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Fabrication and Characterization of Chitosan/Tricalcium Phosphate/Qurcetin Doped Silver Membranes for Guided Bone Regeneration

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Abstract

This research focuses on the fabrication and characterization of chitosan/tricalcium phosphate/quercetin-doped silver membranes for guided bone regeneration (GBR). The study aims to investigate the potential benefits of these membranes in facilitating the regeneration of bone tissue in areas with bone defects or insufficient bone volume, particularly in dental and orthopaedic surgeries, as well as periodontitis. Material characterization using FTIR, XRD, and SEM was conducted to confirm the functional group, the presence of uniform fibres, and a hydrophilic surface. The study also evaluated the material's biocompatibility and its potential application in periodontitis treatment. The study demonstrated the material's hydrophilic nature, biocompatibility, as well as its potential applications in periodontitis treatment due to its compatibility with cell attachment and nourishment, anti-inflammatory properties, and bone-forming ability. The chitosan/tricalcium phosphate/quercetin-doped silver membranes show promise in guided bone regeneration and potential implications in periodontitis treatment, offering a multifunctional approach for enhancing bone tissue regeneration.

Keywords: *Chitosan, FTIR, Guided Bone Regeneration, Periodontitis, SEM, Silver Nanoparticles.*

Introduction

Periodontitis, an inflammation of the periodontium or gingiva, results in pain, swelling, and infection, and affects 11% of the global population in its severe form. It can lead to tooth loss and diminished quality of life [1]. The complex etiology involves subgingival biofilm triggering an immune response, leading to irreversible periodontal tissue destruction in a susceptible host. Effective management requires understanding the disease's pathogenesis, etiology, risk factors, and treatment protocols, with initial treatment typically involving scaling and root planning [2].

Severe infection in periodontitis presents a major challenge, and no tissue-engineered membrane combining natural and synthetic polymers has yet been fabricated to address this issue. Membranes play a key role in guided bone regeneration (GBR) by preventing non-osteogenic tissues from interfering with bone healing. Various membrane materials are being developed and tested to improve biological outcomes, necessitating a thorough understanding of the mechanisms behind bone regeneration [3].

In orthopaedic applications, the need for bone regeneration continues to rise due to donor shortages and the immune rejection of cartilage substitutes [4]. Researchers are addressing these challenges by developing porous scaffolds for bone transplants. Polyvinyl alcohol (PVA) is a promising biocompatible

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and biodegradable polymer for bone regeneration due to its mechanical and bioadhesive properties [5].

Electrospinning is an emerging technique for creating nonwoven, submicron fibre-based membranes, which are useful in applications such as wound healing and tissue engineering. Chitosan, a biopolymer used for electrospun membranes, has properties like biocompatibility, antibacterial activity, and biodegradability, resembling the extracellular matrix (ECM).[6]. Chitosan membranes loaded with active ingredients like nanoparticles can enhance mechanical stability, degradation, and wound healing. Chitosan is widely used for its healing, hemostatic, and antibacterial properties [7].

Recent advances in nanotechnology have enabled the use of silver nanoparticles, known for their broad-spectrum antiviral, antifungal, and antibacterial properties. Silver nanoparticles can disrupt bacterial cell membranes, increase cell membrane permeability, and inhibit DNA replication through the release of reactive oxygen species [8].

Quercetin, a flavonoid found in fruits and vegetables, shows promise in promoting bone health. Studies in animals suggest that quercetin may protect bone tissue, making it a potential additive for bone regeneration membranes [9].

Biopolymers also offer solutions for chronic or hard-to-heal wounds when autologous skin grafts are not viable, helping to promote natural dermal and epidermal healing [10]. This research aims to develop and evaluate silver membranes doped with chitosan, tricalcium phosphate, and quercetin for guided bone regeneration.

Materials and Methodology

Fabrication of Membrane: A 10% w/v polyvinyl alcohol (PVA) solution was blended with 0.5% β-tricalcium phosphate (β-TCP) and 5 mg/mL of quercetin-doped silver oxide (Q-AgO). The mixture was stirred for 24 hours, then loaded into a 5 mL syringe with a 22 G blunt-end needle, charged at 10 kV. Continuous fibres were extruded at a flow rate of 0.9 mL/h and collected on a plate placed 10 cm from the needle tip. The fibres were subjected to further analysis (Figure 1).

