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Fabrication and Characterisation of Chitosan/ Tricalcium Phosphate/ Quercetin Doped Magnesium Membranes for Guided Bone Regeneration

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Abstract

Periodontal disease, characterized by gum inflammation, can lead to bone resorption. Chitosan, derived from chitin in marine crustaceans and fungi, is promising for bone tissue engineering due to its low toxicity. Magnesium ions, essential for bone metabolism, enhance mechanical strength and osteogenesis. This study fabricated and characterized chitosan/tricalcium phosphate/quercetin-doped magnesium membranes for guided bone regeneration. FTIR confirmed the presence of chitosan and magnesium, while SEM images showed increased fibre diameter. A water contact angle of 69° and a slight increase in matrix mineralization indicate the material's hydrophilicity and biocompatibility, making it a potential treatment for periodontitis. The results emphasize the dual potential of these membranes in bone healing and improved patient outcomes, offering novel approaches for regenerative medicine.

Keywords: *Bioactive Membranes, Bone Regeneration Therapies, Chitosan Coatings, Cytocompatibility, Guided Bone Regeneration, Osteogenesis, Regenerative Medicine.*

Introduction

Periodontal disease is a prevalent condition marked by gum inflammation and infection, leading to bone resorption. Researchers are investigating tissue engineering as a treatment method, utilizing nanoparticle-incorporated scaffolds combined with dental pulp stem cells to regenerate lost bone [1]. This approach merges life sciences with materials engineering, focusing on restoring and enhancing tissue function, particularly in bone regeneration. Despite promising advancements, more research is needed to develop alternative materials for guided bone regeneration (GBR), which requires membranes to be biocompatible, effective in cell occlusion, integrative with host tissues, easy to apply clinically, and possess appropriate mechanical properties [2].

A significant challenge in creating effective materials for bone reconstruction is mimicking bone's complex inorganic-organic hybrid structure, which consists of hydroxyapatite nanocrystals and collagen fibrils. To replicate this structure, combining various biomolecules and cells into nanostructured biocomposites is an appealing strategy [3].

Chitosan, a deacetylated polysaccharide from chitin found in marine crustaceans and fungi, is recognized for its biocompatibility, biodegradability, and mucoadhesive properties [4]. It has applications in biomedical and tissue engineering fields and exhibits antimicrobial activity against various microorganisms. Bone, as a specialized connective tissue, can self-heal from minor injuries through the osteogenic differentiation of stem cells [5, 6]. However, larger defects often necessitate grafting. Chitosan-based scaffolds have shown promise in bone tissue engineering, demonstrating low toxicity and no allergic reactions in human applications [7].

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Magnesium ions are integral to bone tissue and are involved in many metabolic processes. They possess mechanical strength, degradation properties, osteogenic potential, and antibacterial effects [8]. In vivo studies with magnesium-doped hydroxyapatite nanoparticles have shown favourable tissue interactions and contributions to regeneration. A magnesium oxide gel has also promoted new bone formation in vivo, while porous magnesium phosphate membranes exhibit biocompatibility and osteogenic potential [9].

Tissue engineering employs biodegradable support matrices with bioactive components and cells to replace damaged tissue [10]. Electrospinning has emerged as a favoured scaffold synthesis method due to its efficiency in producing nanofibers at scale. This technique applies high voltages to generate fibres of various diameters. A bio-composite film made from chitosan and polyvinyl alcohol has demonstrated good antimicrobial properties against oral pathogens and biocompatibility for treating periodontal disease [11, 12]. This study aims to address current gaps in GBR membrane technology by exploring the potential of chitosan/tricalcium phosphate/quercetin-doped magnesium composites for guided bone regeneration. The approach involves fabricating membranes, characterizing them through SEM, FTIR, and re-evaluating biocompatibility and testing the membrane's hydrophilicity and cell viability.

Materials and Methods

Fabrication of Chitosan/TCP/Q-Mg Nanoparticle Membrane via Electrospinning

Chitosan/TCP/Q-Mg nanoparticle membranes for guided bone regeneration were fabricated through electrospinning. Chitosan, derived from the deacetylation of chitin sourced from marine crustaceans, was used in this study. A 10% PVA solution was combined with 3% chitosan in a 6:4 weight ratio, followed by the addition of 0.5% β-TCP and 5 mg/ml Q-MgNP. [13]. This mixture was stirred for 24 hours, loaded into a 5 ml syringe, and extruded through a 20 G needle at 16 kV, maintaining a 10 cm distance from the collector. The process occurred at 25°C and 45% humidity, with fibres collected at a flow rate of 0.9 ml/hr. The membranes were characterized using SEM, FT-IR, XRD, contact angle measurements, and in vitro cell culture assays.

