# Evaluation of Antimicrobial and Cytotoxic Properties of Hyaluronic Acid based injectable Hydrogel incorporated with Dihydroxyacetone Phosphate, Magnesium Nanoparticles and Cissus Quadrangularis

**Ngoubinah Pretty TM[1], Balaji Ganesh S*[2]**
[1]Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai-77, Tamil Nadu, India
[2]Reader, Department of Periodontics, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai - 77, India
**Corresponding Author:** Dr. Balaji Ganesh S
Reader, Department of Periodontics, Saveetha Dental College and Hospital, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai - 77, India
Email Id: balajiganeshs.sdc@saveetha.com

**Abstract**
**INTRODUCTION:** This study explores the development of hyaluronic acid (HA)-based injectable hydrogels enhanced with dibasic ammonium phosphate (DAP) and magnesium (Mg) nanoparticles, further supplemented with Cissus quadrangularis (CQ) extract. The synergistic effects of DAP and Mg nanoparticles promote mineralization and bone matrix formation, while CQ, a traditional medicinal plant, enhances osteoblast proliferation and differentiation. The aim of this study is to evaluate the antimicrobial and cytotoxic properties of hyaluronic acid based injectable hydrogel incorporated with dihydroxyacetone phosphate, magnesium nanoparticles and cissus quadrangularis. **MATERIALS AND METHODS:** Hyaluronic acid-based was prepared. It was methacrylated by adding 20 times the concentration of methacrylic acid. Ag nanoparticles, previously extracted bone graft and DAP and Mg nanoparticles using cissus quadrangularis, were added in optimised quantities to the solution. Then the material was photocrosslinked using the photo initiator 12959 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 hydrogel sample exhibited a zone of inhibition of 32 mm against Staphylococcus aureus, which was equivalent to the control (32 mm) and significantly higher than the standard antibiotic (23 mm). For Enterococcus faecalis, the hydrogel sample exhibited a 28 mm inhibition zone, which was higher than the control (23 mm) but slightly lower than the antibiotic (30 mm). The results indicated that the test gel exhibited a high percentage of cell viability, comparable to the control group. **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 result indicates that the test hydrogel formulation supports cell viability, with only a slight reduction compared to control
**Keywords:** Bone regeneration, Hyaluronic acid hydrogel, Magnesium nanoparticles, Cissus quadrangularis, Osteogenesis

## INTRODUCTION:

Hyaluronic acid is a naturally occurring substance found in various tissues of the human body, including connective tissues, skin, and synovial fluid in joints(1). While it is not a primary component of bone tissue, there is some evidence to suggest that hyaluronic acid may play a role in bone formation and remodeling indirectly through its interactions with other molecules and cells involved in bone health(2). Hyaluronic acid is a major component of the extracellular matrix, which provides structural support to cells in various tissues. In bone, the extracellular matrix plays a critical role in providing a scaffold for bone-forming cells (osteoblasts) and bone-resorbing cells (osteoclasts)(3)(4). Mesenchymal stem cells are multipotent cells that can differentiate into various cell types, including osteoblasts, which are responsible for bone formation(5). Some studies have suggested that hyaluronic acid can influence the differentiation of MSCs toward an osteogenic (bone-forming) lineage(6). It may do so by interacting with cell surface receptors and signaling pathways involved in osteogenesis(7). In the context of bone health, reducing inflammation and supporting tissue repair may indirectly aid in bone formation, especially in cases of bone injury or fractures.

Nanoparticles have garnered significant attention in recent years due to their unique physical and chemical properties, which make them valuable for a wide range of applications in various fields, including medicine, electronics, and environmental science(8,9). Among the various types of nanoparticles, DAP (Diammonium Phosphate) and Mg (Magnesium) nanoparticles have gained prominence for their diverse applications.Cissus Quadrangularis, a well-known medicinal plant, has emerged as a promising candidate for the green synthesis of these nanoparticles.It also highlights the significance of green synthesis methods in nanoparticle production and the unique properties and applications of DAP and Mg nanoparticles(10).

