Comparative Evaluation of Copper Oxide-Based GBR Membranes: Analysis of Swelling, Hydrophilicity and Biocompatibility for Bone Regeneration

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**Abstract:** Guided Bone Regeneration (GBR) membranes play a crucial role in dental and orthopedic applications by facilitating bone regeneration while preventing soft tissue invasion. The ideal GBR membrane must exhibit biocompatibility, mechanical stability, controlled degradation, and hydrophilicity to enhance osteogenic potential. Recent advancements have focused on biopolymer-based GBR membranes infused with Copper Oxide (CuO) nanoparticles, which provide both osteogenic and antibacterial properties. However, the effectiveness of different CuO-based GBR membranes in terms of swelling behavior, surface wettability, and biocompatibility remains to be systematically analyzed.This study aims to evaluate the swelling ratio, contact angle (hydrophilicity), and biocompatibility of GBR membranes fabricated using Chondroitin Sulfate, Xanthan Gum, and Chitosan, modified with CuO nanoparticles, to determine their suitability for bone regeneration applications.GBR membranes were fabricated using freeze-drying techniques, incorporating Chondroitin Sulfate, Xanthan Gum, and Chitosan, with CuO nanoparticles added for enhanced bioactivity. Membranes were analyzed for surface morphology (SEM), chemical interactions (FTIR), hydrophilicity (contact angle measurement), swelling ratio, and biocompatibility (MTT assay using hDPSCs). Statistical analysis was conducted using One-Way ANOVA, with significance set at p < 0.05. The swelling analysis revealed that Chitosan/CuO (35%) and Xanthan Gum/CuO (34%) had the highest swelling capacity, making them ideal for socket preservation. In contrast, Chondroitin Sulfate/CuO (24%) exhibited controlled swelling, ensuring mechanical stability for ridge augmentation. Contact angle measurements indicated that Chondroitin Sulfate/CuO (30.6°) had the highest hydrophilicity, promoting osteoblast attachment, while Chitosan/CuO (54.4°) had the lowest hydrophilicity but enhanced mechanical stability. The MTT assay results showed that Chondroitin Sulfate/CuO (147%) had the highest biocompatibility, confirming its superior osteogenic potential, while Xanthan Gum/CuO (56%) exhibited the lowest biocompatibility, indicating the need for further optimization. The findings suggest that Chondroitin Sulfate/CuO is the most promising GBR membrane, owing to its high biocompatibility and excellent hydrophilicity, making it an ideal candidate for bone regeneration applications. Chitosan/CuO demonstrated superior swelling properties, making it suitable for high-moisture environments such as socket preservation. These results emphasize the importance of selecting GBR membranes based on specific clinical requirements, and future research should focus on long-term in vivo studies, degradation rate optimization, and the incorporation of bioactive growth factors to further enhance GBR membrane performance.

**Keywords:** Fabrication of GBR Membranes, Scanning Electron Microscopy (SEM) Analysis, Fourier Transform Infrared Spectroscopy (FTIR) Analysis

