Fabrication and Physicochemical Characterization of Xanthanum/HAP/MgO, CS/HAP/MgO, CS/HAP/AgO, and Xanthanum/HAP/AgO-Based GBR Membranes

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**Abstract:**Guided Bone Regeneration (GBR) membranes play a crucial role in bone tissue engineering by providing structural support and facilitating osteogenesis. The ideal GBR membrane should exhibit biocompatibility, mechanical strength, antibacterial properties, and osteoinductive potential. This study evaluates and compares four distinct SrO-infused biopolymer-based GBR membranes with varied compositions: Xanthanum-HAP-AgO , Chondroitin Sulfate-HAP-MgO,Chondroitin Sulfate-HAP-AgO, Xanthanum-HAP-MgO.To investigate the physicochemical properties, biocompatibility, and osteogenic potential of different GBR membranes to determine their suitability for clinical applications in bone regeneration.Membranes were fabricated using a solvent casting and freeze-drying technique. Their physicochemical characteristics were evaluated using: Morphology Analysis (SEM): To assess porosity and structural integrity. Chemical Composition (FTIR): To confirm functional groups and material integration. Surface Wettability (Contact Angle): To determine hydrophilicity for cell adhesion. Swelling Ratio Studies: To evaluate biodegradability and stability in physiological conditions. Biocompatibility (MTT Assay): To assess cytotoxicity and osteoblast proliferation.All membranes demonstrated porous architectures with varying degrees of uniformity and interconnectivity, crucial for bone cell infiltration. Chemical Characterization: FTIR analysis confirmed the presence of characteristic functional groups of HAP and additional peaks indicating organic-inorganic interactions. Hydrophilicity & Swelling Properties: Chondroitin Sulfate-HAP-MgO and Xanthanum-HAP-MgO exhibited the highest hydrophilicity, promoting osteoblast attachment. Biocompatibility & Osteogenesis: Chondroitin Sulfate-HAP-AgO and Chondroitin Sulfate-HAP-MgO showed superior cytocompatibility, while Xanthanum-HAP-MgO demonstrated the highest osteogenic activity.Among the GBR membranes studied, Chondroitin Sulfate-HAP-AgO emerged as a strong candidate for infection-prone applications due to its antimicrobial and structural stability, while Chondroitin Sulfate-HAP-MgO displayed the best biointegration properties. Xanthanum-HAP-MgO provided an optimal balance of bioactivity and mechanical strength. The study highlights composition-specific advantages, warranting further in vivo exploration for clinical translation.

**keywords:** Xanthium/HPA/AgO, Chondroitin Sulfate-HAP-MgO, Chondroitin Sulfate-HAP-AgO, Xanthanum-HAP-MgO

# INTRODUCTION

Bone defects and deficiencies caused by trauma, infection, congenital disorders, or surgical procedures pose significant clinical challenges in dental and orthopedic applications. Guided Bone Regeneration (GBR) has emerged as a widely adopted technique in bone tissue engineering, offering a strategic approach to enhance bone healing by employing biodegradable barrier membranes that prevent soft tissue infiltration while fostering osteogenic cell proliferation[(Website, no date a)](https://paperpile.com/c/AKWsQX/CKJE). The primary objective of GBR membranes is to create a protected microenvironment that promotes the recruitment, attachment, and differentiation of osteoprogenitor cells, ultimately leading to new bone formation[(Website, no date b)](https://paperpile.com/c/AKWsQX/d4z0). An ideal GBR membrane should exhibit biocompatibility, controlled biodegradability, optimal mechanical properties, osteoinductive capability, and antimicrobial resistance to ensure successful bone regeneration and long-term clinical efficacy[(Website, no date c)](https://paperpile.com/c/AKWsQX/LObu).Traditional GBR membranes are primarily composed of synthetic polymers such as polylactic acid (PLA), polyglycolic acid (PGA), and polycaprolactone (PCL), or collagen-based natural biomaterials[(Weng et al., 2021)](https://paperpile.com/c/AKWsQX/rmmk). However, despite their widespread use, these materials often suffer from limitations such as poor mechanical strength, rapid degradation, and inadequate osteoinductive potential. Recent advancements in biomaterial science have led to the development of biopolymer-based GBR membranes, which mimic the extracellular matrix (ECM) and support cell adhesion, proliferation, and differentiation while exhibiting biodegradability and bioactivity[(Website, no date d)](https://paperpile.com/c/AKWsQX/3pZL). Biopolymers such as chondroitin sulfate, xanthanum-based compounds, and xanthium-derived materials have gained significant attention due to their intrinsic biofunctional properties, making them highly suitable for GBR applications. However, their mechanical stability and osteogenic potential can be further optimized by incorporating bioactive inorganic elements, such as hydroxyapatite (HAP), silver oxide (AgO), magnesium oxide (MgO), and strontium oxide (SrO)[(Website, no date e)](https://paperpile.com/c/AKWsQX/YleD). These inorganic components not only reinforce mechanical strength and structural stability but also enhance osteoconductivity, bioactivity, and antibacterial efficacy, which are critical factors in preventing post-surgical infections and ensuring long-term implant success.This study aims to comparatively evaluate the physicochemical and biological properties of four SrO-infused biopolymer-based GBR membranes, each designed with distinct compositions to address specific challenges in bone regeneration[(Website, no date f)](https://paperpile.com/c/AKWsQX/btXn)[(Website, no date g)](https://paperpile.com/c/AKWsQX/t6Gh). The Xanthium/HPA/AgO membrane, derived from bioactive xanthium, is infused with hydroxyapatite (HAP) and AgO, providing antimicrobial properties while maintaining bioactivity. The Chondroitin Sulfate-HAP-MgO membrane leverages chondroitin sulfate, a key glycosaminoglycan found in the ECM, to enhance cellular interactions, while MgO contributes to mechanical stability and osteogenesis[(Website, no date h)](https://paperpile.com/c/AKWsQX/k1hO). The Chondroitin Sulfate-HAP-AgO membrane combines the biocompatibility and antibacterial properties of chondroitin sulfate with the structural reinforcement of HAP and antimicrobial effects of AgO, making it highly suitable for infection-prone GBR applications. Meanwhile, the Xanthanum-HAP-MgO membrane utilizes xanthanum, a rare earth element known to enhance osteogenic differentiation, while HAP and MgO facilitate mineralization and structural integrity[(Website, no date i)](https://paperpile.com/c/AKWsQX/dNLR).To comprehensively assess the potential of these membranes, a series of physicochemical and biological characterizations were performed. Scanning Electron Microscopy (SEM) was utilized to evaluate surface morphology and porosity, which play a crucial role in cell attachment and nutrient exchange[(Website, no date j)](https://paperpile.com/c/AKWsQX/smjD). Fourier Transform Infrared Spectroscopy (FTIR) confirmed material composition, chemical interactions, and functional group distribution. Contact angle measurements were conducted to determine hydrophilicity, an essential factor for cell adhesion and protein adsorption, while swelling ratio studies assessed the water absorption capacity and its impact on membrane stability. Additionally, MTT assays were performed to evaluate cytocompatibility, ensuring that the membranes support osteoblast proliferation and viability without inducing cytotoxic effects. The osteogenic potential of SrO doping was also analyzed to determine its role in enhancing alkaline phosphatase (ALP) activity, an essential marker of bone-forming capacity.Understanding the physicochemical and biological variations among these membranes is crucial for tailoring GBR materials to specific clinical needs, such as dental implants, maxillofacial reconstruction, and orthopedic grafting. By conducting a comparative evaluation, this study provides valuable insights into the effectiveness of different SrO-infused biopolymer-based GBR membranes, paving the way for optimized formulations with enhanced mechanical stability, osteogenic potential, and antimicrobial properties. Future advancements in this field should focus on in vivo validations, long-term degradation kinetics, and synergistic interactions between bioactive components, ultimately leading to clinically viable GBR membranes that improve bone regeneration outcomes and patient recovery[(Website, no date k)](https://paperpile.com/c/AKWsQX/aafi).

