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PREPARATION AND CHARACTERIZATION OF HYDROXYAPATITE AND CISSUS QUADRANGULARIS COMPOSITE ENTRAPPED HYDROXYPROPYL METHYLCELLULOSE SCAFFOLD FOR BONE REGENERATION-AN INVITRO STUDY

Snigdha Bharadwaz[1], Balaji Ganesh S[1*], Vishnuvardhan Vengatachalam[2], Gurumoorthy Kaarthikeyan[1]

[1]Department of Periodontics, Saveetha Dental College and hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai-600077, Tamil Nadu, India
[2]Senior lecturer, Department of Prosthodontics, K.S.R Institute of Dental Sciences and Research, Tiruchengode – 637215, Namakkal District, Tamil Nadu, India
*Corresponding author: **Dr. Balaji Ganesh. S**, Reader, Department of Periodontics, Saveetha Dental College and hospitals, Saveetha Institute of Medical and Technical Sciences (SIMATS), Saveetha University, Chennai-600077, Tamil Nadu, India, Email id: balajiganeshs.sdc@saveetha.com
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ABSTRACT

Background: The integration of biomaterials, such as hydroxyapatite (HAp) and natural extracts like Cissus quadrangularis (CQ), into scaffolds can enhance osteogenic properties and support bone healing. This study evaluates the potential of an HAp-CQ composite embedded in a Hydroxypropyl methylcellulose (HPMC) scaffold for bone regeneration.

Material and methods: The HAp-CQ composite was synthesized using a sol-gel method. HPMC scaffolds were prepared through a solvent-casting and particulate-leaching technique, followed by the incorporation of the HAp-CQ composite. To prepare the hydroxypropyl methylcellulose scaffold with the impregnation of Cissus quadrangularis (CQ) and hydroxyapatite (HAp), 1 gram of CQ was dissolved in 10 mL of distilled water and heated at 80°C for 24 hours. Following scaffold formation, FTIR (Fourier Transform Infrared Spectroscopy), contact angle, tensile strength testing and anti-microbial activity was compared between the HAp-CQ and HPMC+HAp+CQ scaffold.

Results: The synthesized HAp-CQ composite displayed improved mechanical properties and bioactivity, with FTIR confirming functional group interactions. The tensile strength of the test group scaffold was 1.51MPa and the contact angle of the test group scaffold showed an average angle of 53.07 degrees.

Conclusion: The present study showed that the HPMC scaffold with the impregnation of CQ and HAp showed high tensile strength and increase in hydrophilicity. The mechanical property was increased and the antimicrobial activity showed an enhanced zone of inhibition.

Keywords: Hydroxyapatite, Cissus quadrangularis, Hydroxypropyl methylcellulose, Bone regeneration, Composite scaffold, Guided regeneration

INTRODUCTION

Periodontitis is a multifactorial inflammatory disease characterized by the progressive destruction of the supporting structures of the teeth, including the periodontal ligament and alveolar bone. It is primarily caused by bacterial infection,It causes the periodontal tissues to break down by inciting an inflammatory reaction. Periodontitis is a significant global health concern, affecting a substantial portion of the adult population. If left untreated, it can result in tooth mobility and eventual loss, severely impacting an individual's oral health, functional capacity, and quality of life. Additionally, periodontitis has been linked to systemic health issues, including cardiovascular disease, diabetes, and respiratory disorders, highlighting the importance of effective management and treatment.[1] Periodontal bone loss is a significant pathological process associated with periodontitis, a chronic inflammatory disease that affects the supporting structures of the teeth. This condition results from the complex interaction between pathogenic bacteria in dental plaque and the host's immune response, leading to inflammation and subsequent destruction of the alveolar bone surrounding the teeth.[2] As the condition worsens, tooth mobility, diminished appearance, and eventually tooth loss may arise from the loss of periodontal attachment and the resorption of alveolar bone.

The prevalence of periodontal disease highlights the need for a deeper understanding of the mechanisms driving bone loss and the development of effective treatment strategies. Periodontal bone regeneration refers to the therapeutic strategies aimed at restoring lost alveolar bone and periodontal tissues resulting from periodontal disease, particularly periodontitis.[3] This condition leads to significant destruction of the supporting structures of teeth, including the alveolar bone, periodontal ligament, and cementum. Given the critical role of alveolar bone in maintaining tooth stability and function, effective regeneration of these tissues is essential for restoring oral health, preventing tooth mobility, and improving overall quality of life. The focus on periodontal bone regeneration has gained momentum due to the increasing prevalence of periodontal disease and its association with systemic health issues, underscoring the need for effective treatment modalities.[4]