FT-IR Analysis

The chemical interactions and functional groups of the membranes were analyzed using Attenuated Total Reflectance FT-IR (Alpha II Bruker spectrometer, 4000-500 cm^{-1}) to assess changes due to nanoparticle incorporation.[14].

Morphology Assessment via SEM

Morphological features were examined using a Field Emission SEM with EDS. Electrospun membranes were punched into 8 mm discs, coated with platinum, and analyzed at 4.5 kX magnification to measure fibre diameter and porosity using Image software.[15].

Wettability Assessment

Contact angles were determined with a goniometer. Membranes were cut into 1 cm^2 squares, and a 50 μL distilled water droplet was placed on each. Contact angles were recorded from three different positions per membrane.[16].

Isolation of Dental Pulp Stem Cells (DPSCs)

DPSCs were isolated from disease-free impacted third molars after obtaining informed consent per ethical guidelines. Teeth were sterilized, sectioned, and pulp extracted, then digested with collagenase/dispase. The cell suspension was cultured in DMEM F12 media with FBS and Penicillin/Streptomycin for expansion.

MTT Assay

Cell viability on the membranes was assessed using an indirect MTT assay. Membrane samples were immersed in DMEM F12 media, and after 24 hours, MTT reagent was added. Formazan crystals formed were dissolved in DMSO, and absorbance was measured at 570 nm.

XRD Analysis

X-ray diffraction (D8 diffractometer, Mg radiation, $\lambda = 1.5406$ Å) was used to evaluate the crystalline structure and verify the presence of magnesium nanoparticles.

In Vitro Osteogenic Potential

The osteogenic activity of the membranes was assessed using Alizarin red staining. MG63 cells were cultured in differentiation media for 14 days, followed by staining with 2% Alizarin Red. Quantitative analysis was performed after dissolving the dye in DMSO and measuring absorbance at 405 nm.

Statistical Analysis

Statistical analysis was performed with all values reported as the mean ± standard error of the mean (SEM) from at least three independent experiments. Differences between groups were assessed using one-way ANOVA, with post hoc comparisons conducted via Scheffe's method, and statistical significance was determined at $p \leq 0.05$.

Results

FTIR

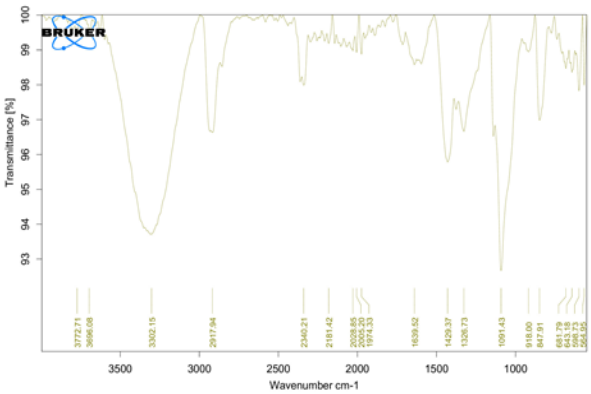

Figure 1. FTIR

Figure 1 presents the FTIR spectrum of MgO nanoparticles synthesized through the combustion method. The spectrum displays a stretching vibration mode around 600-850 cm^{-1}, indicative of Mg–O–Mg bonds. A broad peak observed between 3300-3600 cm^{-1} signifies the formation of the MgO structure. For PVA, the peak at 2917 cm^{-1} corresponds to CH_2 asymmetric stretching, while peaks at 1429 cm^{-1}, 1326 cm^{-1}, and 1091 cm^{-1} are associated with CH_2 bending, C–H deformation, and C–O stretching, respectively.

SEM

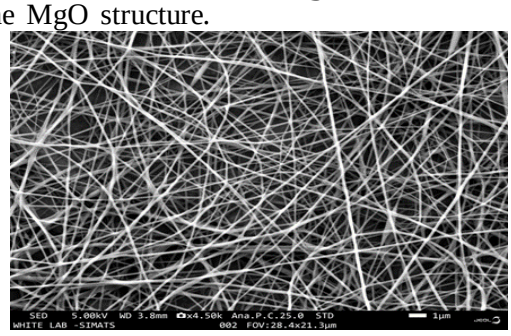

Figure 2. SEM Control Image

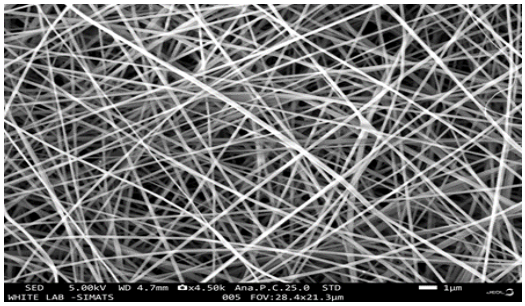

Figure 3. SEM Test Image

SEM results are depicted in Figures 2 and 3 (Figure 2: Control group image, Figure 3: Test group image). The results of Scanning Electron Microscope (SEM) indicate that the porosity has increased, and there has been an increase in fibre diameter.

