Formulation and Evaluation of Novel Chondroitin Sulphate Based Hydrogel Containing Green- Synthesised Magnesium Oxide Nanoparticles for Treatment of Osteomyelitis- An in Vitro Study

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KEYWORDS
osteomyelitis, magnesium oxide nanoparticle, chondroitin sulphate, Musculoskeletal infections, Treatment.

ABSTRACT:
Musculoskeletal infections, particularly osteomyelitis (OM), pose a significant challenge for patients, healthcare providers, and the healthcare system overall.
Osteomyelitis of the jaw is characterized by exposed bone in the mouth that does not heal despite adequate treatment. It involves inflammation of the bone cortex and marrow, typically arising in the jaw following a chronic infection.
Given the persistent nature of osteomyelitis (OM), along with its expensive and prolonged treatment process and high rates of disability, there is an urgent need for better treatment approaches. While some therapies, including vaccines and monoclonal antibodies (mAbs) have been explored, the standard treatment for OM continues to rely on identifying bacterial species, using appropriate antibiotics, removing implants, performing extensive debridement, and administering systemic antibiotics for 4 to 6 weeks. However, issues like inadequate local antibiotic concentrations due to local blood supply and various bacterial resistance mechanisms often limit the effectiveness of systemic treatments. This approach allows for higher concentrations of drugs to be delivered directly to the affected area while minimizing side effects. To lower the reinfection rate in chronic osteomyelitis treatment, the drug delivery system should be biodegradable, eliminating the need for material removal after therapy.
Hence the aim of this study was to formulate and evaluate a novel chondroitin sulphate based hydrogel containing magnesium oxide nanoparticles for treatment of osteomyelitis.

1. Introduction

Musculoskeletal infections, particularly osteomyelitis (OM), pose a significant challenge for patients, healthcare providers, and the healthcare system overall (1).

Osteomyelitis of the jaw is characterized by exposed bone in the mouth that does not heal despite adequate treatment (2). It involves inflammation of the bone cortex and marrow, typically arising in the jaw following a chronic infection (2,3).

Given the persistent nature of osteomyelitis (OM), along with its expensive and prolonged treatment process and high rates of disability, there is an urgent need for better treatment approaches. While some therapies, including vaccines and monoclonal antibodies (mAbs) have been explored (4–6) (7)(8), the standard treatment for OM continues to rely on identifying bacterial species, using appropriate antibiotics, removing implants, performing extensive debridement, and administering systemic antibiotics for 4 to 6 weeks. However, issues like inadequate local antibiotic concentrations due to local blood supply and various bacterial resistance mechanisms often limit the effectiveness of systemic treatments.

Hydrogels, especially thermosensitive ones, are regarded as promising options for localized therapy due to their ability to be molded, their biodegradability, and their compositional and structural similarities to tissues (9)(10). Chondroitin Sulfate (CS) is frequently utilized as a material for tissue regeneration because of its antibacterial properties, pain relief benefits, and ability to promote hemostasis. When combined with antibiotics, CS can enhance their antibacterial effects and combat bacterial resistance (11). Numerous research teams have developed CS-based hydrogels to serve as tissue scaffolds for antibiotic delivery and to support tissue repair, including cardiac repair and bone regeneration (12) (13).

Nanosystems have recently emerged as alternatives to antibiotics for treating bacterial infections (Qi et al. 2017). Nanoparticle (NP)-hydrogel drug delivery systems have gained significant interest for their ability to minimize burst release, allowing for sustained and continuous drug release over extended periods in vivo (14).

Literature indicates that various agents, including antioxidants, bisphosphonates, hormones, and other bone regenerative materials, play a crucial role in enhancing bone regeneration (15). In both in vitro and in vivo studies, the plant-derived polyphenol quercetin has demonstrated a broad range of biological activities, such as anticarcinogenic, anti-inflammatory, and antiviral effects, along with the ability to reduce lipid peroxidation, platelet aggregation, and capillary permeability (16).

Pang et al. investigated the impact of quercetin on the differentiation and proliferation of bone marrow mesenchymal stem cells in mice. Their findings indicated that quercetin significantly enhanced both the proliferation and osteogenic differentiation of these stem cells (17).