Bone regeneration is a critical challenge in orthopedics and dentistry, necessitating the development of effective biomaterials that can promote healing and restore functionality.[5] Traditional approaches to bone repair, such as autografts and allografts, often face limitations including donor site morbidity, risk of infection, and inadequate supply.[6] Consequently, there is an increasing interest in synthetic and natural biomaterials that can serve as scaffolds for bone regeneration, providing not only structural support but also biological cues to enhance healing.[7] Hydroxyapatite (HAp) is one of the most extensively studied biomaterials for bone applications due to its biocompatibility and similarity to natural bone mineral. HAp promotes osteoconduction, allowing for the attachment and growth of bone cells.[8] However, while HAp provides a favorable environment for bone regeneration, it lacks intrinsic biological activity. To overcome this limitation, there is a growing trend to incorporate bioactive compounds into HAp-based materials, enhancing their osteogenic potential. One such compound is Cissus quadrangularis, a medicinal plant known for its traditional use in promoting bone health. Its phytochemicals have demonstrated osteogenic properties, stimulating osteoblast differentiation and enhancing collagen synthesis.[9]

To improve the stability and release profile of the bioactive components, hydroxypropyl methylcellulose (HPMC) is employed as a matrix material. HPMC is a hydrophilic polymer that offers excellent film-forming capabilities and controlled drug release properties.[10] By entrapping HAp and Cissus quadrangularis extract in an HPMC matrix, this study aims to create a composite biomaterial that synergistically combines the structural support of HAp with the bioactive properties of Cissus quadrangularis. This innovative approach has the potential to enhance bone regeneration, making it a promising candidate for clinical applications in treating periodontal bone defects and enhancing healing processes.

MATERIAL AND METHODS

To prepare the hydroxypropyl methycellulose scaffold with the impregnation of Cissus quadrangularis (CQ) and hydroxyapatite (HAp), 1 gram of CQ was dissolved in 10 mL of distilled water and heated at 80°C for 24 hours. This process facilitated the extraction of bioactive compounds from the plant material. After heating, the solution was filtered through Whatman filter paper to obtain a clear CQ extract, which was set aside for later incorporation into the hydroxypropyl methylcellulose matrix. Simultaneously, a 5% hydroxypropyl methylcellulose solution was prepared by dissolving 5 grams of hydroxypropyl methylcellulose in 100 mL of distilled water, and this mixture was kept overnight to ensure complete dissolution. Once fully dissolved, the hydroxypropyl methylcellulose solution was divided into two equal parts. The first 50 mL served as the base membrane scaffold, consisting of pure hydroxypropyl methylcellulose. To the second 50 mL, 50 mg of hydroxyapatite was added, and the mixture was stirred for 5 hours to achieve a uniform dispersion of HA. This HAp-impregnated hydroxypropyl methylcellulose solution

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was then poured into a petri dish and placed in a deep freezer at -20°C for 24 hours to promote solidification. After freezing, the scaffolds were lyophilized to remove any residual: moisture, yielding stable gelatin scaffolds ready for guided tissue regeneration applications. The materials were divided into two groups: the control group consists of hydroxypropyl methylcellulose and the test group consists the addition of cissus quadrangularis and hydroxyapatite(HAp) and hydroxypropyl methylcellulose(HPMC)

CHARACTERIZATION OF THE SCAFFOLDS

Fourier transform infrared (FTIR) spectroscopy

FTIR spectroscopy was used to analyze the scaffolds' functional groups (Perkin Elmer, USA). The scaffold samples were positioned on a crystal surface with attenuated total reflectance, and spectra were recorded between 400 and 4000 cm-1. To look at the existence of different components in the sample, scans were conducted over different sections of the sample.

Tensile strength

The universal testing machine (Instron Electroplus E3000, USA) was used to measure the tensile strength. 10 × 15 mm test samples were used for analysis, with a crosshead speed of 5 mm/min. Forces were applied to the specimens when the scaffolds were fitted into the analyzer. The breaking force was defined as the force at which the scaffold broke. Tensile stress was calculated by multiplying this breaking force by the specimens' area, and tensile strain was defined as the deformation at the breaking force.

Contact angle analysis

Sessile drop method analysis was performed to measure the wettability of the scaffolds. A droplet of distilled water (2 µL) was horizontally dropped on the surface of the scaffolds with the help of a micropipette. Ossila goniometer was used to determine the contact angle of the water drop formed at the interface with the scaffold sample.

Antimicrobial activity

The agar disk diffusion method was used to assess the produced scaffold's antibacterial efficacy against S. mutans. On BHI agar, the bacterial culture was uniformly swabbed. After that, the plates were incubated anaerobically for 24 hours at 37°C. Following incubation, the inhibitory zone's diameter was assessed.