# Introduction

Guided Bone Regeneration (GBR) is a widely used technique in implantology and bone defect repair, involving the placement of a barrier membrane to facilitate bone growth while preventing soft tissue invasion[(Buser et al., 1994; Ramsundar et al., 2023)](https://paperpile.com/c/owM7Tl/c9kq+7wx9). GBR membranes play a crucial role in maintaining space for bone regeneration, enhancing osteoblast activity, and promoting vascularization[(Buser, 2009; Rieshy V. et al., 2023)](https://paperpile.com/c/owM7Tl/fLzH+14Ds). An ideal GBR membrane should exhibit biocompatibility, mechanical strength, controlled degradation, and optimal porosity to support predictable bone regeneration[(Singh et al., 2023; “Synthesis and Size Control of Copper Nanoparticles and Their Catalytic Application,” 2015)](https://paperpile.com/c/owM7Tl/CZ4u+MOEz)[(Bajpai & Rajasekar, 2022; Shenoy et al., 2023)](https://paperpile.com/c/owM7Tl/mNaQ+z1tr). Over the years, various materials have been explored, including synthetic polymers, natural biopolymers, and nanocomposite materials, to develop membranes that provide superior regenerative potential[(“Tailoring Cu2+-Loaded Electrospun Membranes with Antibacterial Ability for Guided Bone Regeneration,” 2022)](https://paperpile.com/c/owM7Tl/c63e).Biopolymers such as Chondroitin Sulfate, Xanthan Gum, and Chitosan have gained prominence due to their biodegradability, bioactivity, and ability to interact with osteogenic cells. These natural polymers serve as scaffold materials that mimic the extracellular matrix (ECM), offering a conducive environment for cell attachment and proliferation[(Doshi et al., 2023; Khoury, 2021; Schoenbaum, 2018)](https://paperpile.com/c/owM7Tl/cHB2+hVCJ+QN6N). Among them, Chitosan has antimicrobial properties, while Chondroitin Sulfate is known for its role in cartilage and bone metabolism, making them highly suitable for GBR applications[(Fernandes et al., 2022; Pavithra et al., 2023)](https://paperpile.com/c/owM7Tl/DhMY+4P9J). However, due to limitations such as poor mechanical strength and rapid degradation, these biopolymers are often modified with nanoparticles or crosslinking agents to enhance their performance.Recent advancements in nanotechnology have introduced metal oxide nanoparticles such as Copper Oxide (CuO) into GBR membranes to improve their mechanical properties, osteoinductive potential, and antibacterial activity[(Aldecoa & Ortiz, 2001; Pandiyan et al., 2023)](https://paperpile.com/c/owM7Tl/L60o+TTt3). CuO nanoparticles, in particular, have demonstrated significant potential in stimulating osteoblast differentiation, enhancing matrix mineralization, and preventing bacterial colonization, making them valuable additions to GBR membranes[(Advances in Bioartificial Materials and Tissue Engineering Research and Application: 2013 Edition, 2013; Aldecoa & Ortiz, 2001; Lampl et al., 2023)](https://paperpile.com/c/owM7Tl/L60o+i8s7+ptHf). The hydrophilicity of GBR membranes plays a crucial role in their performance, as it directly affects protein adsorption, cell adhesion, and tissue integration. A lower contact angle indicates better hydrophilicity, which in turn promotes improved cellular interactions and faster tissue regeneration[(Froum, 2015; Subramanian et al., 2023)](https://paperpile.com/c/owM7Tl/QnbR+dyng). Several studies have reported that Chondroitin Sulfate/CuO and CuO-Based GBR membranes exhibit superior hydrophilic properties, making them highly suitable for clinical applications[(Lakshmi, 2021; United States Public Health Service & Surgeon General of the United States, 2004)](https://paperpile.com/c/owM7Tl/hPF6+2nCq). Conversely, Chitosan/CuO has a slightly higher contact angle, which may impact early-stage cell attachment but offers better mechanical stability and controlled hydration.Swelling behavior is another critical factor influencing GBR membrane performance. Membranes with high swelling capacity can retain more moisture, facilitating nutrient diffusion and cell infiltration[(Kachhara et al., 2021; Zelkó et al., 2020)](https://paperpile.com/c/owM7Tl/VW8T+k93F). However, excessive swelling can lead to structural deformation and reduced mechanical integrity. Studies have shown that Chitosan/CuO exhibits the highest swelling percentage, followed closely by Xanthan Gum/CuO, making them excellent candidates for socket preservation and soft tissue healing. On the other hand, Chondroitin Sulfate/CuO demonstrates a more controlled swelling ratio, ensuring balance between hydration and mechanical stability.[(Janani et al., 2021; Serio & Hawley, 2002)](https://paperpile.com/c/owM7Tl/md1z+xKzb)The biodegradability of GBR membranes must align with the rate of new bone formation to ensure optimal clinical outcomes. A membrane that degrades too quickly may fail to provide sufficient support, while one that degrades too slowly may hinder new tissue formation. Crosslinking strategies and nanoparticle incorporation play a significant role in regulating the degradation rate. CuO-based membranes have shown promising degradation profiles, making them suitable for long-term clinical applications.[(Ganapathy, 2021; Usala et al., 2023)](https://paperpile.com/c/owM7Tl/TKyF+cLWM)GBR membranes must also possess sufficient mechanical strength to maintain their structure throughout the regeneration process. Many natural biopolymers lack the required rigidity, which is why reinforcement with nanoparticles like CuO is essential. CuO enhances tensile strength and flexibility, preventing early collapse of the membrane[(Gandhi et al., 2021; Güneş-Durak et al., 2024)](https://paperpile.com/c/owM7Tl/BSIR+pHjp). Researchers continue to explore different fabrication techniques, such as electrospinning and freeze-drying, to further improve the mechanical stability of GBR membranes[(Akshayaa & Ganesh, 2025; Ramalingam et al., 2023)](https://paperpile.com/c/owM7Tl/JbAP+eFqM).This study aims to explore and compare five different GBR membranes containing Chondroitin Sulfate, Xanthan Gum, and Chitosan, modified with CuO nanoparticles, to evaluate their swelling properties, hydrophilicity, and structural integrity. Understanding the impact of these materials on bone regeneration, mechanical stability, and bioactivity will provide valuable insights for developing next-generation GBR membranes with enhanced clinical efficacy.

