Innovative High Molecular Weight Chitosan, AgO and Hyaluronic Acid Based Gbr Membranes: Fabrication and Physicochemical Insights

P Rithanya1 , K.Dharanya1,a)

1Rithu Health Centre, Trichy, Tamil Nadu, India

Corresponding Author: a)[dharanya17bds@gmail.com](mailto:dharanya17bds@gmail.com)

**ABSTRACT:** Guided bone regeneration (GBR) and guided tissue regeneration (GTR) are essential techniques in periodontal therapy aimed at restoring periodontal attachment, including cementum, periodontal ligament, and alveolar bone lost due to periodontal disease. Barrier membranes play a crucial role in these procedures by preventing non-osteogenic tissues from interfering with bone regeneration. Silver oxide (AgO) nanoparticles, known for their antimicrobial properties, have been incorporated into biomaterials to enhance GBR membrane functionality. This study focuses on the fabrication and physicochemical characterization of AgO-based GBR membranes using various biopolymers such as chitosan, hyaluronic acid, gelatin, and carrageenan.AgO nanoparticles (10 mg) were added to the polymeric solution for the test group. The homogeneous mixture (3 mL) was transferred to six-well plates, followed by the addition of 100 µL of TPP (15%) crosslinking agents. The samples underwent a freeze-drying process at -20°C for 12 hours, followed by storage at -80°C. After lyophilization for 24 hours, the membranes were subjected to physicochemical characterization using Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), contact angle analysis, swelling behavior assessment, and biocompatibility testing. The osteogenic potential of the fabricated membranes was evaluated using alizarin red staining.SEM analysis revealed that the fabricated GBR membranes had a rough and porous structure, with pore sizes averaging 35.3 ± 9 μm. The incorporation of AgO nanoparticles increased porosity and improved membrane hydrophilicity, as evidenced by a contact angle of 80.49°. FTIR analysis confirmed the presence of AgO nanoparticles, with characteristic peaks observed at 3626.40, 2350.90, 1552.93, and 765.55 cm⁻¹. Swelling properties were comparable to the control (HA), indicating suitability for hemostatic applications. Biocompatibility analysis demonstrated over 90% cell viability, and osteogenic studies showed effective bone formation potential, comparable to positive controls.The study successfully fabricated a hybrid resorbable GBR membrane incorporating AgO nanoparticles. The membrane exhibited favorable physicochemical properties, high biocompatibility, and osteoconductive potential, making it a promising material for periodontal and bone regeneration applications.

**KEYWORDS:**Guided Bone Regeneration (GBR), Silver Oxide Nanoparticles, Hybrid Membrane, Osteoconductivity, Biopolymer Scaffold, Bone Regeneration.