Local therapy is quickly emerging as a promising method for treating various conditions, including tumors and osteomyelitis (18). This approach allows for higher concentrations of drugs to be delivered directly to the affected area while minimizing side effects. To lower the reinfection rate in chronic osteomyelitis treatment, the drug delivery system should be biodegradable, eliminating the need for material removal after therapy (19).

Hence the aim of this study was to formulate and evaluate a novel chondroitin sulphate based hydrogel containing magnesium oxide nanoparticles for treatment of osteomyelitis.

2. Methodology

Materials

Chondroitin sulfate with a viscometric molar mass (*Mv*) of 22 kDa, the photoinitiator Irgacure 2959 [2-Hydroxy-4′-(2-hydroxyethoxy)-2-methylpropiophenone], and Quercetin were purchased in required quantities from Sigma Aldrich Lab, Chennai.

Preparation of Double Antibiotic Paste

The formulation of double antibiotic paste (DAP) required equalized quantities (250 mg tablets) of metronidazole and ciprofloxacin which were then mixed with distilled water (1 g/mL).

Green Synthesis of Magnesium Oxide Nanoparticles

In this study green synthesis of MgO2 nanoparticles was done using beetroot extracts. Two small sized beetroots were washed with clean drinking water to eliminate dirt and other impurities present on its surface. They were diced into small pieces were dried on a plate under room temperature. The dried pieces were then powdered using a grinder mixer. 20 gms of this beetroot powder was added to a beaker of 100 ml distilled water which was then boiled and filtered to result in the production of beetroot extract.

0.0169 grams of 1 millimole magnesium nitrate was added to 50ml of distilled. To this mixture, 50ml of the prepared beetroot extract was added. This mixture of beetroot extract and Mg nitrate was placed in an orbital shaker for 32 hours.

The mixture was retrieved from the orbital shaker and equal quantities were poured into six centrifugation tubes. The tubes were placed in the centrifugation machine and centrifuged at 10,000 rpm for 20 minutes. From each tube, the supernatant liquid was separated and disposed off. The residual contents settled at the bottom of each tube (pellet solution) was transferred to a single tube and then refrigerated for future use. The pellet solution was spread out on a petri dish, placed in the hot air oven for 8 hours at a temperature of 100 degrees Celsius. The dried solution obtained after retrieving the petri dish from the oven was scraped out to yield MgO2 NPs.

Preparation of Chondroitin Sulfate Hydrogel

10% CS was prepared by adding 10mg of CS in 100 ml distilled water. This solution was then methacrylated 20 times in concentration using methacrylic acid. Double antibiotic paste (DAP), Quercitin and MgO were added in previously optimized quantities. This mixture was then crosslinked using the photoinitiator Irgacure 2959 to result in the formation of a hydrogel.

Antibacterial assessment of CS Hydrogel

For assessing the antibacterial activity of the CS hydrogel , the agar well diffusion method using Mueller-Hinton agar (MHA) was employed. The microorganisms S.Mutans, S, Aureus, E. faecalis, and C. albicans were inoculated on 0.25 mL of molten MHA on petri plates. Wells of uniform size (6mm) were created on the hardened agar. The contents of the test material were carefully impregnated into discs measuring 6mm in diameter and then placed with precision on the inoculated agar at concentrations of 25,50, and 100 µL per disc. 500mg/ml of Amoxicillin was set as the positive control reference for microorganisms. A blank disc was employed as the negative control. The zone of inhibition was accurately measured to quantify the antimicrobial activity. The solutions were allowed to thoroughly permeate the agar at room temperature for 60 minutes and were then incubated for one whole night at 37◦C. These tests were repeated in duplicate and independently three times, with the zones of inhibition being measured from the plate bases.

Statistical Analysis

The zone of inhibition measurements were entered in a Microsoft Excel sheet and statistical analyses were carried out in SPSS software. In order to measure the difference in antimicrobial activity of the hydrogel against various microorganisms, the Kruskal Wallis test was utilized. The Friedman test was used to compare the antimicrobial activity at various concentrations of test material against the microorganisms.