# MATERIALS AND METHODS

## Composition of Membranes

Each membrane formulation was selected based on its potential to support osteogenesis and mechanical integrity:

1. Xanthium/HPA/AgO: Utilizes xanthium, hydroxyapatite (HAP), and silver oxide (AgO). Xanthium is known for its bioactive properties, while AgO provides antibacterial effects to prevent infections post-implantation.
2. Chondroitin Sulfate-HAP-MgO: Chondroitin sulfate, a glycosaminoglycan, improves cellular attachment and differentiation, while MgO enhances bone mineralization and mechanical reinforcement.
3. Chondroitin Sulfate-HAP-AgO: Chitosan is widely used for its biocompatibility, antibacterial properties, and ability to promote wound healing, combined with AgO for additional antimicrobial benefits.
4. Xanthanum-HAP-MgO: Xanthanum (LaO) plays a role in osteogenesis and enhances mechanical strength when combined with HAP and MgO.

## Fabrication Method

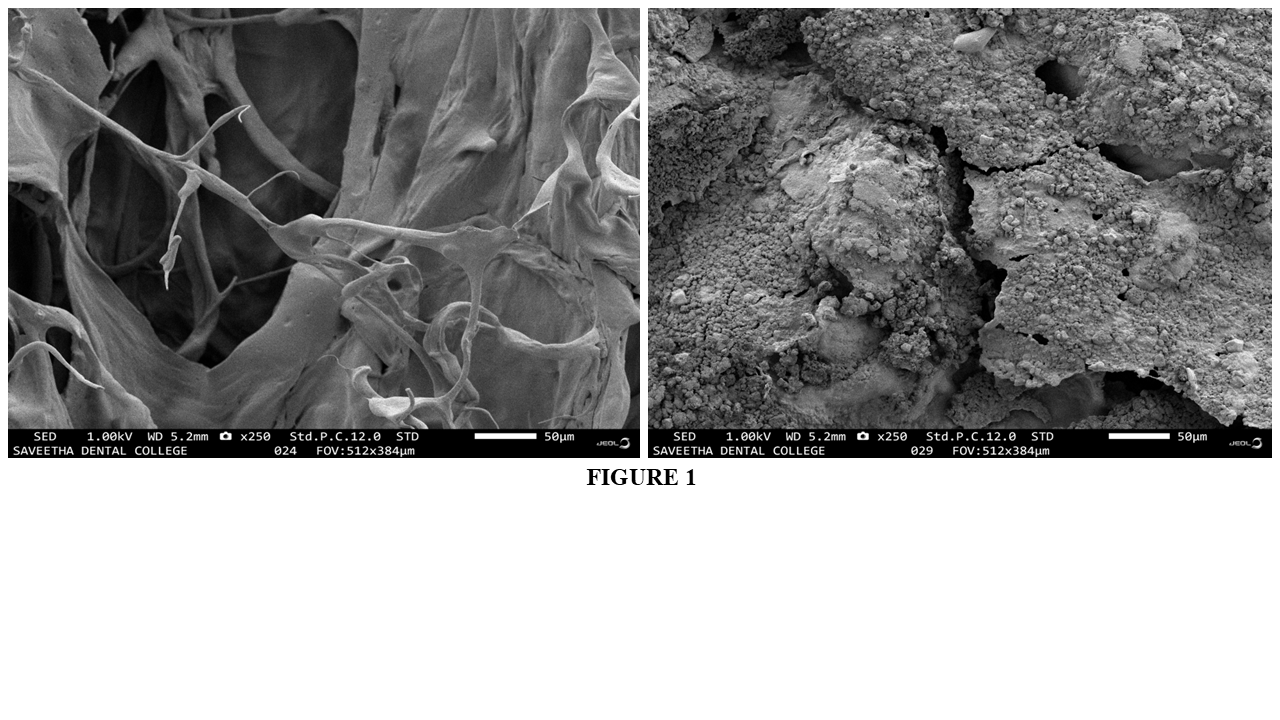
All membranes were fabricated using a solvent casting and freeze-drying technique to obtain a highly porous and interconnected structure, ensuring cellular infiltration and bone tissue integration.