FTIR Analysis: Attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR) was employed to identify chemical interactions and functional group changes in the material after introducing nanoparticles. The analysis was conducted over a wavelength range of 4000–500 cm^{-1} using an Alpha II Bruker spectrometer, validating the predicted functional groups of the scaffolds [11].

SEM Analysis: The morphological characteristics of the GBR membranes were examined using a JSM-IT800 NANO field emission scanning electron microscope (SEM) equipped with JEOL Energetic Dispersive X-ray Spectrometry (EDS). Fibres were extracted from electrospinning sheets using an 8 mm biopsy punch, coated with platinum, and imaged at 2.50kX magnification. SEM micrographs were used to determine the fiber diameter and porosity, analyzed via ImageJ software [12].

XRD Analysis: To confirm the presence of silver nanoparticles within the membrane, X-ray diffraction (XRD) was performed using the D8 diffractometer with Cu radiation ($\lambda = 1.5406$ Å).

Contact Angle: Membrane hydrophilicity was measured by determining the water contact angle using goniometer software. A 1 cm^2 membrane specimen received a 50 µL water droplet, and images were captured to measure the contact angle. The analysis was repeated three times in different positions [13].

Cell Culture: Dental pulp stem cells (DPSCs) were isolated from molars with informed consent and ethical approval from the SIMATS ethics committee. Cells were cultured in Modified Eagle Medium (OMEME12) with penicillin/streptomycin and seeded into 48-well plates at 10,000 cells per well for viability assays.

MTT Assay: A membrane sample containing 1 mg/mL of PVA, β-TCP, and quercetin-doped silver nanoparticles was immersed in DMEM F12 medium for 24 hours [14]. After incubation, 10 μL/100 mL of MTT reagent was added to the cells and incubated at 37°C for 4 hours. Formazan dye was solubilized with DMSO and transferred to a 96-well plate. Absorbance at 570 nm was measured using an ELISA plate reader.

Bone Formation Assay: MG63 and osteoclast cells were cultured for 14 days in DMEMF12 medium containing 10 mM β-glycerophosphate, 0.05 mM ascorbic acid, and silver oxide nanoparticles [15]. Calcium deposition was assessed via alizarin red staining. Cells were washed, incubated with DMSO, and the alizarin red content was measured at 405 nm using a spectrophotometer.

Statistical analysis:

All values are expressed as the mean +/- standard deviation of the mean (SEM) values of at least three separate experiments. Analysis of variance (ANOVA) was used in one way to assess for significant differences. Scheffe's approach was used in many comparisons. At p<0.05, statistical significance was established.