# INTRODUCTION

Guided Tissue Regeneration (GTR) and Guided Bone Regeneration (GBR) membranes play a crucial role in periodontal therapy and bone augmentation by acting as physical barriers that prevent soft tissue invasion into bone defects.[(Lynch, 2008)](https://paperpile.com/c/tpFG38/pSDX) These membranes facilitate the proper regeneration of bone and periodontal structures, promoting the healing process.[(Khang, 2017)](https://paperpile.com/c/tpFG38/kciy) Various materials, including natural polymers like collagen, chitosan, and gelatin, as well as synthetic options such as polytetrafluoroethylene (PTFE) and polylactic acid (PLA), have been used in membrane fabrication. [(Briguglio et al., 2013)](https://paperpile.com/c/tpFG38/jOFC)However, conventional membranes present several challenges, such as bacterial adherence, rapid degradation, and limited bone-filling capacity, which can compromise their clinical efficacy. [(Rakhmatia et al., 2013)](https://paperpile.com/c/tpFG38/wmXB)[(Chokkattu et al., 2023; Dharman et al., 2023; Govindaraj & Shanmugam, 2023)](https://paperpile.com/c/tpFG38/WP90r+GpgzE+0HGh9)To overcome these limitations, researchers are exploring innovative biomaterials that offer enhanced regenerative and antibacterial properties. [(Kabilamurthi et al., 2021)](https://paperpile.com/c/tpFG38/yUxf)Natural polysaccharides such as hyaluronic acid (HA) and carrageenan have shown significant potential due to their biocompatibility and ability to support cell adhesion, proliferation, and differentiation.[(Kalluri & Duan, 2022)](https://paperpile.com/c/tpFG38/Tko3) High molecular weight HA possesses anti-inflammatory properties, while carrageenan plays a role in osteogenesis, making it a valuable addition to GBR membranes. Additionally, gelatin, a denatured form of collagen, is widely used in tissue engineering because of its ability to promote cell attachment and tissue regeneration.[(Oshida, 2021)](https://paperpile.com/c/tpFG38/eA8H)[(Rajeshkumar & Lakshmi, 2021; Sivakumar et al., 2021)](https://paperpile.com/c/tpFG38/zanXQ+7s5Id)One of the major concerns in GBR procedures is bacterial colonization, which can lead to premature membrane degradation and implant failure. [(Rothamel et al., 2014)](https://paperpile.com/c/tpFG38/UkL3)Microbial contamination at the surgical site may hinder bone regeneration by interfering with osteoblast activity and increasing the risk of infection.[(Mathew & Varun Menon, 2018)](https://paperpile.com/c/tpFG38/2Qgg) To address this, silver nanoparticles (AgNPs) and silver oxide (AgO) have been incorporated into GBR membranes due to their strong antimicrobial properties.[(Shin et al., 2025)](https://paperpile.com/c/tpFG38/dKg0)[(Doshi et al., 2023; Lampl et al., 2023; Pandiyan et al., 2023)](https://paperpile.com/c/tpFG38/EKcQp+Fcen2+RrXRv) These nanoparticles help reduce bacterial adherence, minimize infection risks, and enhance wound healing, thereby improving the overall success of periodontal and implant treatments.[(Y. Zhu et al., 2022)](https://paperpile.com/c/tpFG38/8182)[(Gandhi et al., 2021; Katyal et al., 2023; Priyadharshini et al., 2023)](https://paperpile.com/c/tpFG38/SnDSL+pWuPc+u6PYf)Recent advancements in biomaterial technology have led to the development of biomimetic, nanofibrous, and functionally graded membranes that closely replicate the extracellular matrix (ECM). setghiyaBy mimicking the ECM structure, these next-generation membranes provide improved mechanical strength, controlled degradation rates, and better cellular interactions, making them more effective in supporting tissue regeneration.