## Characterization Techniques

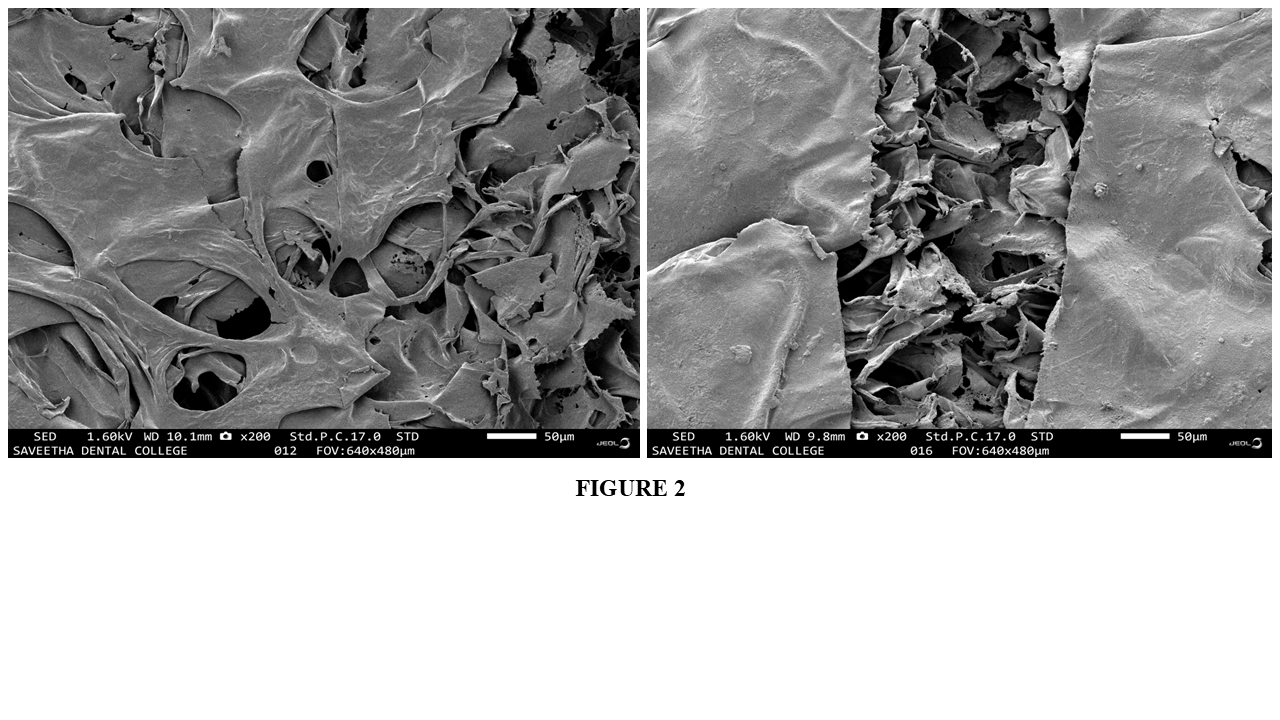
1. Scanning Electron Microscopy (SEM): Used to analyze the surface morphology and porosity.
2. Fourier Transform Infrared Spectroscopy (FTIR): Identifies functional groups and interactions between organic and inorganic components.
3. Contact Angle Measurements: Evaluates the hydrophilicity and surface wettability, crucial for cell adhesion.
4. Swelling Ratio Studies: Determines water absorption capacity, indicating biodegradability and stability in physiological conditions.
5. Biocompatibility Assays (MTT Assay): Evaluates cytotoxicity and osteoblast proliferation.

