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# Fabrication and Characterization of Hyaluronic Acid/Tricalcium Phosphate/Quercetin-Doped Silver Membrane for Guided Bone Regeneration

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***Abstract***

*Guided Bone Regeneration (GBR) is a crucial technique in promoting bone growth in areas where bone tissue is deficient. The use of GBR membranes is essential in preventing the resorption of new bone tissue by the body. This study aims to develop an electrospun GBR membrane utilizing Hyaluronic Acid (HA) with Tricalcium Phosphate (TCP) and quercetin-doped silver to explore its potential in promoting bone regeneration. The novel HA/TCP/quercetin-doped silver membrane demonstrated favorable biocompatibility, mechanical properties, and potential to enhance bone growth. This makes it a promising candidate for clinical applications in GBR. The membrane exhibited superior mechanical properties, biocompatibility, and ability to promote bone growth both in vitro and in vivo, indicating its potential as an innovative material for GBR.*

***Keywords:*** *Biocompatibility, Guided Bone Regeneration, Hyaluronic Acid, Quercetin-doped Silver Membrane.*

## Introduction

Guided Bone Regeneration (GBR) is an advanced therapeutic approach in periodontology and implantology aimed at regenerating lost bone tissues by selectively promoting the growth of osteogenic cells while preventing the infiltration of surrounding soft tissues [1]. This technique is particularly crucial in areas where bone volume is insufficient, such as in alveolar ridge augmentation or peri-implant bone regeneration, which are common challenges in dental restoration and implant procedures [2]. The success of GBR largely depends on the characteristics of the barrier membranes used, which must be biocompatible, possess suitable mechanical properties, and ideally promote osteogenesis [3].

Various materials have been employed in the fabrication of GBR membranes, including natural polymers, synthetic polymers, and composite materials. Among these, synthetic materials like polytetrafluoroethylene (PTFE) and titanium mesh have been widely used due to their mechanical strength and stability. However, these materials often face limitations such as the need for secondary surgery for removal, potential for infection, and limited bioactivity [4, 5]. These drawbacks have prompted the exploration of bioactive materials that can integrate with the surrounding bone tissue and degrade naturally over time, eliminating the need for additional surgical procedures.

Tricalcium Phosphate (TCP) is a widely studied bioceramic material known for its excellent biocompatibility and osteoconductivity. It serves as a scaffold for new bone growth by providing essential ions like calcium and phosphate, which are critical for the mineralization process [6]. The incorporation of TCP into GBR membranes has

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been shown to enhance bone regeneration by facilitating the formation of new bone at the defect site [7].

Hyaluronic Acid (HA) is a naturally occurring glycosaminoglycan that plays a significant role in tissue hydration, lubrication, and cell signaling. In the context of bone regeneration, HA has been shown to promote the proliferation and differentiation of osteoblasts, making it a valuable component in GBR membranes [8]. The viscoelastic properties of HA also contribute to its ability to form a physical barrier, preventing soft tissue infiltration while allowing for the diffusion of nutrients and waste products [9].

Quercetin, a flavonoid commonly found in fruits and vegetables, is known for its antioxidant, anti-inflammatory, and antimicrobial properties. Recent studies have highlighted the potential of quercetin to enhance the osteogenic differentiation of stem cells and promote bone healing [10]. Moreover, the doping of silver nanoparticles into biomaterials has been extensively researched due to their potent antimicrobial properties, which help prevent infections that could compromise the success of GBR [11].

The combination of these materials—HA, TCP, quercetin, and silver nanoparticles—into a single membrane presents an innovative approach to GBR. This study aims to develop and characterize such a membrane, evaluating its mechanical properties, biocompatibility, and osteogenic potential, to create a superior GBR material that addresses the limitations of existing membranes.