Cissus Quadrangularis, commonly known as Veldt Grape or Hadjod, is a perennial plant native to Asia and Africa. It has a long history of use in traditional medicine for its various health benefits, including bone healing, pain relief, and anti-inflammatory properties(11). The plant contains bioactive compounds, such as flavonoids, polyphenols, and alkaloids, which make it an ideal candidate for nanoparticle synthesis (12). DAP (Diammonium Phosphate) is a crucial fertilizer in agriculture due to its high phosphorus and nitrogen content. The synthesis of DAP nanoparticles using Cissus Quadrangularis can enhance nutrient uptake by plants, leading to improved crop yield and reduced environmental pollution (13). These nanoparticles may also find applications in controlled-release fertilizers, promoting sustainable agriculture. Magnesium (Mg) is an essential element for plant growth and development. Mg nanoparticles synthesized from Cissus Quadrangularis can serve as effective nutrient carriers for plants(14). They offer improved solubility and bioavailability, potentially leading to enhanced magnesium uptake and healthier plant growth. Additionally, Mg nanoparticles have applications in pharmaceuticals and as catalysts in chemical reactions(15).

Periodontal bony defects are classified into 'intraosseous' ('suprabony') or 'intraosseous' ('infrabony') according to the location of the base of the defect compared to the coronal part of the residual alveolar crest(16). Intraosseous defects are generally considered more challenging to treat and are thought to be associated with a higher risk of periodontal progression. The emergence and advancement of periodontal regenerative procedures have improved the clinician's ability to manage intraosseous defects(17). The aim of this study is to evaluate the antimicrobial and cytotoxic properties of hyaluronic acid based injectable hydrogel incorporated with dihydroxyacetone phosphate, magnesium nanoparticles and cissus quadrangularis

**MATERIALS AND METHODS:**

Hyaluronic acid-based was prepared. It was methacrylated by adding 20 times the concentration of methacrylic acid. Ag nanoparticles, previously extracted bone graft and DAP and Mg nanoparticles using cissus quadrangularis, were added in optimised quantities to the solution. Then the material was photocrosslinked using the photo initiator 12959 to form the gel. We used a photoinitiator called 12959. The methacrylate group in the gelatin methacrylate will be chemically cross linked with each other on photoactivation by 12959 This forms the gel. The material was then freeze dried using a lyophilizer.(figure1) The lyophilized material was then analyzed for antimicrobial properties by testing bacterial/fungal growth around the sample and cell compatibility by MTT assay.

