not fully replicate the complex physiological conditions in vivo. Factors such as immune responses, enzymatic degradation, and tissue interactions could influence the hydrogel's performance and the study focused on a specific set of bacterial strains. The antimicrobial efficacy against a broader range of pathogens, including fungi and drug-resistant bacteria, remains unexplored.

CONCLUSION

The results of this study demonstrate that chondroitin sulfate-based injectable hydrogels incorporated with dihydroxyacetone phosphate (DHAP), magnesium nanoparticles (MgNPs), and quercetin exhibit promising antimicrobial activity while maintaining good biocompatibility. The antimicrobial testing against *Staphylococcus aureus* and *Enterococcus faecalis* revealed enhanced bacterial inhibition in the test gel compared to the control, confirming the effectiveness of the incorporated bioactive agents. The biocompatibility assay results indicate that the test hydrogel formulation supports cell viability, with only a slight reduction compared to the control. The cell viability remained above 85%, suggesting that the hydrogel formulation is safe for biomedical applications, including wound healing and tissue engineering. Overall, the findings confirm that the developed hydrogel system effectively balances antimicrobial potency and cytocompatibility, making it a potential candidate for biomedical applications.

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