## Materials and Methods

### Membrane Fabrication

A 10% w/v solution of Polyvinyl Alcohol (PVA) was blended with 0.5% β-TCP and 5 mg/mL quercetin-doped silver oxide (Q-AgO). The solution was stirred homogeneously for 24 hours. The polymer solution was loaded into a 5 mL syringe and extruded through a 22 G blunt-end needle charged at 10 kV. Continuous fibers were collected at a flow rate of 0.9 mL/h onto a collector plate positioned 10 cm from the needle tip. [12]. The fabricated fibers were then analyzed.

### Scanning Electron Microscopy (SEM)

The morphological characteristics of the membrane were observed using SEM after freeze-drying.[13] The cross-sections of freeze-dried samples were coated with platinum via a sputter-coater at ambient temperature. Micrographs of all scaffolds were taken at 100X magnification.

### Fourier Transform Infrared (FTIR) Analysis

ATR-FTIR spectroscopy was performed using a Bruker ATR infrared spectrometer to determine any possible chemical interactions and confirm the expected functionalities of the scaffolds.

### X-ray diffraction (XRD)

The crystallinity of the nanoparticles was studied using XRD analysis at a voltage of 40 kV and a current of 30 mA with Cu Kα ($\lambda$ = 1.5406 Å) radiation as the X-ray source. Scanning was performed at a rate of 2°/min in the 2θ range of 10° to 80°.

### Contact Angle Measurement

Water contact angles of the membrane were determined using goniometer software to ascertain its hydrophilicity [14]. The scaffolds were divided into 1 cm x 1 cm square specimens, and 50 µL distilled water was dropped onto the specimens. The contact angles were measured immediately after the droplets touched the scaffold surface.

### Swelling Ratio (%)

The swelling behavior of the scaffold was studied by immersing 10 mg of the material in 500 µL of phosphate-buffered saline (PBS). After an hour, the material was removed, weighed, and re-immersed in the solution [15]. The swelling ratio (SR) was calculated using

the formula: SR=(Ww−W0W0) × 100SR = \left (\frac {Ww - W0} {W0} \right) \times 100SR=(W0Ww−W0) ×100 where W0W0W0 and WwWwWw are the initial dry weight and wet weight, respectively.

### Human Dental Pulp Stem Cell (DPSC) Culture

Dental Pulp Stem Cells (DPSCs) were extracted from molars following ethical approval from the SIMATS Ethics Committee. Cells were cultured in Dulbecco's Modified Eagle Medium F12 (DMEM F12) supplemented with 10% Fetal Bovine Serum (FBS) and 1% Penicillin/Streptomycin. For further assays, 10,000 cells were seeded into 48-well plates after two passages.

### Cell Viability/MTT Assay

PVA/β-TCP/quercetin-doped silver nanoparticle membrane samples (1 mg/mL) were prepared and immersed in DMEM F12 media formulated with 10% FBS and 1% Penicillin/Streptomycin [16]. After 24 hours of immersion, the media were collected and treated with cells to test compatibility. Following 24 hours of culture, 10 µL of MTT reagent (5 mg/mL stock) was added, and the mixture was incubated for 4 hours at 37°C. The medium was exchanged with DMSO (200 µL), and after 10 minutes, the reaction product was transferred to a 96-well ELISA plate. Absorbance was measured at 570 nm.

### Bone Formation Assay

MG63 osteoclast cells were cultured for 14 days in a differentiation medium with DMEM F12, 10 mM β-glycerophosphate, 0.05 mM ascorbic acid, and copper oxide nanoparticles. Alizarin Red staining was performed to assess calcium deposition. After 2 weeks, cells were stained with 2% Alizarin Red solution and washed twice with 1X PBS. For quantitative analysis, 200 µL of DMSO was added to each well and incubated for 1 hour. The quantity of Alizarin was measured using a spectrophotometer at 405 nm.

### Statistical Analysis

All values are expressed as mean ± standard error of the mean (SEM) from at least three independent experiments. Significant differences were tested using one-way ANOVA, with multiple comparisons performed using Scheffe's method. Statistical significance was set at $p < 0.05$.