3. Results

The mean zone of inhibition measurements for different pathogenic bacteria Strep. Mutans, Staph. Aureus, E. Faecalis, and C. albicans and control antibiotics (Cap. Amoxicillin 500 mg) are presented in Table 1, Figure 1, and Graph 1. The highest levels of antimicrobial activity was observed against S.Mutans at the concentration of 100µl, and the lowest values were observed at the concentration of 25µl for all microorganisms. The overall value differed significantly with the antibiotic control except for Streptococcus Mutans at 100 µl.

Table 1: Zone of inhibition produced by various concentrations of hydrogels

Test concentration of hydrogel	Zone of Inhibition in mm Mean +/- SD			
	S.Aureus	S.Mutans	E.Faecalis	C.Albicans
25µl	8.00 ± 1.00 mm	8.00 ± 0.50 mm	8.00 ± 1.10 mm	8.00 ± 1.20 mm
50 µl	14.00 ± 1.00 mm	18.00 ± 1.00 mm	14 ± 1.00 mm	9.00 ± 1.00 mm
100 µl	19.00 ± 1.00 mm	21.00 ± 1.50 mm	19.00 ± 1.50 mm	17.00 ± 1.50 mm
Antibiotic (Control)	44.00 ± 1.00 mm	24.00 ± 1.00mm	45.00 ± 1.00mm	12.00 ± 1.00mm
P Value	0.003	0.001	0.004	0.005

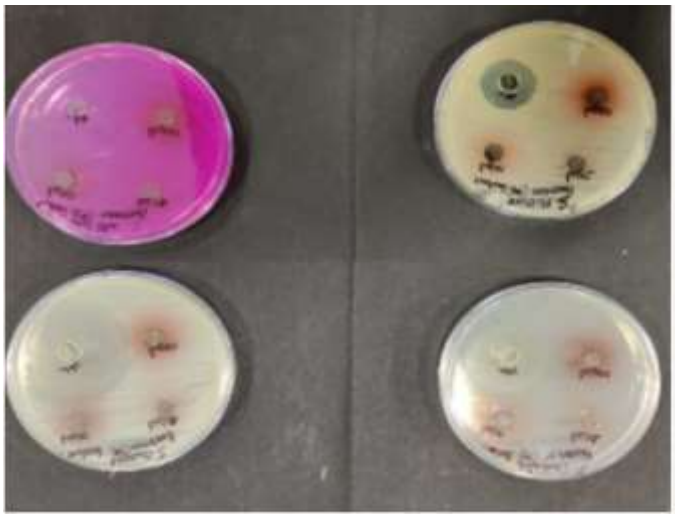
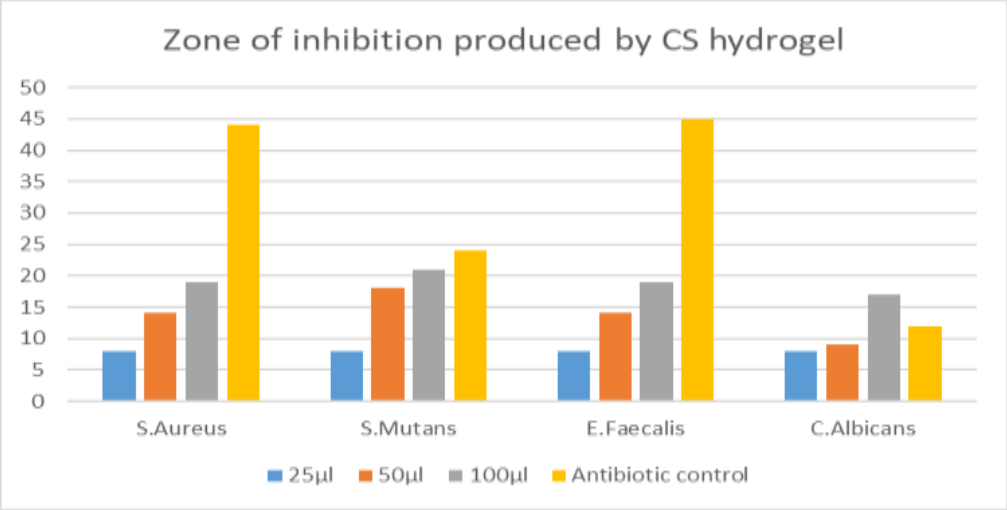