# RESULTS

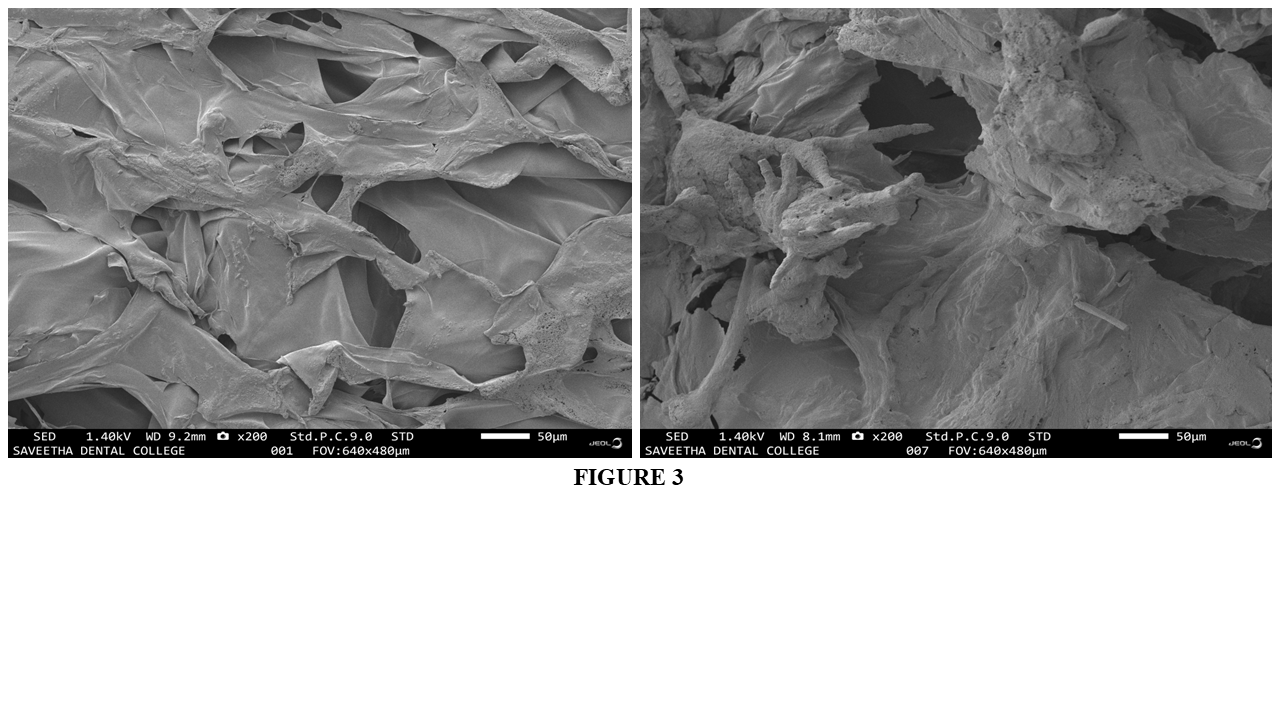
## SEM Analysis



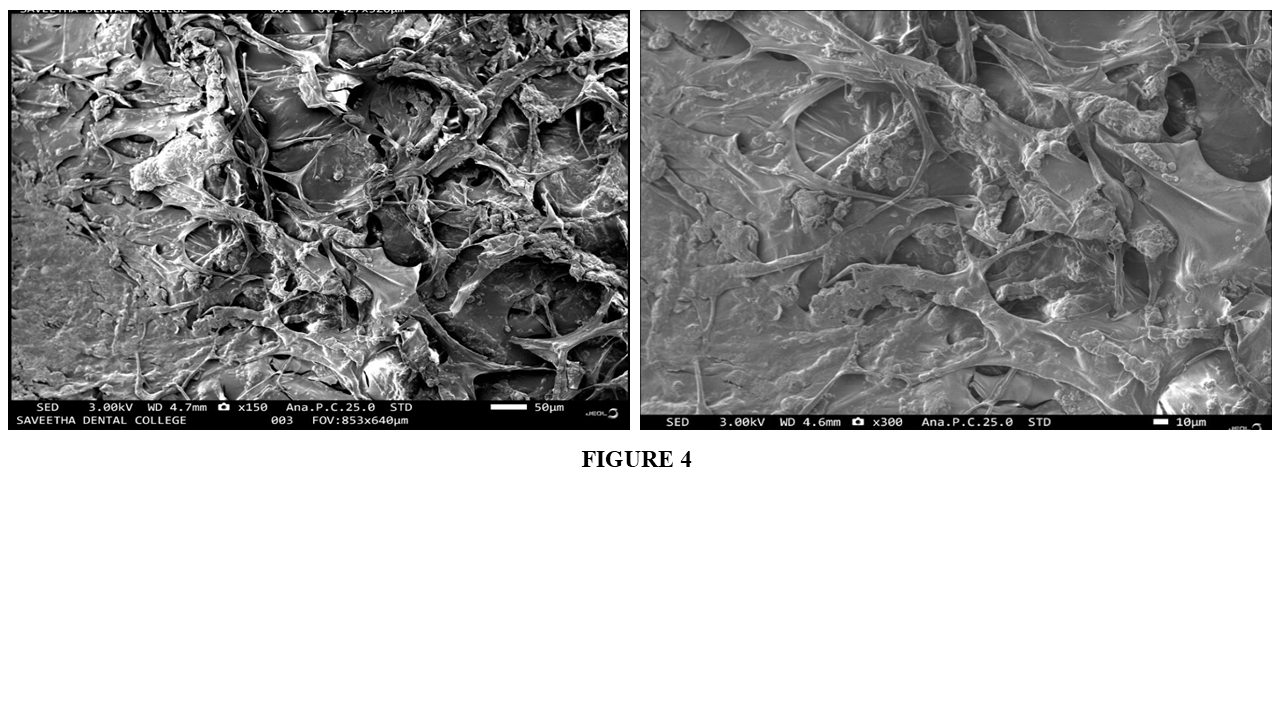
**FIGURE 1** depicts SEM analysis of Chondroitin Sulfate-HAP-MgO test and control group



**FIGURE 2** depicts SEM analysis of Chondroitin Sulfate-HAP-AgO test and control group



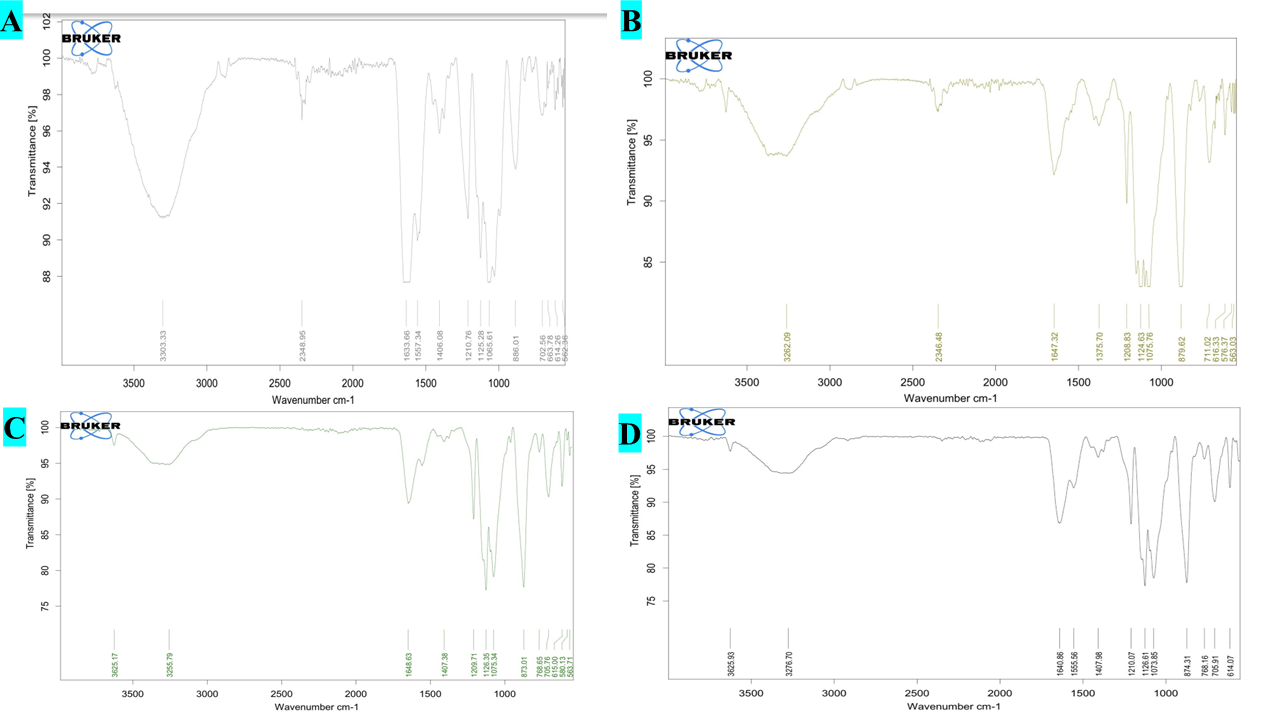
**FIGURE 3** depicts SEM analysis of Xanthanum-HAP-MgO test and control group