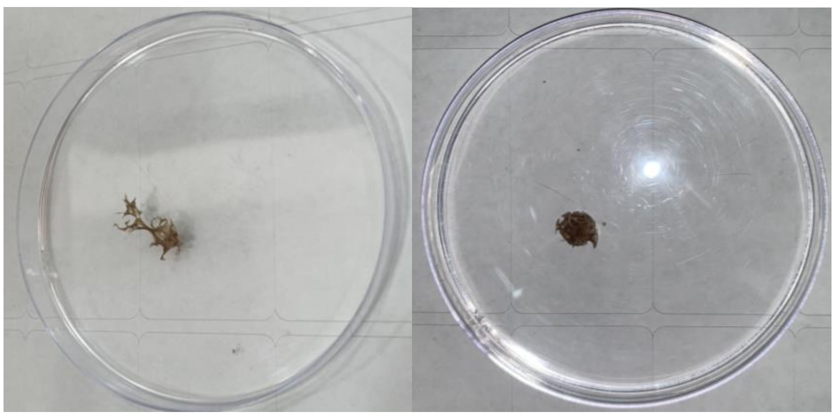

**FIGURE 1: Depict the image of dehydrated test gel and control gel**

**RESULTS:**

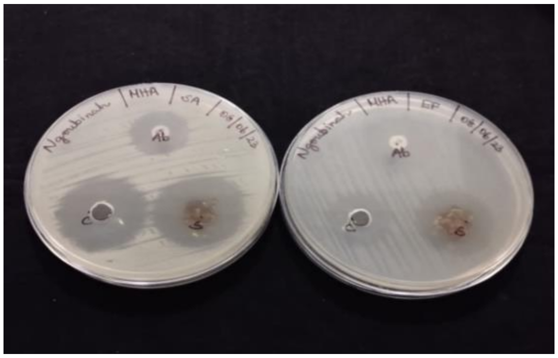

**FIGURE 2: culture plate for staphylococcus Aureus and E.Faecalis**

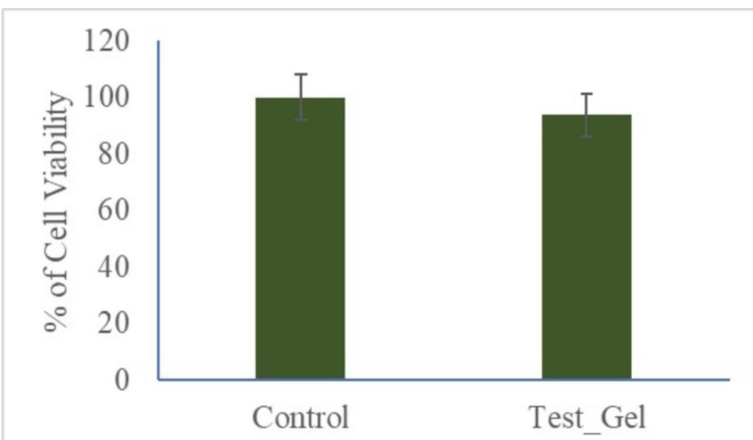


**FIGURE 3: Results of compatibility test on control and test gel**

The hydrogel sample exhibited a zone of inhibition of **32 mm** against *Staphylococcus aureus*, which was equivalent to the control (**32 mm**) and significantly higher than the standard antibiotic (**23 mm**). This suggests that the combination of DAP, Mg nanoparticles, and *Cissus quadrangularis* extract within the hydrogel formulation effectively inhibited bacterial growth, comparable to conventional antimicrobial treatments. For *Enterococcus faecalis*, the hydrogel sample exhibited a **28 mm** inhibition zone, which was higher than the control (**23 mm**) but slightly lower than the antibiotic (**30 mm**). This indicates that the hydrogel formulation was effective against *E. faecalis*, although its antimicrobial potency was slightly reduced compared to the antibiotic standard. (figure 2)

The biocompatibility of the developed hyaluronic acid (HA)-based injectable hydrogel incorporated with doxycycline (DAP), magnesium (Mg) nanoparticles, and *Cissus quadrangularis* extract was evaluated using a cell viability assay. The results, as depicted in the bar graph, indicate that the test gel exhibited a high percentage of cell viability, comparable to the control group. (figure 3)

## DISCUSSION:

The antimicrobial efficacy of the hyaluronic acid (HA)-based injectable hydrogels incorporated with doxycycline (DAP) and magnesium (Mg) nanoparticles was assessed against *Staphylococcus aureus* and *Enterococcus faecalis*, two common pathogens associated with bone infections. The results were compared with standard antibiotics and a control group, as shown in the zone of inhibition measurements(18). This suggests that the combination of DAP, Mg nanoparticles, and *Cissus quadrangularis* extract within the hydrogel formulation effectively inhibited bacterial growth, comparable to conventional antimicrobial treatments. The synergistic effect of these components likely contributed to the enhanced antibacterial activity, where DAP acted as a potent antibiotic while Mg nanoparticles exerted their bactericidal effects through membrane disruption and reactive oxygen species (ROS) generation(19). Additionally, the phytochemical constituents of *Cissus quadrangularis*, known for their antimicrobial properties, may have further strengthened the antibacterial potential of the hydrogel.

Our study results indicate that the hydrogel formulation was effective against *E. faecalis*, although its antimicrobial potency was slightly reduced compared to the antibiotic standard. The observed antibacterial effect suggests that while the hydrogel formulation can inhibit *E. faecalis*, optimization of DAP release kinetics or Mg nanoparticle concentration might further enhance its effectiveness. The overall antimicrobial test results validate the ability of the developed HA-based injectable hydrogel to function as a bioactive material with significant antibacterial properties. The comparable or superior performance against *S. aureus* and its substantial efficacy against *E. faecalis* highlight its potential application in preventing infections in bone regeneration procedures. Furthermore, the sustained release of antimicrobial agents from the hydrogel matrix could contribute to prolonged protection against bacterial colonization, reducing the risk of implant-associated infections.Future studies should focus on optimizing the hydrogel formulation to maximize antimicrobial effectiveness while ensuring biocompatibility. Additionally, in vivo investigations are necessary to confirm its long-term efficacy in clinical settings and its role in promoting both antibacterial protection and bone regeneration.