Figure 1: Zone of inhibition produced on culture medium against S. Mutans, S. Aureus, E.Faecalis, C.Albicans



Graph 1: Zone of inhibition produced by various concentrations of hydrogels

4. Discussion

Hydrogels are hydrophilic polymer networks that can absorb significant amounts of water, allowing them to swell and shrink in a way that enables controlled drug release. Their porous structure and compatibility with water make them ideal biocompatible carriers for drug delivery.

They have a wide range of applications in various biomedical fields, as they can be shaped into different physical forms, including nanoparticles, microparticles, slabs, films, and coatings. The United States is the largest producer of hydrogels and is anticipated to maintain this status for several more years.(20).

Hydrogels are innovative and sophisticated drug delivery systems designed to meet specific needs for targeted drug delivery and controlled release. They can be manipulated for drug release at the desired location using enzymatic, hydrolytic, or environmental stimuli (21).

However, there are disadvantages to using hydrogels. One major issue is that most drugs are hydrophobic, making them difficult to incorporate into the hydrophilic polymer core of the hydrogels. This is a challenge since many effective drugs for treating diseases are hydrophobic. Additionally, hydrogels often have weak tensile strength, which can lead to premature drug release before reaching the target site.

Jung et al. conducted an animal study to evaluate the antibacterial effects and bone healing capabilities of an in situ gelling alginate (ALG)/hyaluronic acid (HA) hydrogel that contained vancomycin (an antibiotic) and bone morphogenetic protein-2 (BMP-2, a growth factor) for treating osteomyelitis. The study showed that the ALG/HA hydrogel effectively inhibited the growth of Staphylococcus aureus at the osteomyelitis site and promoted bone regeneration without the need for additional bone grafts. (22).

Tao et al. developed a chitosan-based thermosensitive hydrogel to create a local drug delivery system using vancomycin nanoparticles (VCM-NPs) for osteomyelitis treatment. The VCM-NPs demonstrated high encapsulation efficiency and drug loading, with values of 60.1±2.1% and 24.1±0.84%, respectively. When incorporated into the chitosan gel, the VCM-NPs preserved their particle size and morphology. The hydrogel maintained its injectability and thermosensitivity, as confirmed by injectability tests and rheological measurements. The VCM-NPs/gel system provided sustained release of vancomycin over a period of 26 days (23).

Zhou et al. developed an injectable carboxymethyl chitosan hydrogel scaffold created through coordination bonds for antibacterial and osteogenic applications in osteomyelitis. They found that this hydrogel effectively regulated antibacterial responses and promoted osteogenesis in infected environments without the need for additional additives.((24).

In a study by Zhang et al., the effectiveness of vancomycin-loaded silk fibroin microspheres in an injectable hydrogel for treating chronic osteomyelitis was evaluated. The hydrogel demonstrated significant antibacterial activity against both Escherichia coli and Staphylococcus aureus, maintaining its antibacterial effect for 10

days. Injecting the vancomycin-loaded silk fibroin microspheres encapsulated in the hydrogel into the infected rat tibia reduced the bone infection and enhanced bone regeneration compared to other treatment groups (25).

Wei et al. created an antibacterial bone graft by immobilizing levofloxacin hydrochloride-loaded mesoporous silica microspheres onto the surface of a porous scaffold. Antibacterial tests showed that the material exhibited effective antibacterial activity against both gram-positive (Staphylococcus aureus) and gram-negative (Escherichia coli) bacteria. Additionally, biosafety assessments indicated that the material met the necessary biosafety standards (26-30).

Extensive research has been undertaken to address this issue through local drug delivery systems. These systems enhance tissue concentration of antibiotics, reduce toxicity, and improve efficacy. Non-biodegradable carriers loaded with antibiotics have been successfully utilized for this purpose (18).

5. Conclusion

Within the limitations of this study, it can be concluded that formulation of a hydrogel containing nanoparticles, antibiotics, and flavonoids can have a synergistic effect on the treatment of osteomyelitis. However further studies examining its efficacy in vivo need to be conducted.