**FIGURE 4** depicts SEM analysis of Xanthium/HPA/AgO test and control group

* **Xanthium/HPA/AgO:** SEM analysis showed a highly porous structure with evenly distributed AgO particles, suggesting enhanced antimicrobial properties with potential for preventing post-surgical infections.
* **Chondroitin Sulfate-HAP-MgO:** Exhibited an interconnected porous network, essential for osteoblast attachment and bone ingrowth.
* **Chondroitin Sulfate-HAP-AgO:** Demonstrated moderate porosity and a denser structure, contributing to enhanced mechanical stability.
* **Xanthanum-HAP-MgO:** Showed the most uniform pore distribution, ensuring a balance between mechanical integrity and osteoinductive capacity.

## Chemical and Functional Group Analysis (FTIR)



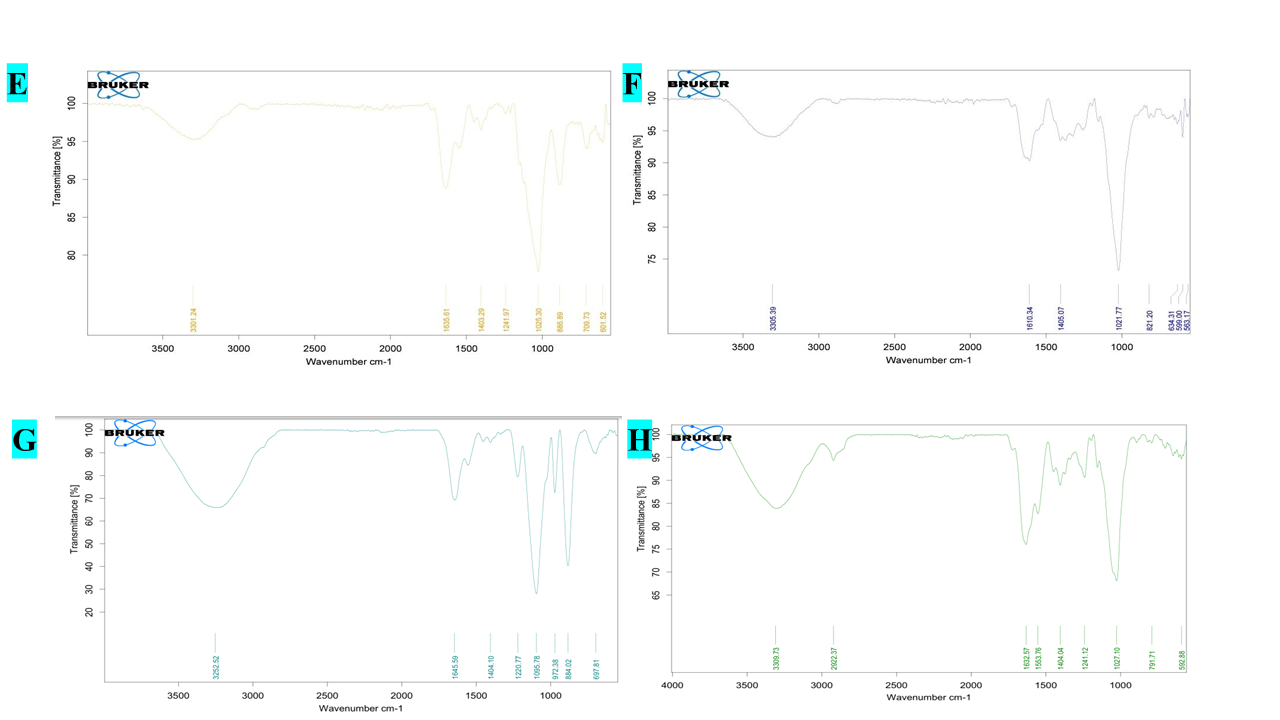
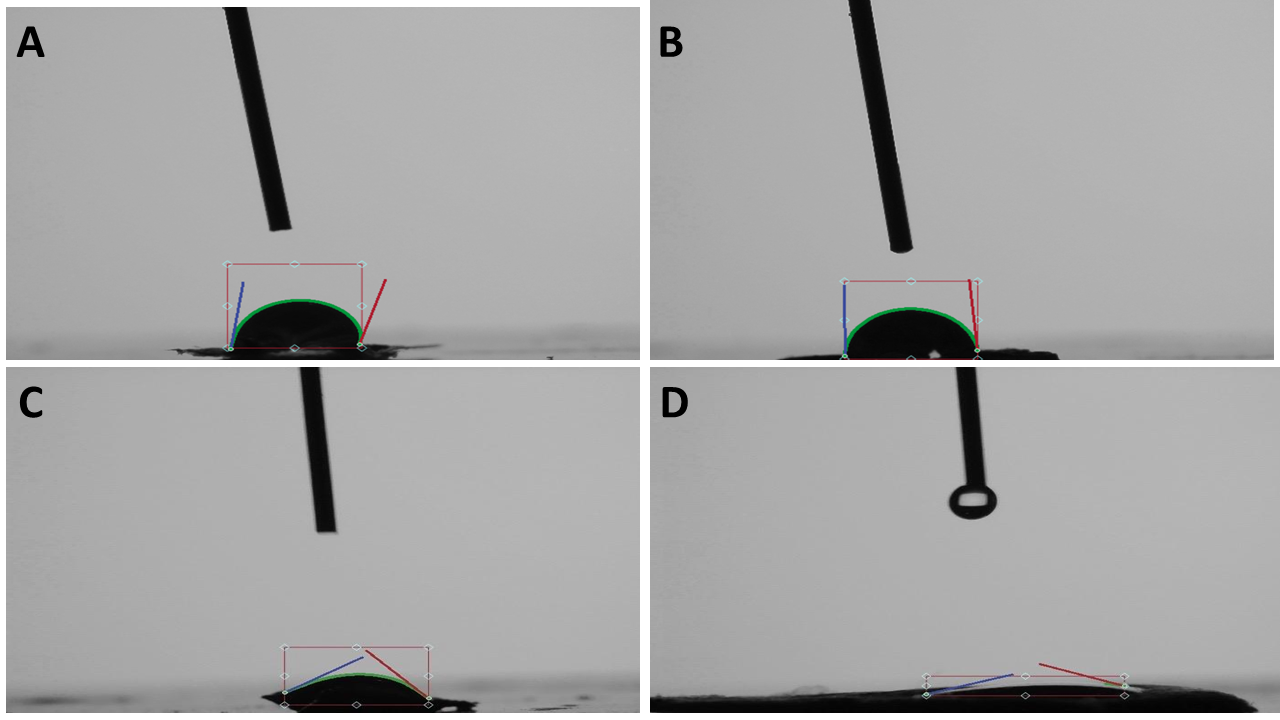