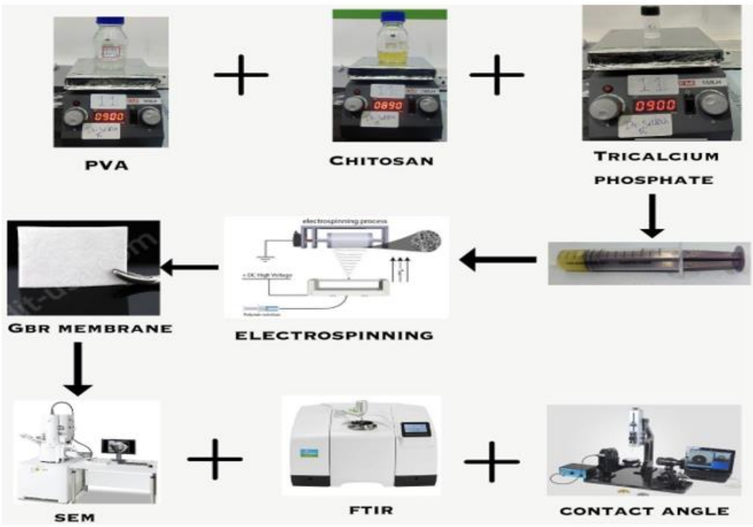

Figure 1. Methodology Utilized for Analyzing the Material

Results

FTIR

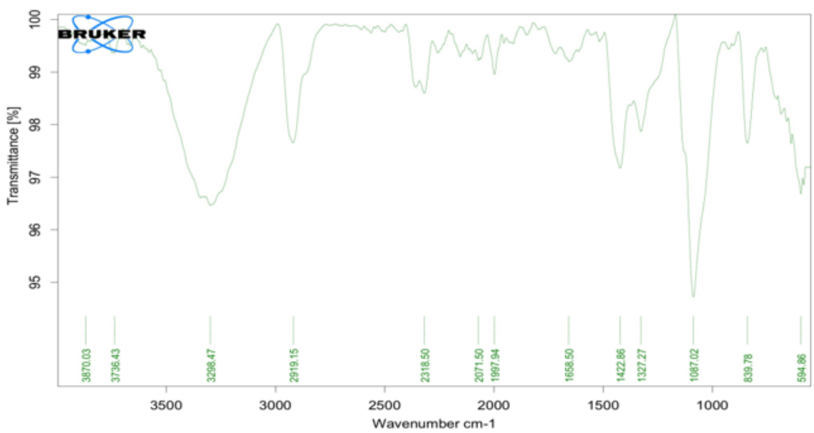

Figure 2. FTIR Analysis

The FTIR spectrum (Figure 2) indicates the successful incorporation of chitosan, tricalcium phosphate, and quercetin into the membrane. The broad peak around 3288 cm^{-1} confirms the presence of chitosan, with its characteristic O-H and N-H stretching vibrations. The peaks around 1658 cm^{-1} and 1422 cm^{-1} further support the presence of chitosan and quercetin, showing Amide I and II bands along with C=C stretching from the aromatic rings. The peaks around 1077 cm^{-1} and 594 cm^{1} confirm the presence of tricalcium phosphate, attributed to P-O stretching and bending vibrations.

The spectrum suggests that the membrane was successfully fabricated with the intended components, each contributing characteristic vibrational modes to the FTIR spectrum. This composition is expected to provide the desired properties for guided bone regeneration, combining the biocompatibility of chitosan, the osteoconductive nature of tricalcium phosphate, and the antioxidant properties of quercetin, potentially enhanced by the presence of silver nanoparticles.

SEM

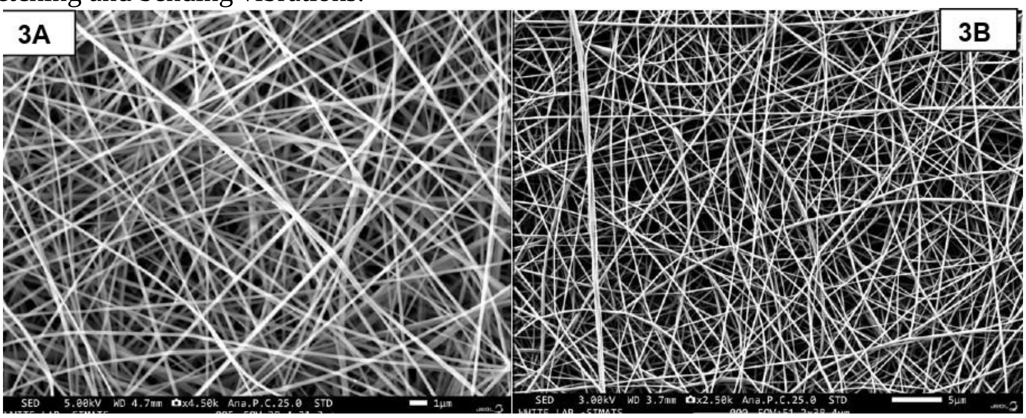

Figure 3. SEM Analysis of Control and Test Groups

The SEM analysis (Figure 3) helps to analyse the surface structure and variations between the control group and the test group. It was visible by the SEM image, and with the addition of Q-AgO nanoparticles in the test group, a reduced porosity was observed than in the control group. In the nanoparticle-incorporated membrane, the diameter of the fibres is also reduced to a great extent. Pore size was found to be 1-2 microns, and nanofiber size was found to be 100-150 nm. This indicates that the membrane is compatible with cell attachment and nutrient flow.

XRD

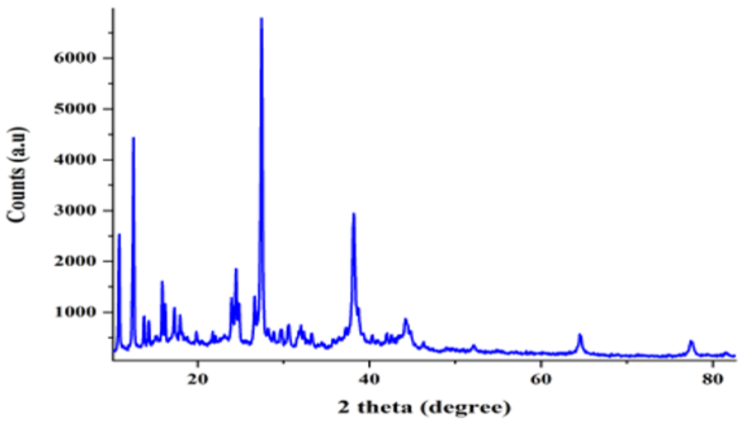

Figure 4. X-ray diffraction spectroscopy

The crystal nature of the membrane has been analysed using XRD analysis. The XRD pattern of the sample is shown in Figure 4. The peaks observed in the as-prepared sample are positioned at 2θ values of 38.19, 64.74, and 77.46, respectively. The results obtained are in perfect agreement with the face-centred cubic structure of AgO. The crystalline characteristics and phase purity of the synthesized material are indicated by the production of strong and sharp peaks and the lack of other peaks.