RESULTS

The graph shows the Fourier-transform infrared (FTIR) spectra of two samples, labeled as HPMC (in green) and HAp-HPMC (in red). The x-axis represents the wavenumber (cm^{-1}), which is a measure of the frequency of infrared light absorbed by the sample, while the y-axis shows the transmittance (%) of infrared light through the sample. The differences between the HPMC and HAp-HPMC spectra suggest that HAp (likely hydroxyapatite) has modified the structure or composition of HPMC (hydroxypropyl methylcellulose). The green line represents the pure HPMC spectrum. The characteristic peaks here would be attributed to HPMC's molecular structure, such as C-H, C-O, and O-H stretches, depending on their wavenumbers. The red line represents the HAp-HPMC complex, showing peaks that differ from pure HPMC. The presence of HAp might introduce additional peaks or shift existing ones due to interactions between HPMC and HAp, indicating potential bonding or structural changes. To precisely interpret each peak, the wavenumber positions could be compared with a reference table of common functional groups. For instance, peaks around 1000-1200 cm^{-1} might correspond to C-O stretches, while those near 1400-1600 cm^{-1} could be related to C=C or C=O bonds (Figure 1).

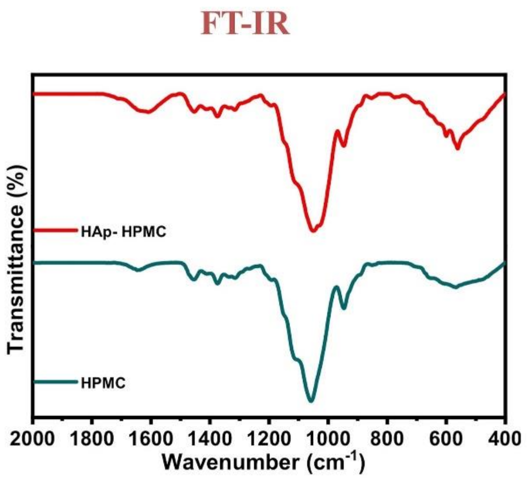

Figure 1. FTIR analysis

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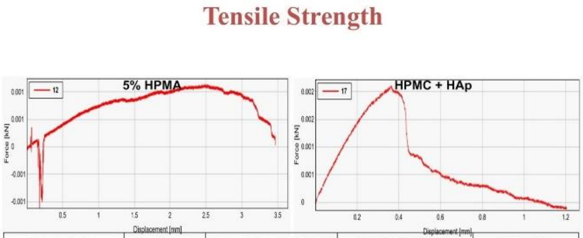
Tensile strength was measured in newtons and it was tested using the INSTRON 3000 universal testing machine. The tensile stress for HPMC (control group) was 0.81MPa with tensile strength 8.67% at break and tensile stress of 0.12% at break, whereas with the addition of HPMC+HAp (test group) was 1.51MPa with tensile strength 3% at break and tensile stress of 0.06% at break this proves that an enhanced property of tensile strength in the test group was observed. The tensile strength of the scaffolds of HPMC and HPMC+HAp mechanical properties of material is increased (Figure 2).



Materials Name	Maximum Force [N]	Tensile stress at Tensile strength [MPa]	Tensile strain (Displacement) at Break (Standard) [%]	Tensile stress at Break (Standard) [MPa]
HPMC	1.13	0.81	8.67	0.12
HPMC+HAp	2.11	1.51	3.00	0.06

Figure 2. Tensile strength analysis

Contact angle was measured using an ossila goniometer. Test group had a contact angle of 37.75 degrees and the control group had an angle of 53.07 degrees. Hydrophilic property is more in the test group compared to the control group, which shows the test scaffold has good wettability. (Figure 3).

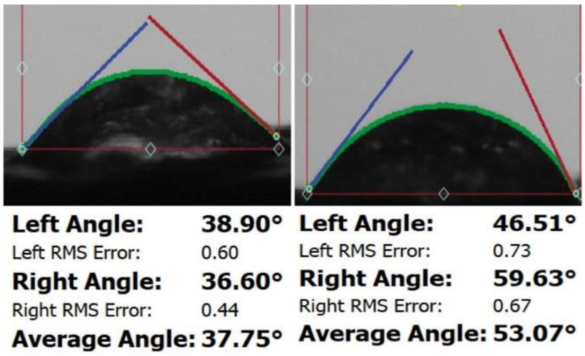


Figure 3. Contact angle analysis

The zone of diameter of S.mutans in the control group was 9 mm whereas in the test group it was 15 mm. The antibacterial activity in a cultured medium that shows the zone of inhibition of S.mutans, amoxicillin and erythromycin has been used as the antibody medium (Figure 4).