Contact Angle

The water contact angle is shown in Figure 4. The Water Contact angle of the control group was found to be 20°, and on studying the contact angle for the test group, it was revealed that the water contact angle was approximately 69°, which indicated that the hydrophilicity had decreased. However, the hydrophilic nature of the membrane is still intact (as contact angle <90°).

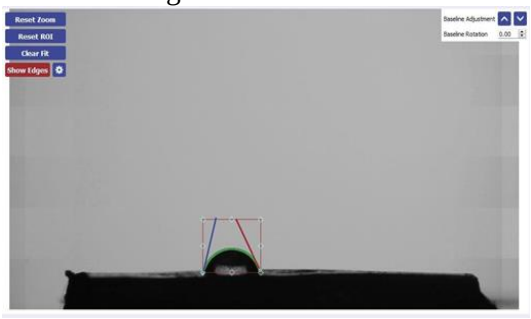

Figure 4. Water Contact Angle

MTT Assay

Figure 5 shows the MTT Assay. The cell viability of the prepared nanomembrane was found to be 89.0%. The PVA membrane shows 100 % cell viability. The membrane retains viability on doping with chitosan and Magnesium.

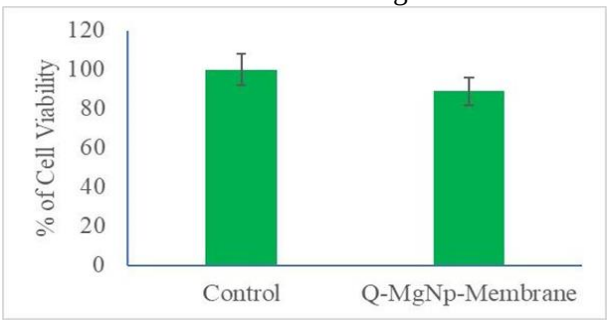

Figure 5. MTT Assay

XRD

The results of X-Ray Diffraction are displayed in Figure 6. As observed in the figure, MgO peaks - 15.97 (degree), 30.614, 38.74, 42.09. According to Balamurugan et al., the cubic structure of MgO can be seen in the XRD pattern, and the crystallite size is 22 nm (15). According to Ercan et al., the XRD data showed that the grain size ranged from 26 to 37 nm.

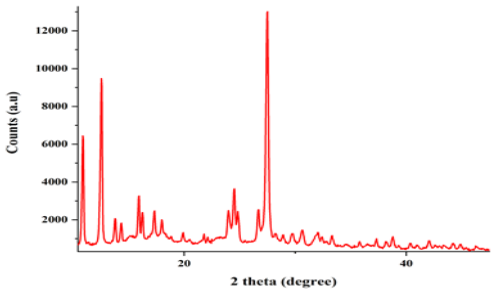

Figure 6. XRD

Bone Formation Assay

Figure 7 shows the bone formation assay using alizarin red staining to assess magnesium-based matrix mineralization. Quercetin, an osteoconductive control, demonstrated a non-significant increase in matrix mineralization with Q-MgO and Q-MgO/B-TCP groups. Our study suggests that surface-coated quercetin on nanoparticles enhances cell absorption and mineralization compared to bulk quercetin.

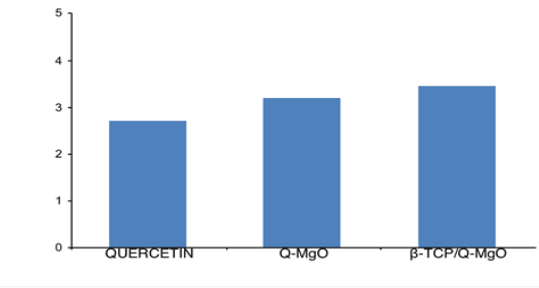

Figure 7. Bone Formation Assay

Discussion

In the study of chitosan scaffolds, it was found that these scaffolds exhibited interconnected open pores with sizes ranging from 300 to 400 µm, a feature that is critical for facilitating cell infiltration and nutrient exchange. When graphene (Gn) was blended into the scaffolds, the pore size was reduced to 250–300 µm, suggesting a denser network that might influence mechanical properties and cell behaviour. The addition of $nSiO_2$ particles further decreased the pore size to 200–250 µm, indicating a potential increase in the material's structural integrity and possibly altering the scaffold's degradation rate and interaction with cells [17, 18].