References:

[1] Website.
[2] Reid IR. Osteonecrosis of the jaw: who gets it, and why? Bone. 2009 Jan;44(1):4–10.
[3] Yeoh SC, MacMahon S, Schifter M. Chronic suppurative osteomyelitis of the mandible: case report. Aust Dent J. 2005 Sep;50(3):200–3.
[4] Mouzopoulos G, Kanakaris NK, Kontakis G, Obakponovwe O, Townsend R, Giannoudis PV. Management of bone infections in adults: the surgeon's and microbiologist's perspectives. Injury. 2011 Dec;42 Suppl 5:S18–23.
[5] Rouha H, Badarau A, Visram ZC, Battles MB, Prinz B, Magyarics Z, et al. Five birds, one stone: neutralization of α-hemolysin and 4 bi-component leukocidins of Staphylococcus aureus with a single human monoclonal antibody. MAbs. 2015;7(1):243–54.
[6] Fowler VG Jr, Proctor RA. Where does a Staphylococcus aureus vaccine stand? Clin Microbiol Infect. 2014 May;20 Suppl 5(0 5):66–75.
[7] Thammavongsa V, Rauch S, Kim HK, Missiakas DM, Schneewind O. Protein A-neutralizing monoclonal antibody protects neonatal mice against Staphylococcus aureus. Vaccine. 2015 Jan 15;33(4):523–6.
[8] Varshney AK, Kuzmicheva GA, Lin J, Sunley KM, Bowling RA Jr, Kwan TY, et al. A natural human monoclonal antibody targeting Staphylococcus Protein A protects against Staphylococcus aureus bacteremia. PLoS One. 2018 Jan 24;13(1):e0190537.
[9] Cheng J, Amin D, Latona J, Heber-Katz E, Messersmith PB. Supramolecular Polymer Hydrogels for Drug-Induced Tissue Regeneration. ACS Nano. 2019 May 28;13(5):5493–501.
[10] Annamalai RT, Hong X, Schott NG, Tiruchinapally G, Levi B, Stegemann JP. Injectable osteogenic microtissues containing mesenchymal stromal cells conformally fill and repair critical-size defects. Biomaterials. 2019 Jul;208:32–44.
[11] Delezuk JAM, Ramírez-Herrera DE, Esteban-Fernández de Ávila B, Wang J. Chitosan-based water-propelled micromotors with strong antibacterial activity. Nanoscale. 2017 Feb 9;9(6):2195–200.
[12] Tripodo G, Trapani A, Rosato A, Di Franco C, Tamma R, Trapani G, et al. Hydrogels for biomedical applications from glycol chitosan and PEG diglycidyl ether exhibit pro-angiogenic and antibacterial activity. Carbohydr Polym. 2018 Oct 15;198:124–30.
[13] Mahanta AK, Senapati S, Paliwal P, Krishnamurthy S, Hemalatha S, Maiti P. Nanoparticle-Induced Controlled Drug Delivery Using Chitosan-Based Hydrogel and Scaffold: Application to Bone Regeneration. Mol Pharm. 2019 Jan 7;16(1):327–38.
[14] Zheng L, Li C 'e, Huang X, Lin X, Lin W, Yang F, et al. Thermosensitive hydrogels for sustained-release of sorafenib and selenium nanoparticles for localized synergistic chemoradiotherapy. Biomaterials. 2019 Sep;216:119220.
[15] Wong SK, Chin KY, Ima-Nirwana S. Quercetin as an Agent for Protecting the Bone: A Review of the Current Evidence. Int J Mol Sci [Internet]. 2020 Sep 3;21(17). Available from: http://dx.doi.org/10.3390/ijms21176448
[16] Yurteri A, Yildirim A, Çelik ZE, Vatansev H, Durmaz MS. The effect of quercetin on bone healing in an experimental rat model. Jt Dis Relat Surg. 2023 Apr 26;34(2):365–73.