[(Mathew & Varun Menon, 2018; Yuan, 1992)](https://paperpile.com/c/tpFG38/2Qgg+MMJE) The incorporation of nanotechnology also enhances the physical and biological properties of these membranes, ensuring their long-term stability in clinical applications.[(Motta & Migliaresi, 2006)](https://paperpile.com/c/tpFG38/9fzn)[(Janani et al., 2021; Kachhara et al., 2021; Subramanian et al., 2023)](https://paperpile.com/c/tpFG38/Kb65b+xg5j8+HZ89E)Moreover, the integration of osteoconductive and osteoinductive materials in GBR membranes has been explored to enhance bone regeneration.[(Harikrishnan et al., 2021)](https://paperpile.com/c/tpFG38/OlUx) These bioactive materials stimulate osteoblast differentiation, promote new bone formation, and improve overall treatment outcomes.[(Harikrishnan et al., 2021; Kabilamurthi et al., 2021)](https://paperpile.com/c/tpFG38/OlUx+yUxf)[(Pavithra et al., 2023; Shenoy et al., 2023; Thomas & Jain, 2023)](https://paperpile.com/c/tpFG38/PAaBR+csPJE+oeDn2) For example, deproteinized bovine bone mineral, when combined with a specialized collagen membrane, has been shown to significantly enhance periodontal regeneration by promoting the formation of new cementum, periodontal ligament, and alveolar bone.Hyaluronic acid, a primary component of the ECM, plays a vital role in tissue repair by regulating inflammation, cell migration, and angiogenesis. [(Fernandes-Cunha et al., 2023)](https://paperpile.com/c/tpFG38/fnJd)The molecular weight of HA significantly influences its biological properties, with high molecular weight HA exhibiting anti-inflammatory effects and low molecular weight HA inducing pro-inflammatory responses. [(Gopal, Rohinikumar, & Nesappan, 2020; Gopal, Rohinikumar, N, et al., 2020)](https://paperpile.com/c/tpFG38/elbw+R63N)Research suggests that high molecular weight HA can bind to cellular receptors such as CD44, suppress inflammatory pathways, and downregulate the expression of inflammatory mediators like NF-κB, MMPs, and IL-8.[(Kokubo, 2008)](https://paperpile.com/c/tpFG38/exWY) These properties make HA a valuable addition to GBR membranes for periodontal regeneration.Advancements in biomaterials and regenerative medicine continue to drive improvements in GBR membranes. [(Aldecoa & Ortiz, 2001; Mathew & Varun Menon, 2018; Yuan, 1992)](https://paperpile.com/c/tpFG38/2Qgg+MMJE+D89l)[(Ramsundar et al., 2023; Rieshy et al., 2023; Singh et al., 2023)](https://paperpile.com/c/tpFG38/uEY2U+YRWVO+AunVd)Future research is focused on developing multifunctional, resorbable membranes that integrate antibacterial properties and enhanced regenerative capabilities.[(Gopal, Rohinikumar, & Nesappan, 2020)](https://paperpile.com/c/tpFG38/elbw) By combining bioactive molecules, osteogenic materials, and nanotechnology, researchers aim to create GBR membranes that not only prevent infection but also optimize bone and tissue regeneration.[(Miguel Oliveira & Reis, 2019)](https://paperpile.com/c/tpFG38/oDMZ) These innovations hold great promise for improving the clinical success of periodontal and maxillofacial treatments, ultimately benefiting patients with severe bone and tissue defects.[(Rajeshkumar & Lakshmi, 2021; Sivakumar et al., 2021)](https://paperpile.com/c/tpFG38/zanXQ+7s5Id)