Chitosan coating is a highly effective method for reducing the initial degradation rate of magnesium alloys, a finding that underscores the importance of chitosan as a protective barrier in biomedical applications. The slower degradation rate is particularly advantageous for maintaining the structural integrity of magnesium implants during the early stages of bone healing. Furthermore, this approach is supported by another study on chitosan-magnesium composites, which not only confirmed the reduction in degradation but also provided compelling evidence of new bone formation around the chitosan-coated Mg-6%Zn-10%Ca (PO_4) composite implant. This study revealed that the chitosan coating significantly enhances the integration of bone tissues, a critical factor for successful bone regeneration. Additionally, the chitosan layer acts as an efficient corrosion-resistant barrier, reducing the hydrogen release from the magnesium matrix during corrosion, which is crucial for minimizing inflammatory responses and ensuring the biocompatibility of the implant [19].

Further research on asymmetric collagen-chitosan membranes highlighted the unique structural composition of these membranes, which consist of a loose collagen layer paired with a dense chitosan layer. This bilayer design is particularly beneficial for guided bone regeneration (GBR), as the dense chitosan layer can act as a barrier to cell infiltration while the loose collagen layer promotes cellular interaction and tissue integration. SEM imaging provided visual confirmation of this structure, and subsequent in vitro studies showed that aspirin-loaded chitosan nanoparticles within these chitosan-collagen membranes (ACS-CCM) could significantly enhance the proliferation of bone mesenchymal stem cells (BMSCs). This finding suggests that the incorporation of bioactive compounds such as aspirin within the scaffold matrix can further enhance the scaffold's osteogenic potential [20].

The performance of magnesium membranes in vivo was particularly noteworthy. The membrane, which was bordered by magnesium metal and ions, was gradually replaced by new bone tissue during the resorption process. This replacement process is ideal for GBR, as it ensures that the membrane provides support only as long as needed before being fully resorbed, leaving behind only healthy tissue. The in vivo performance investigation revealed that the healing response and tissue regeneration facilitated by the magnesium membrane were comparable to those observed with a resorbable collagen membrane, suggesting that magnesium membranes possess all the necessary characteristics for use as a barrier membrane in GBR treatments [21].

Moreover, magnesium ions (Mg^{2+}) play a vital role in the healing process by acting as cofactors for protein and collagen synthesis, which are essential for tissue regeneration. Mg^{2+} ions are also involved in regulating the function of the integrin family, which affects cell differentiation, wound healing, and blood clotting. This multifaceted role of magnesium ions makes them invaluable in the context of bone healing and regeneration, as they contribute to the overall effectiveness of the membrane [22]. The use of magnesium membrane shields in treating compromised extraction sockets has shown significant promise, particularly due to the development of these membranes. The magnesium membrane is unique in its ability to offer a combination of mechanical stiffness, which is critical for promoting osteogenic formation, and malleability, which allows it to conform to defect spaces. Additionally, its resorbability ensures that it does not need to be removed after healing, thereby reducing the need for additional surgical interventions [23].

However, challenges remain, particularly concerning the cytocompatibility of magnesium membranes. A study comparing untreated magnesium and magnesium coated with physical vapour deposition (PVD) found that both types exhibited weak cytocompatibility, with notable formation of gas bubbles in vitro [24]. These gas bubbles, which have been previously identified as indicators of material degradation, were particularly pronounced in the magnesium-coated sample (MGCo), suggesting that the coating may have adversely affected the membrane's performance.

This observation underscores the need for further refinement in the coating processes to enhance the cytocompatibility of magnesium membranes (24). On a positive note, a magnesium-reinforced PLA membrane demonstrated superior mechanical properties compared to a non-reinforced PLA membrane, with these advantages persisting for at least three weeks of immersion. This finding highlights the potential of magnesium reinforcement to improve the mechanical stability of biodegradable membranes, which is crucial for their performance in dynamic biological environments [25].

In summary, while significant progress has been made in developing chitosan-magnesium

composite materials and membranes for bone regeneration, ongoing research is needed to optimize their properties. The incorporation of bioactive compounds, improvement of coating techniques, and careful balance of mechanical and biological properties will be essential to fully realize the potential of these materials in clinical applications.

Conclusion

Chitosan-magnesium composite materials and membranes demonstrate significant potential for advancing bone regeneration therapies, offering a favourable balance of structural support, osteoinductivity, and controlled degradation. While challenges like cytocompatibility issues with certain magnesium coatings persist, the promising results—such as reduced degradation rates and enhanced osteogenesis—underscore their potential for clinical application. Continued research and refinement are needed to optimize these materials for successful use in regenerative medicine, particularly for treating bone defects and compromised extraction sockets.

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Conflict of Interest

Nil.

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