The biocompatibility of the developed hyaluronic acid (HA)-based injectable hydrogel incorporated with doxycycline (DAP), magnesium (Mg) nanoparticles, and *Cissus quadrangularis* extract was evaluated using a cell viability assay. The results, as depicted in the bar graph, indicate that the test gel exhibited a high percentage of cell viability, comparable to the control group(20). Cell viability is a crucial parameter in determining the cytocompatibility of biomaterials intended for bone regeneration. The results demonstrate that the test gel maintained a viability percentage close to 100%, with no significant cytotoxic effects observed. This suggests that the incorporation of DAP, Mg nanoparticles, and *Cissus quadrangularis* does not negatively impact cell survival, making the hydrogel a promising candidate for biomedical applications. The presence of Mg nanoparticles, which are known to play a role in osteogenesis and cell proliferation, likely contributed to the favorable cellular response(21). Additionally, *Cissus quadrangularis*, a medicinal plant with known antioxidant and anti-inflammatory properties, may have further enhanced cell viability by reducing oxidative stress and promoting a conducive environment for cellular growth(22).

The results also suggest that the sustained release of bioactive components from the hydrogel does not induce toxicity, which is a critical consideration for in vivo applications. The comparable viability between the test gel and control further reinforces the potential of this formulation for clinical translation. Since cytocompatibility is an essential prerequisite for bone tissue engineering, these findings strongly support the suitability of the HA-based hydrogel as a biocompatible scaffold for bone regeneration. Future studies should focus on assessing long-term cellular responses, proliferation, and differentiation potential in osteogenic environments. Additionally, in vivo studies will be necessary to confirm tissue integration, biodegradability, and overall safety of the hydrogel in bone repair applications.

## CONCLUSION:

The results of this study demonstrate that hyaluronic acid based injectable hydrogel incorporated with dihydroxyacetone phosphate, magnesium nanoparticles and cissus quadrangularis present a promising approach for enhancing bone regeneration. 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 result indicates that the test hydrogel formulation supports cell viability, with only a slight reduction compared to control. The hydrogel production appears to be safe for use in biomedical applications, such as tissue engineering and wound healing, as the cell viability stays over 85%. All things considered, the results show that the created hydrogel system successfully strikes a compromise between cytocompatibility and antibacterial potency, making it a viable option for biomedical uses.