# MATERIALS AND METHODS

## Fabrication of Scaffolds

The scaffolds were fabricated using a blend of 1% high molecular weight hyaluronic acid, 0.5% carrageenan, and 1% gelatin, mixed in a 6:1:3 ratio. For the experimental group, 10 mg of silver oxide (AgO) nanoparticles were incorporated into the mixture. A total of 3 mL of the homogeneous solution was poured into six-well plates, followed by the addition of 100 μL of 15% sodium tripolyphosphate (TPP) as a crosslinking agent. The prepared samples were subjected to a two-step freezing process, initially at -20°C for 12 hours, then at -80°C overnight. After freezing, the samples were lyophilized for 24 hours and stored under dry conditions for further experimentation.

## SEM Analysis

Scanning electron microscopy (SEM) was used to analyze the morphological characteristics of the scaffolds after freeze-drying. The cross-sections of the freeze-dried scaffolds were coated with platinum using a sputter-coater at room temperature to enhance image resolution. SEM images of the scaffolds were captured at 100X magnification to assess their microstructure and surface topology.

## Fourier Transform Infrared (FT-IR) Spectroscopy

The chemical composition and functional groups present in the scaffolds were determined using attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR). The analysis was conducted with a Bruker ATR infrared spectrometer to identify any chemical interactions between scaffold components. The FT-IR spectra confirmed the presence of characteristic functional groups, verifying the expected chemical structure of the fabricated scaffolds.

## Contact Angle Measurement

The hydrophilicity of the scaffolds was evaluated by measuring their water contact angles using goniometer software. The scaffolds were cut into 1 cm × 1 cm square specimens and placed on the testing plate. A 50 μL droplet of distilled water was carefully deposited onto each scaffold, and images were captured immediately (within 2 seconds) to record the contact angles. Each scaffold was measured at three different positions to ensure accuracy.

## Swelling Ratio of Scaffolds

The swelling properties of the scaffolds were assessed by determining their water uptake capacity. Freeze-dried scaffold samples (10 mg) were immersed in 500 μL of phosphate-buffered saline (PBS) at 37°C. After 24 hours, the samples were removed from PBS, gently blotted with a Kimwipe to eliminate excess surface moisture, and weighed. The swelling ratio (SR) was calculated using the formula:where represents the initial dry weight of the scaffold, and denotes the wet weight after immersion. All experiments were conducted six times to ensure reproducibility.

## Dental Pulp Stem Cell (hDPSC) Culture

Human dental pulp stem cells (hDPSCs) were obtained following ethical approval from the SIMATS ethics committee and informed patient consent. The cells were isolated from molars and cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. After two passages, 10,000 cells per well were seeded in 48-well plates for cell viability and compatibility assays.

## Bone Formation Assay

Osteogenic differentiation of the scaffolds was assessed using MG-63 osteoblast cells, which were cultured for 14 days in a differentiation medium containing the test solution, 10 mM β-glycerophosphate, 0.05 mM ascorbic acid, and DMEM/F12. Calcium deposition was evaluated using alizarin red staining. After 14 days, the cells were stained with a 2% alizarin red solution for 10 minutes, followed by two washes with PBS. For quantitative analysis, 200 μL of DMSO was added to each well, and the plates were incubated for one hour. The alizarin red concentration was measured using a spectrophotometer at 405 nm.