Contact Angle

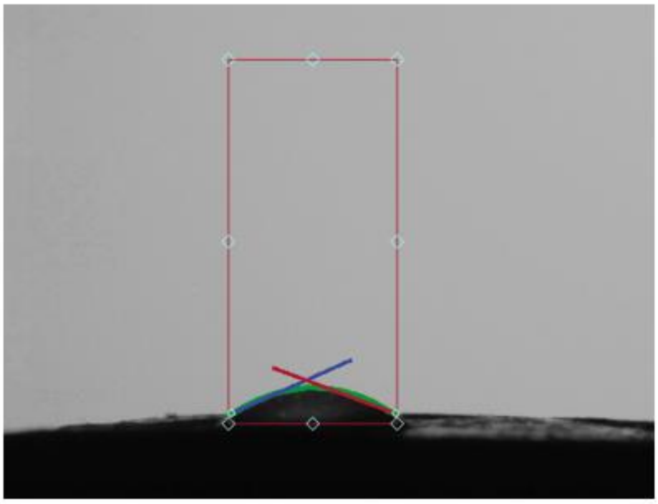

Figure 5. Water Contact Angle of Membrane

Contact angle analysis of the membrane helps to analyse the membrane's hydrophilicity. The contact angle of PVA is found to be 20°. The contact angle of the synthesised membrane was found to be 22.37, which is comparable with that of PVA. A contact angle below 90 ° indicates the hydrophilic nature of the substance, hence proving the hydrophilic and binding nature of the membrane obtained (Figure 5).

Cell Viability/MTT

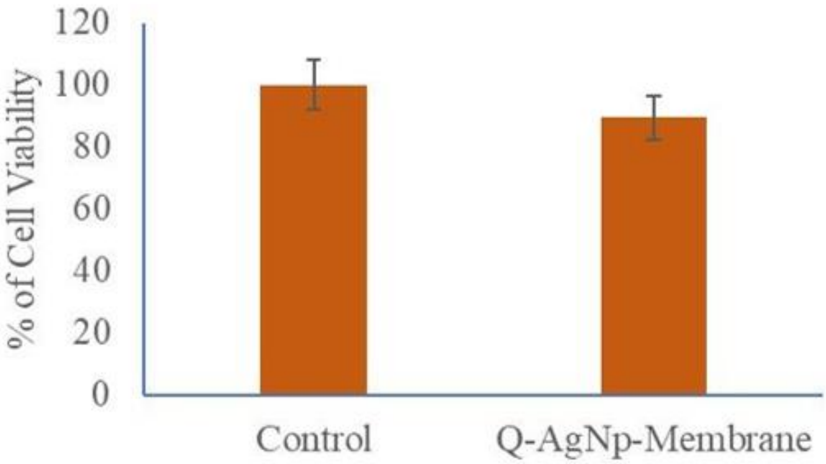

Figure 6. MTT Assay

The cell viability of the region with the membrane was checked, and it was found to be 89.5%, which is not very low in comparison to that of the control group. The control group membrane had PVA and chitosan in it, whereas the test group had silver nanoparticles incorporated in the membrane (Figure 6).