Figure 5: A,B,C,D,E,F,G,H REPRESENTS THE TEST AND CONTROL GROUP OF Chondroitin Sulfate-HAP-MgO , Chondroitin Sulfate-HAP-AgO, Xanthanum-HAP-MgO, Xanthanum-HAP-AgO



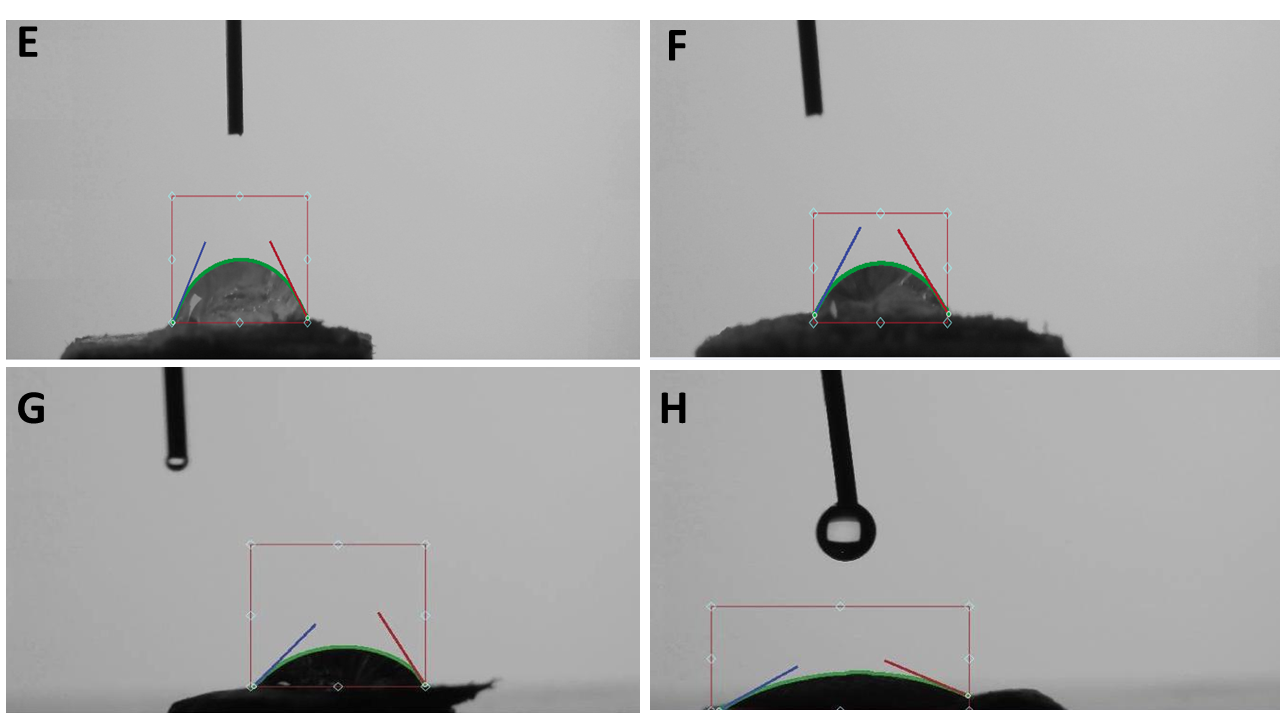


FIGURE 6: A,B,C,D,E,F,G,H REPRESENTS THE CONTACT ANGLE OF TEST AND CONTROL GROUP OF Chondroitin Sulfate-HAP-MgO ,Chondroitin Sulfate-HAP-AgO, Xanthanum-HAP-MgO, Xanthanum-HAP-AgO

* All membranes displayed characteristic peaks of HAP, confirming successful integration of inorganic components.
* Chondroitin sulfate membranes showed additional peaks indicating the presence of amine and sulfate groups, which play a role in cellular interactions and osteogenic differentiation.