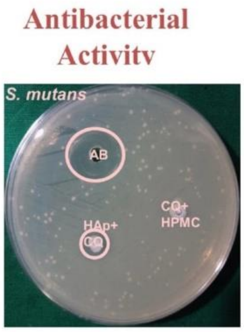


Figure 4. Antimicrobial activity

DISCUSSION

The development of hydroxyapatite (HA) Cissus quadrangularis (CQ) composite entrapped in hydroxypropyl methylcellulose (HPMC) scaffolds represents a significant advancement in the field of bone tissue engineering. Hydroxypropyl Methylcellulose (HPMC) is a versatile polymer widely used in biomedical applications due to its excellent biocompatibility, biodegradability, and ability to form hydrogels. In the context of bone tissue engineering, HPMC serves as a crucial scaffold material that provides mechanical support while allowing for the controlled release of bioactive agents. Its hydrophilic nature promotes the absorption of water, which is essential for maintaining a conducive environment for cell proliferation and differentiation. Additionally, the ability of HPMC to form a gel-like structure enhances the scaffold's porosity, facilitating nutrient diffusion and waste removal key factors in promoting osteogenesis. The incorporation of HPMC with Hydroxyapatite and Cissus quadrangularis not only enhances the scaffold's structural integrity but also enables the sustained release of bioactive compounds from the Cissus quadrangularis, potentially amplifying its osteogenic effects. Cissus quadrangularis is believed to possess anti-inflammatory and antioxidant properties that may enhance the healing process.[11] Its bioactive constituents are thought to stimulate osteoblast activity and promote the synthesis of bone matrix, crucial for effective bone regeneration.[12] Cissus quadrangularis was found to be useful for tissue engineering. Previous study showed that the cissus quadrangularis exhibits better microbial activity suitable for biomedical application.[13]
Contact angle measurements help determine the surface characteristics, with higher angles indicating more hydrophobic surfaces and lower angles indicating more hydrophilic surfaces. One crucial

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factor that indicates this composite's wettability and, in turn, its biological performance is its contact angle. Greater hydrophilicity, which is necessary to encourage cell attachment and proliferation, is shown by a smaller contact angle.[14] The material's overall wettability can be increased by encasing HAp in HPMC, a hydrophilic polymer. Surface morphology and composition are also pivotal in determining the contact angle. The ratio of HAp to Cissus quadrangularis and HPMC affects the overall surface characteristics, influencing porosity and roughness.[15] These structural features can enhance surface area contact with cells and fluids, promoting better adhesion and integration with host tissue. Additionally, factors such as processing conditions and the method of composite formation can significantly alter the surface properties, necessitating careful optimization during material development. This study shows that the contact angle in the test group is better than the control group.

The addition of HAp to HPMC improves the material's tensile strength and maximum load capacity, making it stronger but also more brittle. The reduced strain at break indicates that the composite material is less stretchable and breaks at lower deformation, a common trade-off when reinforcing materials with rigid particles like HAp.[16] This analysis suggests that the HPMC+HAp composite may be more suitable for applications requiring higher strength with less concern for flexibility. Furthermore, intriguing insights are revealed when this composite is contrasted with other biomaterials, including chitosan-based composites and poly(lactic-co-glycolic acid) (PLGA). Although PLGA-based composites frequently exhibit greater tensile strength (between 50 and 70 MPa), they might not have the osteoconductivity and bioactivity that HAp provides.[17] Conversely, chitosan composites may offer superior bioactivity, but they frequently lack the mechanical strength needed for load-bearing applications.[18] In conclusion, while the tensile strength of HAp-Cissus quadrangularis-HPMC composites may not reach the levels of some synthetic polymers, the balance of mechanical properties, biocompatibility, and bioactivity positions it as a promising candidate for bone regeneration.

Overall, the HAp-CQ-HPMC composite scaffold demonstrates significant promise for clinical applications in bone regeneration. However, further research is necessary to optimize the formulation, including variations in the HAp and CQ ratios and the exploration of additional bioactive agents that could enhance its properties. Long-term studies assessing the scaffold's performance and integration with surrounding bone, as well as potential immunological responses, are essential for determining its clinical viability. This innovative approach could pave the way for more effective treatments of bone defects and ultimately improve patient outcomes in dental applications. despite current limitations in biocompatibility, mechanical strength, and regulatory pathways.

CONCLUSION

The present study showed that the HPMC scaffold with the impregnation of cissus quadrangularis and hydroxyapatite showed high tensile strength and increased hydrophilicity. In conclusion, the antimicrobial activity is more evident in HPMC+HAp as compared to HPMC control group, the tensile strength is more in HPMC+HAp. Cissus quadrangularis entrapped in a hydroxypropyl methylcellulose matrix holds promise as a good scaffold for bone regeneration.

DECLARATIONS

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Conflict of Interest

The authors declare no conflict of interest.

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Ethics approval and consent to participate
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Consent for publication
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Declaration of competing interest
The authors declare None of the authors have any relevant financial relationship(s) with a commercial interest.

Data Availability
Not applicable

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