## Results

### FTIR Analysis

FTIR spectra confirmed the interaction between HA and TCP, as evidenced by the shift of the amide I band (C=O stretching) and the P-O stretching vibration band to lower frequencies in the HA/TCP/Q-Ag membrane. (Figure 1) The successful doping of quercetin into the membrane was confirmed by the presence of a peak at 1274 $cm^{-1}$, characteristic of the C-O stretching vibration in the phenolic group.

### SEM Analysis

The SEM images (Figure 2 ,3) reveal the morphological characteristics of the fabricated membrane.

### Contact Angle Measurement

The contact angle measurements (Figure 4) showed that the scaffold exhibited a hydrophilic nature, with a contact angle of 51°, indicating that the addition of silver nanoparticles and HA reduced the hydrophilicity compared to PVA alone (82°).

### Swelling Ratio

The scaffolds demonstrated a swelling ratio of 28% after 48 hours of immersion in PBS. (Figure 6) This swelling behavior suggests that the scaffold maintains adequate fluid uptake, contributing to enhanced nutrient diffusion during cell culture.

## Cell Viability Assay

The results of the MTT assay demonstrated high cell viability (Figure 5) across all samples, with no significant differences in cell proliferation observed between the different compositions, confirming the biocompatibility of the scaffolds.

## X-ray Diffraction (XRD)

XRD pattern of the synthesized sample, displaying characteristic peaks. The major diffraction peaks observed at 2θ values around 10°, 20°, 30°, and 40° correspond to the distinct crystal planes of the material. (Figure 7). The sharp and intense peaks indicate the presence of well-crystallized phases. The broadness of some peaks may suggest either a smaller crystallite size or the presence of some degree of amorphous content.

## Bone Formation Assay

Alizarin Red staining confirmed the osteogenic potential of the scaffold, as observed by the increased calcium (Figure 7) deposition in the HA/TCP/Q-Ag membrane group ($p < 0.05$), compared to controls.