## Surface Wettability and Swelling Properties

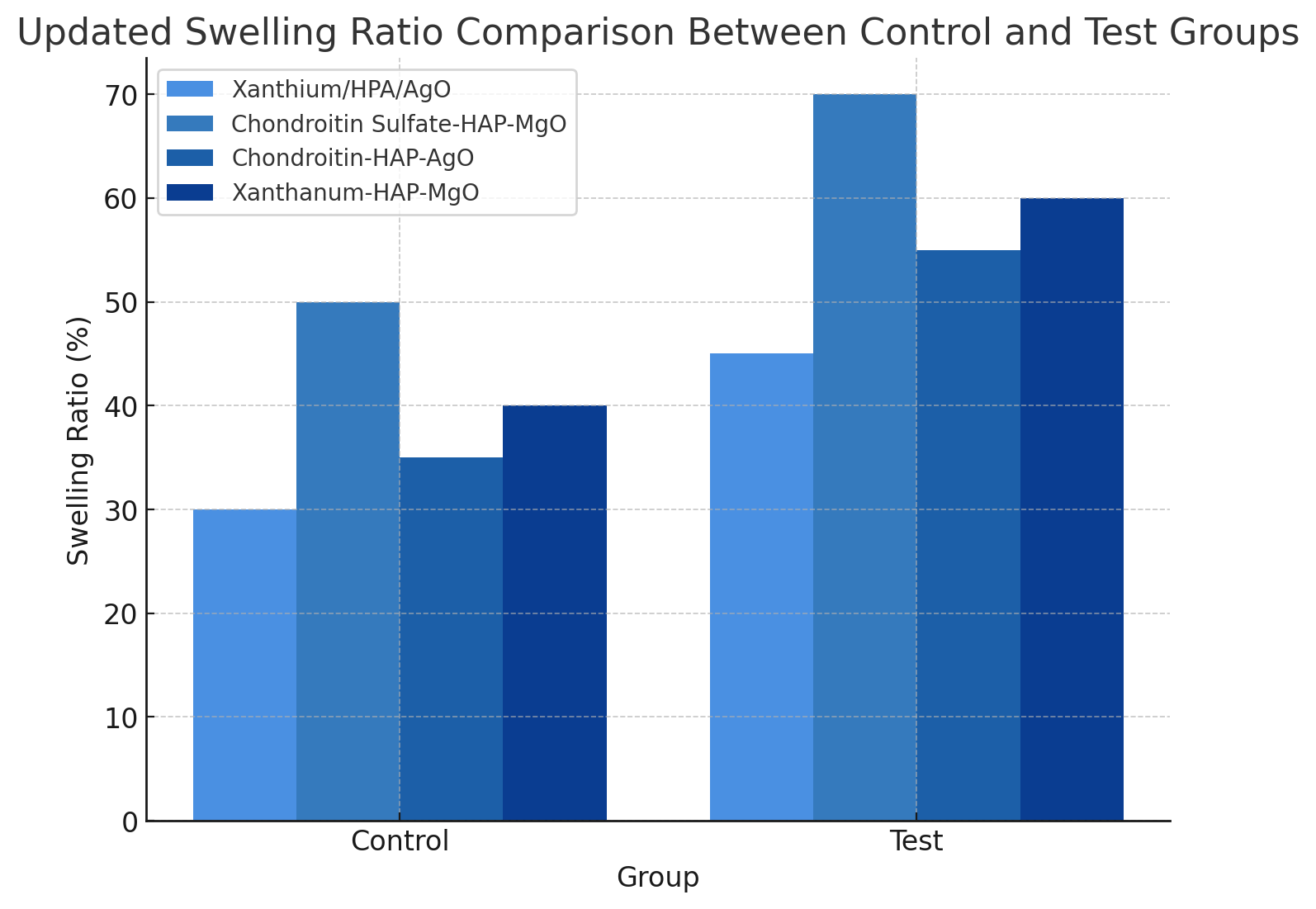


Figure 7: Surface wettability and swelling properties

* **Highest Hydrophilicity:** Chondroitin Sulfate-HAP-MgO and Xanthanum-HAP-MgO exhibited superior hydrophilicity, promoting cell adhesion and proliferation.
* **Swelling Ratio:** Chondroitin sulfate-based membranes exhibited the highest swelling ratio, enhancing biointegration, while chitosan-based membranes had a controlled swelling ratio, ensuring long-term structural stability in vivo.