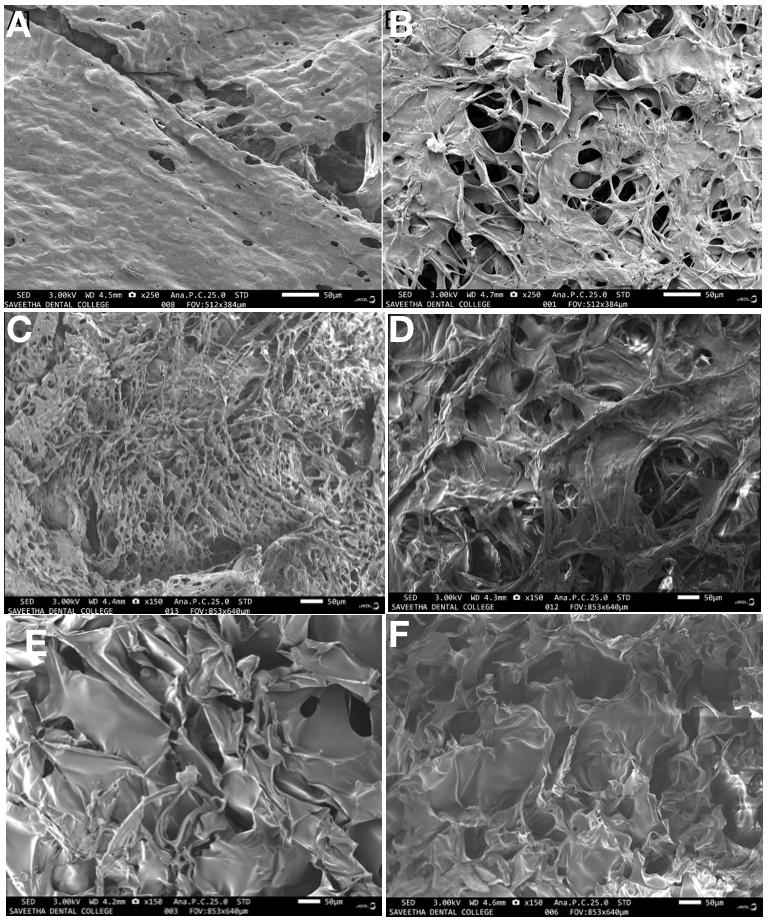
## Biocompatibility Analysis (MTT Assay)

To evaluate the biocompatibility of the scaffolds, 100 mg of 5 mm cylindrical blocks were immersed in DMEM-low glucose medium supplemented with 10% FBS and 1% penicillin-streptomycin. Media samples were collected after 24 hours and seven days of immersion and used to treat cultured cells. Following 24 hours of incubation, 10 μL of MTT reagent (5 mg/mL stock) was added to each well, and the plates were incubated for an additional four hours at 37°C. The medium was then replaced with 200 μL of dimethyl sulfoxide (DMSO) and left to stand for 10 minutes. The reaction product was transferred to a 96-well ELISA plate, and absorbance at 590 nm (A590) was measured using an ELISA plate reader.

# Statistical Analysis

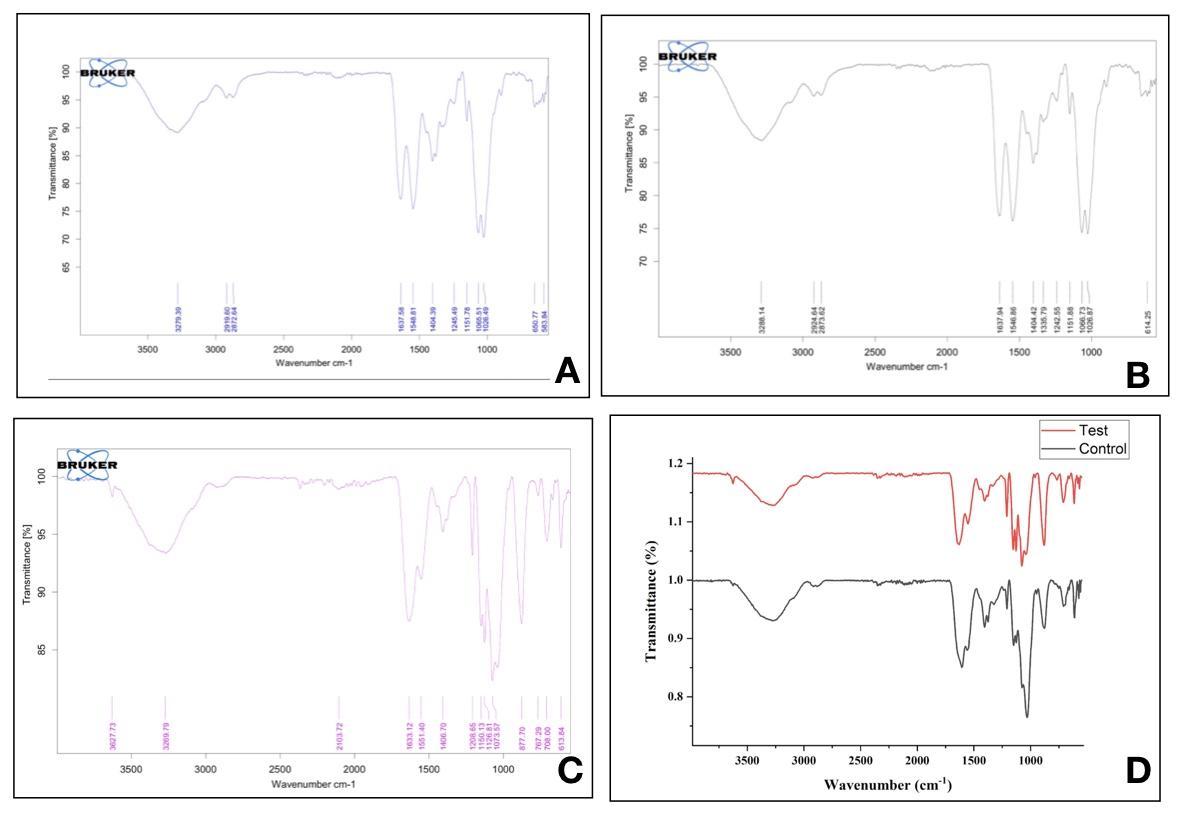
All experimental values were presented as mean ± standard error of the mean (SEM), calculated from at least three independent experiments. One-way analysis of variance (ANOVA) was employed to determine statistical significance, and multiple comparisons were conducted using Scheffe’s post hoc method. A significance level of p < 0.05 was considered statistically significant.