[17] Pang XG, Cong Y, Bao NR, Li YG, Zhao JN. Quercetin Stimulates Bone Marrow Mesenchymal Stem Cell Differentiation through an Estrogen Receptor-Mediated Pathway. Biomed Res Int. 2018 Mar 15;2018:4178021.

[18] Nandi SK, Bandyopadhyay S, Das P, Samanta I, Mukherjee P, Roy S, et al. Understanding osteomyelitis and its treatment through local drug delivery system. Biotechnol Adv. 2016 Dec;34(8):1305–17.

[19] Cheng CA, Deng T, Lin FC, Cai Y, Zink JI. Supramolecular Nanomachines as Stimuli-Responsive Gatekeepers on Mesoporous Silica Nanoparticles for Antibiotic and Cancer Drug Delivery. Theranostics. 2019 May 18;9(11):3341–64.

[20] Narayanaswamy R, Torchilin VP. Hydrogels and Their Applications in Targeted Drug Delivery. Molecules [Internet]. 2019 Feb 8;24(3). Available from: http://dx.doi.org/10.3390/molecules24030603

[21] Moncada-Basualto M, Matsuhiro B, Mansilla A, Lapier M, Maya JD, Olea-Azar C. Supramolecular hydrogels of β-cyclodextrin linked to calcium homopoly-l-guluronate for release of coumarins with trypanocidal activity. Carbohydr Polym. 2019 Jan 15;204:170–81.

[22] Jung SW, Oh SH, Lee IS, Byun JH, Lee JH. Gelling Hydrogel with Anti-Bacterial Activity and Bone Healing Property for Treatment of Osteomyelitis. Tissue Eng Regen Med. 2019 Oct;16(5):479–90.

[23] Tao J, Zhang Y, Shen A, Yang Y, Diao L, Wang L, et al. Injectable Chitosan-Based Thermosensitive Hydrogel/ Nanoparticle-Loaded System for Local Delivery of Vancomycin in the Treatment of Osteomyelitis. Int J Nanomedicine. 2020 Aug 6;15:5855–71.

[24] Zhou T, Zhou H, Wang F, Zhang P, Shang J, Shi L. An injectable carboxymethyl chitosan hydrogel scaffold formed via coordination bond for antibacterial and osteogenesis in osteomyelitis. Carbohydr Polym. 2024 Jan 15;324:121466.

[25] Zhang P, Sun Y, Yang H, Liu D, Zhang F, Zhang Y, et al. Vancomycin-loaded silk fibroin microspheres in an injectable hydrogel for chronic osteomyelitis therapy. Front Bioeng Biotechnol. 2023 Jun 1;11:1163933.

[26] Wei J, Wang Y, Jiang J, Yan Y, Fan D, Yang X, et al. Development of an Antibacterial Bone Graft by Immobilization of Levofloxacin Hydrochloride-Loaded Mesoporous Silica Microspheres on a Porous Scaffold Surface. J Biomed Nanotechnol. 2019 May 1;15(5):1097–105.

[27] Biosynthesis And Characterization of Silver Nanoparticles Derived from Ethanol and Aqueous Extract of Tarragon. (2023). Journal of Population Therapeutics and Clinical Pharmacology, 30(13), 403-407. https://doi.org/10.47750/jptcp.2023.30.13.041

[28] Rajasekar N, Mohanraj KG, Mary Martin T, K MS. Advanced Dental Care: β-Chitosan Zinc Oxide Nanoparticles Targeting Cariogenic Microorganisms. Cureus. 2024 Aug 6;16(8):e66296. doi: 10.7759/cureus.66296. PMID: 39238748; PMCID: PMC11376470.

[29] Vairavel M, Devaraj E, Shanmugam R. An eco-friendly synthesis of Enterococcus sp.-mediated gold nanoparticle induces cytotoxicity in human colorectal cancer cells. Environ Sci Pollut Res Int. 2020 Mar;27(8):8166-8175. doi: 10.1007/s11356-019-07511-x. Epub 2020 Jan 3. PMID: 31900772.

[30] Ramamurthy J. Evaluation of Antimicrobial Activity of Nanoformulated Grape Seed Oil against Oral Microbes: An In Vitro Study. World J Dent 2024;15(1):44-47.