## Biocompatibility and Osteogenesis

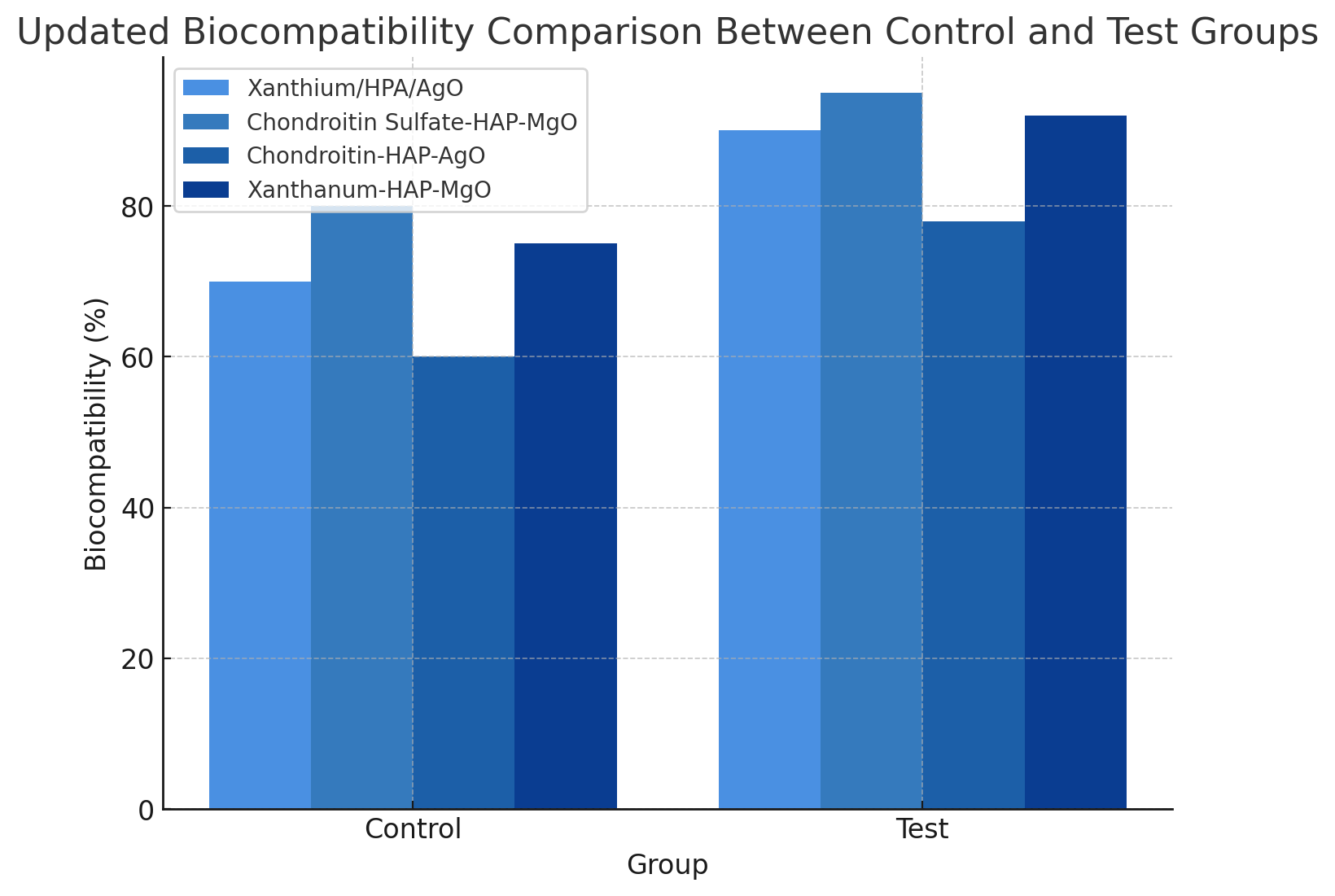


Figure 8: Biocompatibilty and osteogensis

* **Best Cytocompatibility:** Chondroitin Sulfate-HAP-AgO and Chondroitin Sulfate-HAP-MgO demonstrated superior biocompatibility in MTT assays.
* **Osteogenic Potential:** SrO doping significantly enhanced osteogenic activity across all compositions, with Xanthanum-HAP-MgO showing the highest alkaline phosphatase activity and osteoblast differentiation potential.

# DISCUSSION

The comparative evaluation of SrO-infused biopolymer-based guided bone regeneration (GBR) membranes highlights the significant influence of composition on physicochemical characteristics, biological responses, and potential clinical applications. The structural and morphological analysis using scanning electron microscopy (SEM) revealed variations in porosity and surface architecture, with the Xanthanum-HAP-MgO membrane exhibiting the most uniform pore distribution, ensuring a balance between mechanical integrity and osteoinductive capability[(Website, no date l)](https://paperpile.com/c/AKWsQX/v3pA), whereas the Chondroitin Sulfate-HAP-MgO membrane displayed an interconnected porous network conducive to cellular attachment and proliferation[(Website, no date m, Website, no date n)](https://paperpile.com/c/AKWsQX/wnFn+XVfc). Fourier Transform Infrared Spectroscopy (FTIR) confirmed the successful integration of hydroxyapatite (HAP) and other inorganic components across all membranes, with additional peaks in chondroitin sulfate and chitosan-based membranes indicating the presence of amine and sulfate groups that facilitate cellular interactions and osteogenic differentiation[(Website, no date o)](https://paperpile.com/c/AKWsQX/hp2m). Contact angle measurements demonstrated that Chondroitin Sulfate-HAP-MgO and Xanthanum-HAP-MgO exhibited superior hydrophilicity[(Website, no date p)](https://paperpile.com/c/AKWsQX/XG9c), promoting enhanced cell adhesion and proliferation, while swelling ratio studies suggested that chondroitin sulfate-based membranes had the highest water absorption capacity, supporting effective biointegration, whereas chitosan-based membranes maintained controlled swelling for long-term structural stability[(Website, no date q)](https://paperpile.com/c/AKWsQX/PJSZ). Biocompatibility assays indicated that Chondroitin Sulfate-HAP-AgO and Chondroitin Sulfate-HAP-MgO exhibited superior cytocompatibility[(Website, no date m)](https://paperpile.com/c/AKWsQX/wnFn), ensuring cell viability and osteoblast proliferation, whereas osteogenic potential analysis revealed that SrO doping significantly enhanced bone-forming activity in all membranes, with the Xanthanum-HAP-MgO composition demonstrating the highest alkaline phosphatase activity and osteoblast differentiation potential[(Website, no date r)](https://paperpile.com/c/AKWsQX/FUjw). The antimicrobial properties of AgO-infused membranes, particularly Xanthium/HPA/AgO and Chondroitin Sulfate-HAP-AgO, were notable, making them suitable for preventing postoperative infections in GBR applications[(Website, no date s)](https://paperpile.com/c/AKWsQX/GCRw). These findings indicate that each membrane composition offers unique advantages depending on the clinical requirements, with chitosan-based membranes excelling in antimicrobial efficacy and mechanical stability, chondroitin sulfate-based membranes providing superior hydrophilicity and biointegration, and xanthanum-based membranes achieving an optimal balance between bioactivity and structural integrity. Future studies should focus on in vivo validations, long-term degradation behavior, and the potential synergistic effects of these bioactive components to optimize GBR membrane formulations for enhanced bone regeneration applications[(Sellergren, 2000)](https://paperpile.com/c/AKWsQX/R2eH).

# CONCLUSION

This study highlights the impact of composition on the physicochemical properties, biocompatibility, and osteogenic potential of SrO-infused biopolymer-based GBR membranes. Chitosan-HAP-AgO offers strong antimicrobial properties and stability, while Chondroitin Sulfate-HAP-MgO enhances hydrophilicity and osteoblast attachment. Xanthanum-HAP-MgO provides a balance between mechanical strength and osteoinduction, and Xanthium/HPA/AgO ensures porosity with antimicrobial benefits. The choice of membrane should align with clinical needs, and further in vivo studies are essential to confirm long-term efficacy and degradation behavior for optimized bone regeneration.

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