# RESULTS AND DISCUSSION

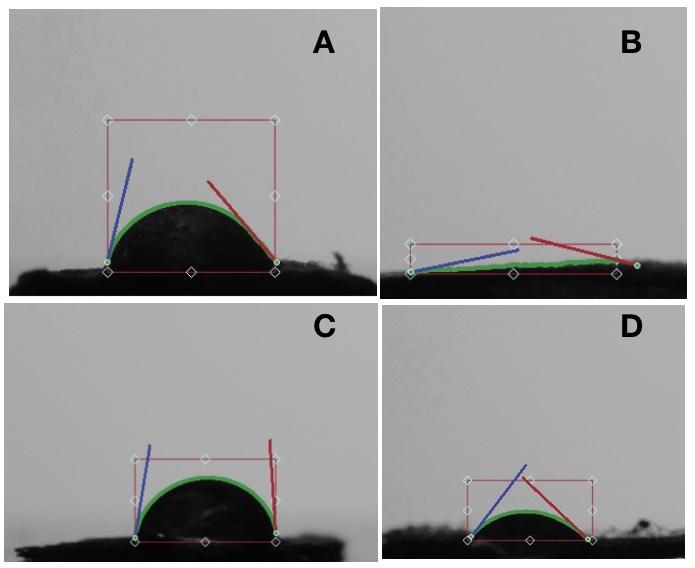


**Figure 1:** Figure representing the SEM analysis of the following study groups:

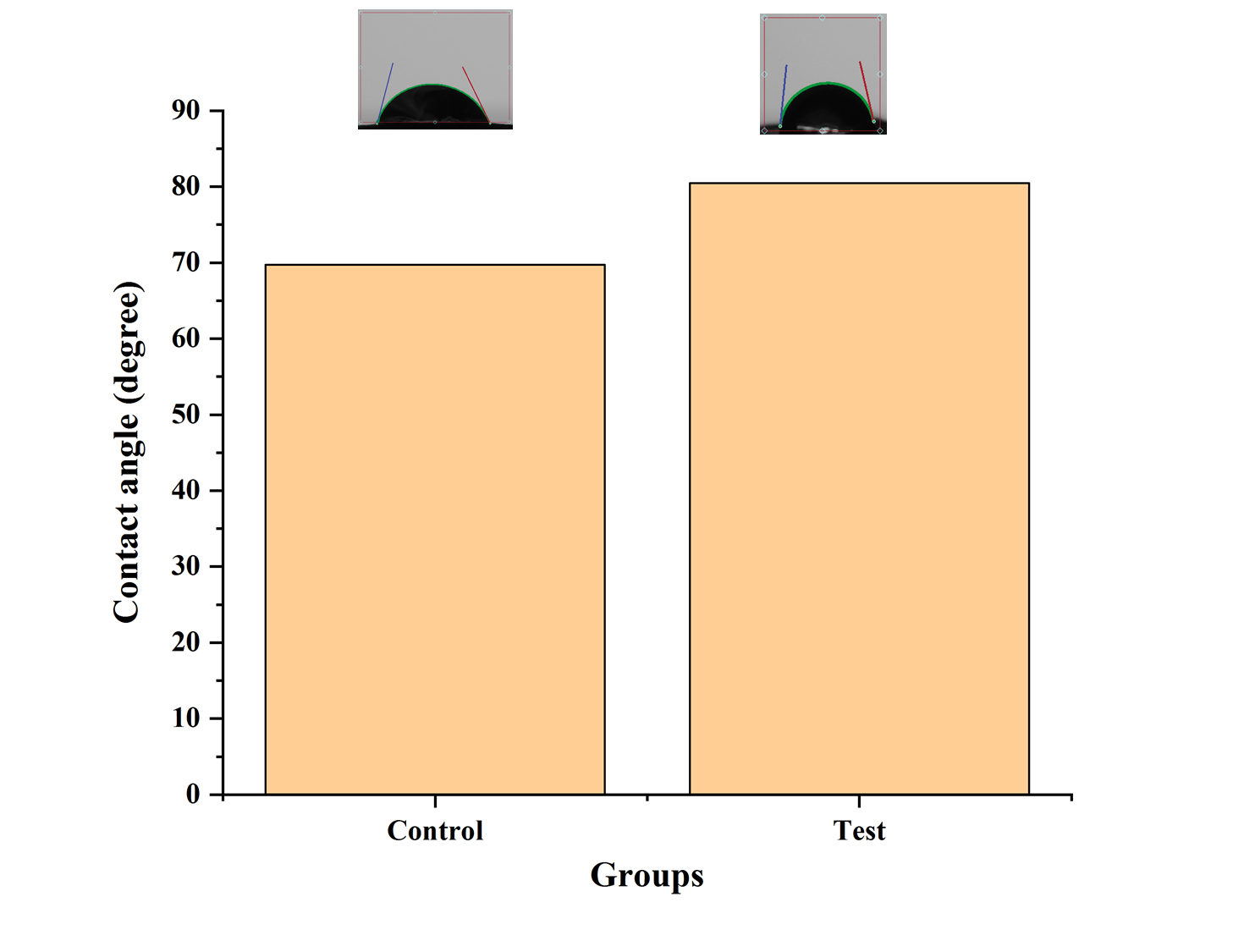
A- SEM analysis of Hyaluronic acid and AgO, B- SEM analysis of Hyaluronic acid, C - SEM analysis of Hyaluronic acid, D- SEM of AgO particles, E- SEM of chitosan particles, F- SEM of AgO nanoparticles. The material's surface morphology was described by the use of a scanning electron microscope or SEM. The pictures were taken at 250x magnification. From the SEM analysis results we can conclude that our Test sample (HA+ AgO) gave rough and porous material with 50μm porosity.The material can undergo various surface characterizations to make it porous [14]. Figure 1 A & B depicts the SEM analysis of the control and test groups, respectively. The porosity of the fabricated GBR membrane is 35.3 ± 9 μm and with the addition of AgO nanoparticles the porosity decreased. It is seen that the Ago-based group has a comparatively less porous and evenly distributed manner, whereas the low Hyaluronic acid has more porosity and is scattered manner. This addition in AgO decrease in porosity is highly evident that AgO-based particles have higher cell adherence, increased cell migration, increased cell penetrating efficiency and a high flow of nutrition. Given that it provides information on the manufactured membranes' morphology and topography, scanning electron microscopy (SEM) is one of the essential methods for characterizing membranes. The GBR membrane's SEM analysis study helps us to know some surface characteristics like surface roughness and sparse distribution of tiny surface porosities. The ability to partition cellular microenvironments in vitro is made possible by porous membranes, which also permit the physical and biochemical interaction between cells—a trait that is frequently required to replicate physiological processes and also the passage of materials into and out of cells, including ions, water, and other tiny molecules, is facilitated by membrane pores.



**Figure 2:** Figure representing the FTIR values of the study groups: A- FTIR on chitosan based GBR, B- FTIR on AgO based nanoparticles, C- FTIR on Hyaluronic acid based GBR, D- FTIR value comparing HA+AgO and HA.