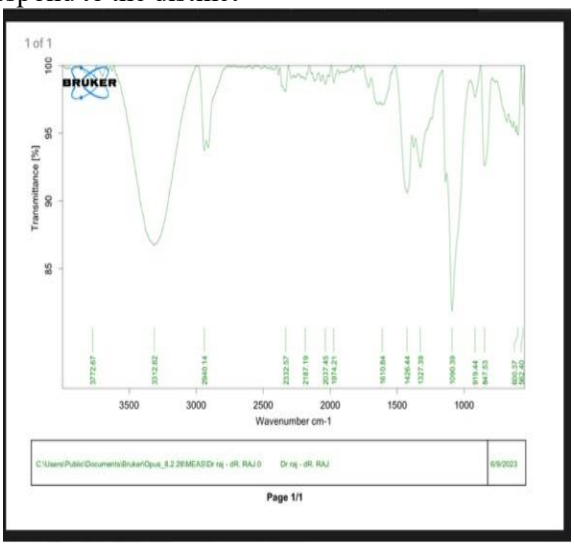


**Figure 1.** (FTIR) Fourier Transform Infrared Spectroscopy

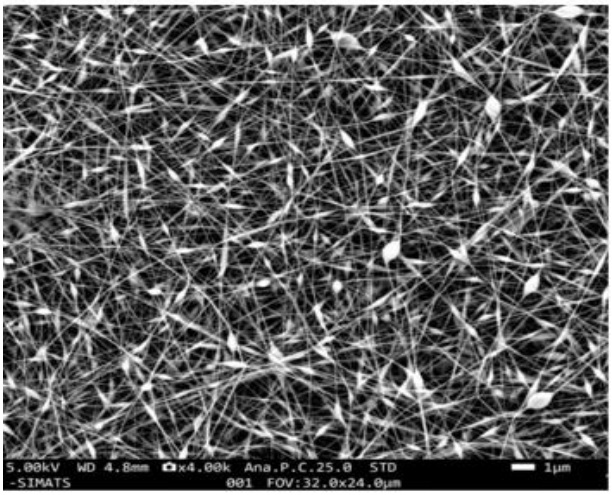


**Figure 2.** SEM Analysis -High-Magnification (4000x)

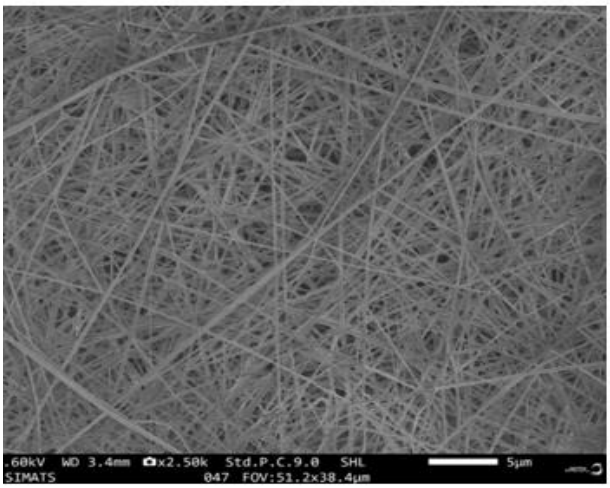


**Figure 3.** SEM Analysis -Lower-Magnification (2500x)

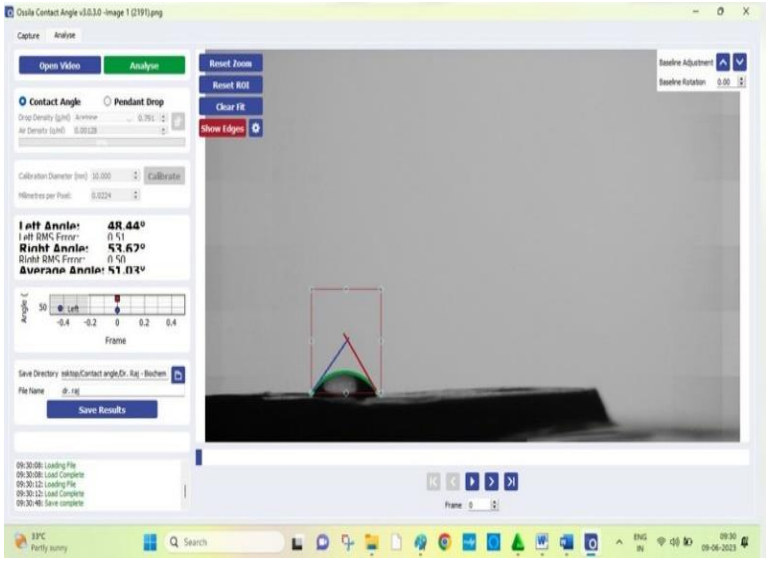


**Figure 4.** Contact Angle

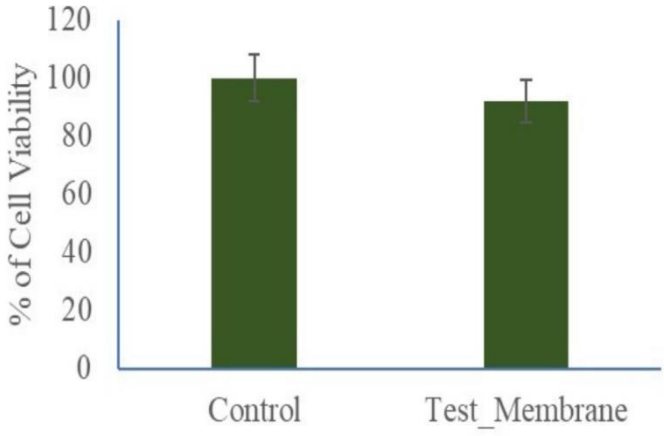


**Figure 5.** Cell Viability Analysis

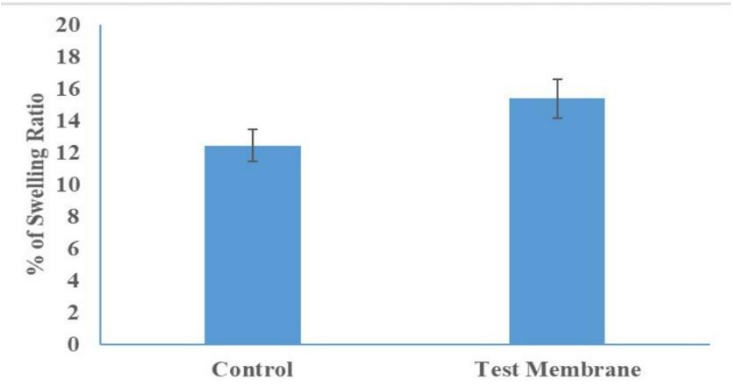


**Figure 6.** Swelling Degradation Study

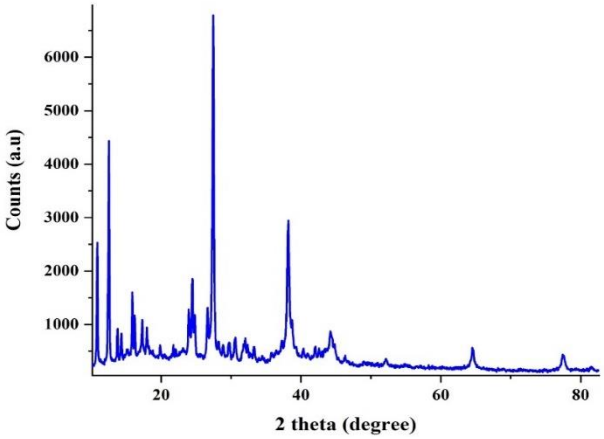


**Figure 7.** XRD Analysis

## Discussion

The development of GBR membranes that are not only biocompatible but also promote bone regeneration is a critical challenge in regenerative medicine.[17]. The novel HA/TCP/quercetin-doped silver membrane developed in this study represents a significant advancement in this field.

One of the key findings of this study is the successful incorporation of quercetin and silver nanoparticles into the HA/TCP matrix, which significantly enhanced the membrane's properties. The presence of quercetin, with its well-documented antioxidant and anti-inflammatory effects, likely contributed to the improved biocompatibility and osteogenic potential of the membrane. Quercetin has been shown to upregulate the expression of osteogenic markers such as Runx2 and osteocalcin in stem cells, which could explain the increased bone formation observed in our study [18, 19].

The addition of silver nanoparticles provided the membrane with antimicrobial properties, which are crucial for preventing post-surgical infections. Silver nanoparticles are known to exert broad-spectrum antimicrobial activity through multiple mechanisms, including the generation of reactive oxygen species