## REFERENCES:

1. Xing Y, Qiu L, Liu D, Dai S, Sheu CL. The role of smart polymeric biomaterials in bone regeneration: a review. Front Bioeng Biotechnol. 2023 Aug 17;11:1240861.
2. Agarwal R, García AJ. Biomaterial strategies for engineering implants for enhanced osseointegration and bone repair. Adv Drug Deliv Rev. 2015 Nov 1;94:53–62.
3. Boateng J. Therapeutic Dressings and Wound Healing Applications. John Wiley & Sons; 2020. 452 p.
4. Bs A, Ganesh S B, Eswaramoorthy R, G K. Hyaluronic Acid Hydrogel Combined With Ocimum sanctum and Tendon-Derived Extracellular Matrix Enhances Tenogenesis in Periodontal Ligament Stem Cells. Cureus. 2024 Feb;16(2):e54060.
5. Atila D, Kumaravel V. Advances in antimicrobial hydrogels for dental tissue engineering: regenerative strategies for endodontics and periodontics. Biomater Sci. 2023 Oct 10;11(20):6711–47.
6. Taokaew S, Kaewkong W, Kriangkrai W. Recent Development of Functional Chitosan-Based Hydrogels for Pharmaceutical and Biomedical Applications. Gels [Internet]. 2023 Mar 27;9(4). Available from: http://dx.doi.org/10.3390/gels9040277
7. Cao H, Duan L, Zhang Y, Cao J, Zhang K. Current hydrogel advances in physicochemical and biological response-driven biomedical application diversity. Signal Transduct Target Ther. 2021 Dec 16;6(1):426.
8. Shukla AK, Iravani S. Green Synthesis, Characterization and Applications of Nanoparticles. Elsevier; 2018. 552 p.
9. Siddhardha B, Dyavaiah M, Kasinathan K. Model Organisms to Study Biological Activities and Toxicity of Nanoparticles. Springer Nature; 2020. 466 p.
10. Yayintas OT, Demir N, Canbolat F, Ayna TK, Pehlivan M. Characterization, biological activity, and anticancer effect of green-synthesized gold nanoparticles using Nasturtium officinale L. BMC Complement Med Ther. 2024 Oct 1;24(1):346.
11. Liao L, Zhu W, Tao C, Li D, Mao M. Cissus quadrangularis L extract-loaded tricalcium phosphate reinforced natural polymer composite for guided bone regeneration. J Mater Sci Mater Med. 2023 Jul 19;34(7):33.
12. Toor RH, Malik S, Qamar H, Batool F, Tariq M, Nasir Z, et al. Osteogenic potential of hexane and dichloromethane fraction of Cissus quadrangularis on murine preosteoblast cell line MC3T3-E1 (subclone 4). J Cell Physiol. 2019 Dec;234(12):23082–96.
13. Muthusami S, Senthilkumar K, Vignesh C, Ilangovan R, Stanley J, Selvamurugan N, et al. Effects of Cissus quadrangularis on the proliferation, differentiation and matrix mineralization of human osteoblast like SaOS-2 cells. J Cell Biochem. 2011 Apr;112(4):1035–45.
14. Herrmann A, Haag R, Schedler U. Hydrogels and Their Role in Biosensing Applications.

Adv Healthc Mater. 2021 Jun;10(11):e2100062.
15. Taokaew S, Kriangkrai W. Correction: Taokaew, S.; Kriangkrai, W. Chitinase-Assisted Bioconversion of Chitinous Waste for Development of Value-Added Chito-Oligosaccharides Products. 2023, , 87. Biology (Basel) [Internet]. 2023 May 8;12(5). Available from: http://dx.doi.org/10.3390/biology12050689
16. Panda S, Ramamoorthi S, Jayakumar ND, Sankari M, Varghese SS. Platelet rich fibrin and alloplast in the treatment of intrabony defect. J Pharm Bioallied Sci. 2014 Apr;6(2):127–31.
17. Panda S, Jayakumar ND, Sankari M, Varghese SS, Kumar DS. Platelet rich fibrin and xenograft in treatment of intrabony defect. Contemp Clin Dent. 2014 Oct;5(4):550–4.
18. Stan D, Ruta LL, Bocancia-Mateescu LA, Mirica AC, Stan D, Micutz M, et al. Formulation and Comprehensive Evaluation of Biohybrid Hydrogel Membranes Containing Doxycycline or Silver Nanoparticles. Pharmaceutics [Internet]. 2023 Nov 28;15(12). Available from: http://dx.doi.org/10.3390/pharmaceutics15122696
19. Kumar S, Madihally SV. Role of Chitosan and Chitosan-Based Nanomaterials in Plant Sciences. Academic Press; 2022. 556 p.
20. Ganesh S, S G, Madhu K. Commercially Pure Titanium Implants With Selenium and Hyaluronic Acid Coating for Dental Applications. Cureus. 2024 Jan;16(1):e52984.
21. Suresh N, Kaarthikeyan G. Green Synthesis and the Evaluation of Osteogenic Potential of Novel Europium-Doped-Monetite Calcium Phosphate by Cissus quadrangularis. Cureus. 2024 Apr;16(4):e59202.
22. Subramaniam D, Sekaran S. In Vitro Biocompatibility Assessment of a Novel Membrane Containing Magnesium-Chitosan/Carboxymethyl Cellulose and Alginate Intended for Bone Tissue Regeneration. Cureus. 2024 Feb;16(2):e54597.