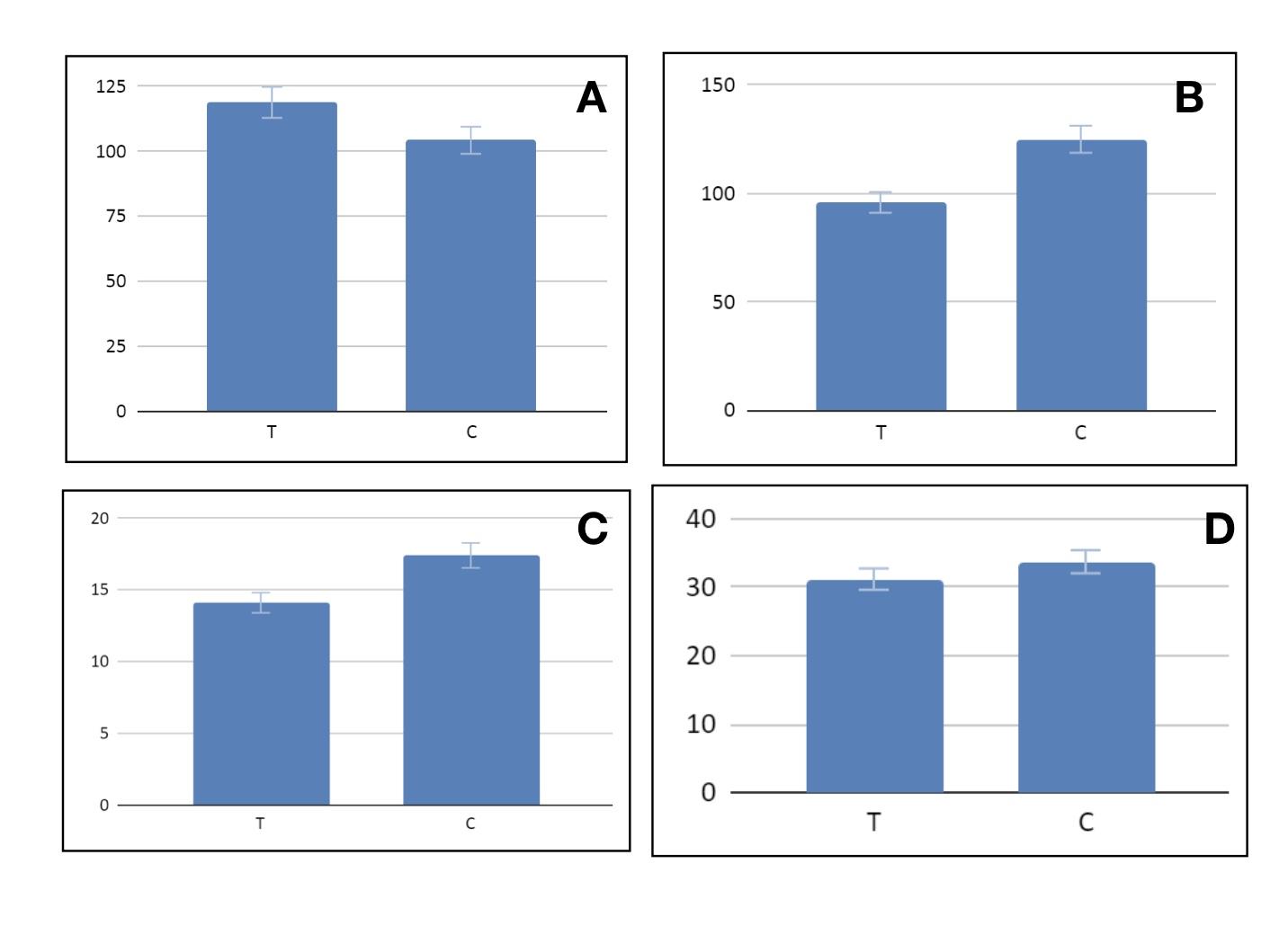


**Figure 3:** Representing the contact angle of the following: A- contact angle of Chitosan based GBR, B-contact angle of AgO based GBR, C-contact angle of AgO nanoparticles, D- contact angle of Hyaluronic acid.



**Figure 4:** Wettability andContact angle results of control (HA) and test (HA + AgO) group GBR membrane. With the addition of AgO nanoparticles a reduction of hydrophilicity in the test group from 69.74° to 80.49° was observed.

Figure 3 represents the contact angle of the control group which was 69.74° and test group 80.49°. Wettability is the propensity of a fluid to spread out or stick to a solid surface when additional immiscible fluids are present. Relative permeability curves, dispersion, capillary pressure, irreducible water saturation, oil displacement, and reducible oil saturation are all impacted by wettability. The material was found to be less hydrophilic when compared to the control. The material has to undergo various surface treatments to increase its hydrophilicity and make it efficient to use the material as a GBR membrane. In membrane science, contact angle is crucial because it indicates a membrane's hydrophilicity and wetting behavior. Because of its high water flux and low fouling, a hydrophilic membrane is preferred. Given that water makes up all living things, hydrophilic molecules are crucial to the formation of cells and living organisms. Hydrophilicity is a necessary property for many proteins, polysaccharides, and other compounds inside the cell to support the cell's operations. So by addition of AgO to HA (L) the hydrophilicity was 80.49° and the control value (HA) was 69.74°.



**Figure 5:** Figure respresenting the swelling test and compactibility test of the follwing groups: A- compactibility between Chitosan and AgO, B- compactibility between HA and AgO, C- swelling between HA and AgO and D- swelling between Chitosan and AgO.

**Table1:** Table 1 represents the contact angle of the following: Chitosan and Ago synthesised particles and Hyaluronic acid and AgO based GBR.

|  |  |
| --- | --- |
| **GROUPS** | **CONTACT ANGLE VALUES** |
| 2% Chitosan + AgO | 62.91 |
| 2% Chitosan | 12.71 |
| HA (M) + AgO | 58.94 |
| HA (M) | 60.31 |
| 2% hyaluronic acid | 83.58 |
| 2% hyaluronic acid +AgO | 48 |