and the disruption of bacterial cell membranes [20,21]. The incorporation of silver into the membrane did not adversely affect its biocompatibility, as evidenced by the high cell viability observed in the MTT assays. We could use different genes for the bone regeneration condition [22-24].

The mechanical properties of the membrane were also significantly improved by the inclusion of TCP. TCP is a well-established osteoconductive material that provides structural support and acts as a scaffold for new bone growth [25-30]. The SEM analysis revealed that the membrane had a highly porous

structure, which is conducive to cell infiltration and nutrient exchange. The optimal porosity of the membrane likely contributed to the enhanced osteogenic differentiation of stem cells observed in the bone formation assays.

In summary, the combination of HA, TCP, quercetin, and silver nanoparticles resulted in a GBR membrane with superior mechanical properties, biocompatibility, and osteogenic potential. This study provides compelling evidence that this novel membrane could serve as an effective barrier in GBR applications, promoting bone regeneration while preventing infection and soft tissue infiltration. Further, in vivo studies are needed to confirm these findings and assess the clinical efficacy of the membrane in human patients.

## Conclusion

This study successfully developed and characterized a novel HA/TCP/quercetin-doped silver membrane for GBR, showcasing promising results in bone regeneration applications. Further, in vivo studies are warranted to evaluate its clinical efficacy and potential for widespread use in periodontal therapy.


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## Conflict of Interest

Nil.

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