Bone Formation Assay

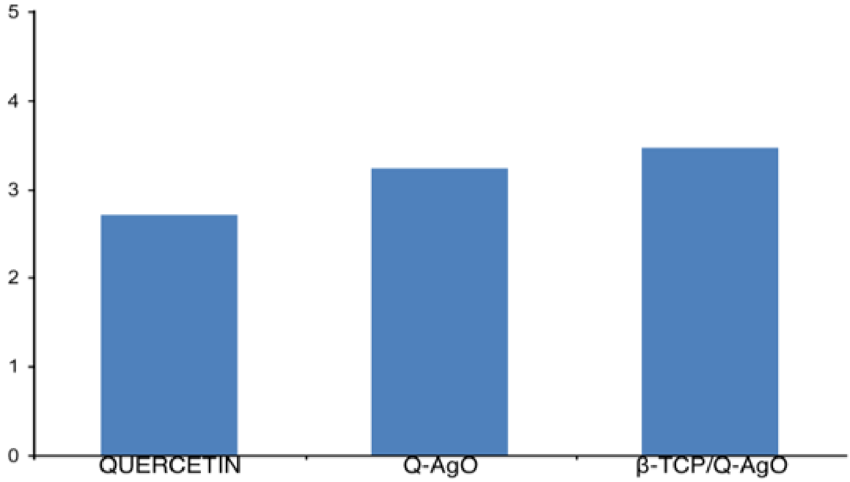

Figure 7. Bone Forming Assay

Alizarin red staining shows the calcium-based matrix mineralisation during the osteogenesis process. Quercetin acts as a positive control, as previous studies have proven to be osteoconductive (Figure 7). The test group Q-AgO nanoparticle and the Q-AgO/B-TCP have shown a non-significant increase in matrix mineralisation than the positive control. Although previous studies have reported that AgO nanoparticles have little effect on bone formation, an increased bone formation was observed in our study. This can be due to the effect of quercetin coated on the surface of the nanoparticles. As the nanoparticles can be internalised by the cell more than the bulk quercetin, there can be enhanced matrix mineralisation.

Discussion

Titanium implants are pivotal in dental and orthopedic applications, especially when combined with guided bone regeneration (GBR) techniques. GBR uses membranes to separate non-osteogenic tissues, promoting an optimal environment for bone repair [16]. The development of asymmetric membranes, which cater to different tissue interfaces, has gained traction. A study by Ma et al. (2015) developed an asymmetric bioabsorbable chitosan membrane, demonstrating excellent biocompatibility and biodegradability, critical for minimizing adverse reactions and supporting healing [17]. The versatility of chitosan is also evident in its application for wound dressing, as demonstrated by Fwu-Long Mi et al. (2003), utilizing phase separation to create asymmetric chitosan membranes.

Incorporating nano-hydroxyapatite (nHA) into chitosan membranes is another major advancement. Studies by Elgali et al. (2017) and Huang et al. (2018) demonstrated that nHA/CS composites enhance mechanical strength and osteogenic activity. SEM and mechanical testing confirmed the firm integration of nHA spheres within the chitosan matrix, essential for maintaining these enhanced properties throughout bone regeneration [18].

Guided tissue regeneration (GTR) and GBR both aim to promote tissue regeneration using biodegradable membranes. Poly (lactic-co-glycolic acid) (PLGA) membranes, patterned with thermal nanoimprinting techniques, exemplify the potential of customizing membrane surfaces for specific regenerative purposes. Studies using scanning electron and laser microscopy assessed surface topographies, while contact angle analysis revealed crucial insights into hydrophilicity—key for cell adhesion and proliferation [19].

Hydrophilicity is influenced by material composition. For instance, the addition of β-tricalcium phosphate (β-TCP) nanoparticles into polycaprolactone/poly (glycerol sebacate) (PCL/PGS) nanofibers increased hydrophilicity, promoting cell adhesion. Similarly, adding chitosan to PCL/PGS further reduced contact angles, enhancing membrane performance in bone regeneration [20, 21].

These findings highlight the potential to tailor GBR membranes through material integration and advanced fabrication techniques. Future research on asymmetric membranes and nanotechnology-enhanced composites, such as nHA/CS, could significantly improve the osteogenic potential of these membranes, offering better clinical outcomes for regenerative therapies.

Conclusion

The integration of titanium implants with guided bone regeneration (GBR) has marked a significant advancement in regenerative medicine, particularly through the use of specialized membranes. The development of asymmetric, bioabsorbable, and nanocomposite membranes, such as those incorporating chitosan and nano-hydroxyapatite, has enhanced the biocompatibility, mechanical strength, and osteogenic potential of these materials. Moreover, innovations like thermal nanoimprinting and the incorporation of hydrophilic materials have further optimised the surface properties of GBR membranes, promoting better cell adhesion and proliferation. These advancements underscore the importance of interdisciplinary research in creating more effective and adaptable GBR membranes, which are crucial for improving clinical outcomes in dental and orthopaedic applications. As this field continues to evolve, the potential for even more refined and patient-specific solutions remains a promising frontier in regenerative therapies.

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Conflict of Interest

Nil.

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