Guided Bone Regeneration (GBR) membranes play a crucial role in dental implantology and alveolar bone regeneration by acting as a barrier against soft tissue infiltration. [(Waddington & Sloan, 2016)](https://paperpile.com/c/tpFG38/hfyD)The study aimed to evaluate the properties of bioabsorbable membranes modified with silver oxide (AgO) and hyaluronic acid (HA).[(Li et al., 2021)](https://paperpile.com/c/tpFG38/kTEC) [(Buchmann, 2010)](https://paperpile.com/c/tpFG38/YouB)SEM analysis revealed that the AgO-based membranes exhibited higher porosity compared to the control group, which enhanced cell adhesion, migration, and penetration efficiency. [(Frank-Kamenetskaya et al., 2019; Motta & Migliaresi, 2006)](https://paperpile.com/c/tpFG38/9fzn+xtN9)The porosity values were measured at over 50 µm, ideal for facilitating bone growth.[(Fernandes et al., 2022)](https://paperpile.com/c/tpFG38/rT0h) Additionally, the presence of AgO nanoparticles was confirmed through FTIR analysis, which detected spectral peaks at 754 cm⁻¹, indicating successful incorporation into the chitosan membrane . [(M. Zhu et al., 2023)](https://paperpile.com/c/tpFG38/W071)Hydrophilicity is a key factor in membrane performance, as it influences cell attachment and tissue integration. Contact angle measurements demonstrated that AgO nanoparticles reduced the angle from 62.91° in the control group to 12.71°, significantly enhancing hydrophilicity. Similarly, HA was found to be inherently hydrophilic, with a contact angle of 83.58°, which further improved when combined with AgO. These findings suggest that AgO and HA modifications improve the membrane’s wettability, making it more suitable for guided tissue regeneration. Additionally, the swelling test showed that the addition of carrageenan increased swelling from 12% to 35%, indicating its potential use in socket preservation or chemo-static-based membrane treatments .Biocompatibility is a critical requirement for GBR membranes, and the study confirmed that AgO-based membranes exhibited high compatibility with oral tissues. The biocompatibility test showed that the control group scored approximately 120%, while the AgO-based membranes scored around 110%, confirming their suitability for clinical applications. Furthermore, the incorporation of HA and carrageenan improved cell viability and proliferation, as observed in previous studies where carrageenan enhanced osteoblast differentiation. Bone formation assays also demonstrated that HA-based membranes supported significant bone growth, reaching up to 8 mm, comparable to previously reported studies using HA in rabbit calvarial defect models.The GBR membranes’ mechanical properties were also assessed, particularly their ability to maintain space for bone regeneration. [(Weng et al., 2021)](https://paperpile.com/c/tpFG38/MeCi) The study found that non-bioabsorbable membranes provide better mechanical strength but require a second surgical procedure for removal. Conversely, bioabsorbable membranes, such as those modified with AgO and HA, offer an alternative that eliminates the need for secondary surgeries while maintaining structural integrity. [(Gama et al., 2016)](https://paperpile.com/c/tpFG38/Bn8r)The introduction of AgO nanoparticles also provided antimicrobial properties, although further research is needed to confirm their long-term effectiveness in preventing post-surgical infections.While the modified GBR membranes demonstrated promising results in terms of porosity, hydrophilicity, biocompatibility, and mechanical strength, some limitations remain.[(Chen et al., 2022; Li et al., 2021)](https://paperpile.com/c/tpFG38/kTEC+Oetw) The handling properties of bioabsorbable membranes need further improvement to ensure tight adaptation to bone surfaces and prevent connective tissue infiltration.Additionally, long-term clinical trials are required to evaluate the stability and effectiveness of AgO- and HA-modified membranes in real-world applications.Despite these challenges, the combination of HA and AgO in GBR membranes presents a promising alternative to conventional materials, potentially enhancing bone regeneration outcomes while reducing patient morbidity associated with non-bioabsorbable membranes.

# CONCLUSION

The findings of this study demonstrate the potential of AgO-infused chitosan particles in enhancing the properties of guided bone regeneration (GBR) membranes. A significant reduction in contact angle was observed, which positively influenced FTIR and SEM readings, as well as swelling and compatibility. These changes indicate that AgO-based chitosan particles improve membrane hydrophilicity and tissue integration, making them suitable candidates for future dental applications. The ability of these modified membranes to facilitate bone healing and regeneration further supports their potential in clinical use.Similarly, AgO-infused hyaluronic acid (HA) particles exhibited improved wettability, as reflected in reduced contact angle measurements and enhanced SEM and FTIR results. The swelling and compatibility of these membranes suggest their efficiency in GBR applications, particularly in promoting bone growth. The findings reinforce that AgO-based chitosan and HA membranes could be effectively utilized for dental treatments, offering a promising alternative to conventional GBR materials. The improved hydrophilicity and bioactivity of these membranes make them well-suited for use in guided tissue regeneration and bone repair procedures.The study successfully developed a hybrid scaffold incorporating AgO nanoparticles, which demonstrated enhanced bone formation. The L-HA/AgO hybrid material exhibited superior osteoconductive properties, making it a suitable candidate for resorbable GBR membranes. However, further investigations are required to understand the intracellular responses.

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