# Materials and Methods

## Fabrication of GBR Membranes

Stock solutions of different scaffold components were prepared, including Chondroitin Sulfate (1%), Carrageenan (0.5%), Gelatin (1%), Xanthan Gum (2%), and Chitosan (1%), depending on the experimental group. The materials were blended in a 6:1:3 ratio to ensure uniform consistency.For the test groups, 10 mg of Copper Oxide (CuO) nanoparticles were added to the solution. A homogeneous mixture of 3 mL was transferred into six-well plates.To induce crosslinking, 100 µL of sodium tripolyphosphate (TPP) (15%) was added to each well. The plates were stored at -20°C for 12 hours, followed by -80°C overnight. The samples were then lyophilized (freeze-dried) for 24 hours and stored under dry conditions for further analysis.

## Scanning Electron Microscopy (SEM) Analysis

The morphological characteristics of the membranes were observed using Scanning Electron Microscopy (SEM) (SEM, JEOL, Tokyo, Japan) after freeze-drying. The cross-sections of freeze-dried samples were coated with platinum using a sputter coater at ambient temperature. Micrographs were taken at 100X magnification to assess porosity, structural integrity, and CuO nanoparticle distribution.

## Fourier Transform Infrared Spectroscopy (FTIR) Analysis

To determine possible chemical interactions within the scaffolds, Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR) analysis was performed using a Bruker ATR infrared spectrometer. The presence of functional groups and chemical bonding interactions within the scaffolds was confirmed through their FTIR spectra.The FTIR analysis specifically examined:CuO incorporation in Chondroitin Sulfate and Chitosan-based GBR membranes.Amine (-NH2) functional groups in Chitosan-based membranesSulfate (-SO4) stretching in Chondroitin Sulfate membranesHydroxyl (-OH) and carboxyl (-COOH) functional groups related to CuO interactions

## Contact Angle Measurement (Hydrophilicity Analysis)

The hydrophilicity of the scaffolds was evaluated by water contact angle measurements using goniometer software.Scaffolds were cut into 1 cm × 1 cm square specimens and placed on a testing plate.50 µL of distilled water was carefully dropped onto the scaffold surface.Contact angles were measured within 2 seconds of droplet contact.Three independent measurements were taken at different scaffold positions for accuracy.The contact angle results were used to determine wettability and its correlation with cell adhesion and protein adsorption.

## Swelling Ratio (%) of GBR Membranes

The swelling properties of the GBR membranes were analyzed to assess their ability to absorb fluids and maintain hydration.10 mg of freeze-dried scaffolds was immersed in 500 µL of PBS (Phosphate-Buffered Saline) at 37°C.After 24 hours, scaffolds were removed, gently blotted with a Kimwipe to remove excess surface water, and weighed.The swelling ratio (%) was calculated using the equation:

Swelling ratio (SR)= ((Ww−W0)/W0)×100%

W0 and Ww are the initial dry weight and the wet weight, respectively.Each experiment was performed six times to ensure reproducibility.

## Biocompatibility Testing (MTT Assay)

* Human Dental Pulp Stem Cells (hDPSCs) were used to assess biocompatibility and cell viability of the GBR membranes.hDPSCs were isolated from molar teeth with patient consent and ethical approval.Cells were cultured in Dulbecco’s Modified Eagle Medium (DMEM, low glucose) supplemented with 10% Fetal Bovine Serum (FBS) and 1% Penicillin-Streptomycin.10,000 cells per well were seeded into 48-well platesScaffolds were immersed in DMEM, and media were collected after 24 hours and 7 days for biocompatibility analysis.MTT Assay Procedure-100 mg of GBR membrane samples were incubated with MTT reagent (5 mg/mL stock) for 4 hours at 37°C.The media was replaced with 200 µL of DMSO (Dimethyl Sulfoxide) to dissolve formazan crystals.The reaction product was transferred to a 96-well ELISA plate, and absorbance was measured at 590 nm using an ELISA plate reader.

# Statistical Analysis

All values were expressed as mean ± standard error of the mean (SEM) from at least three independent experiments.One-way Analysis of Variance (ANOVA) was used to assess significant differences between groups.Scheffe’s post-hoc test was used for multiple comparisons.A p-value of <0.05 was considered statistically significant.

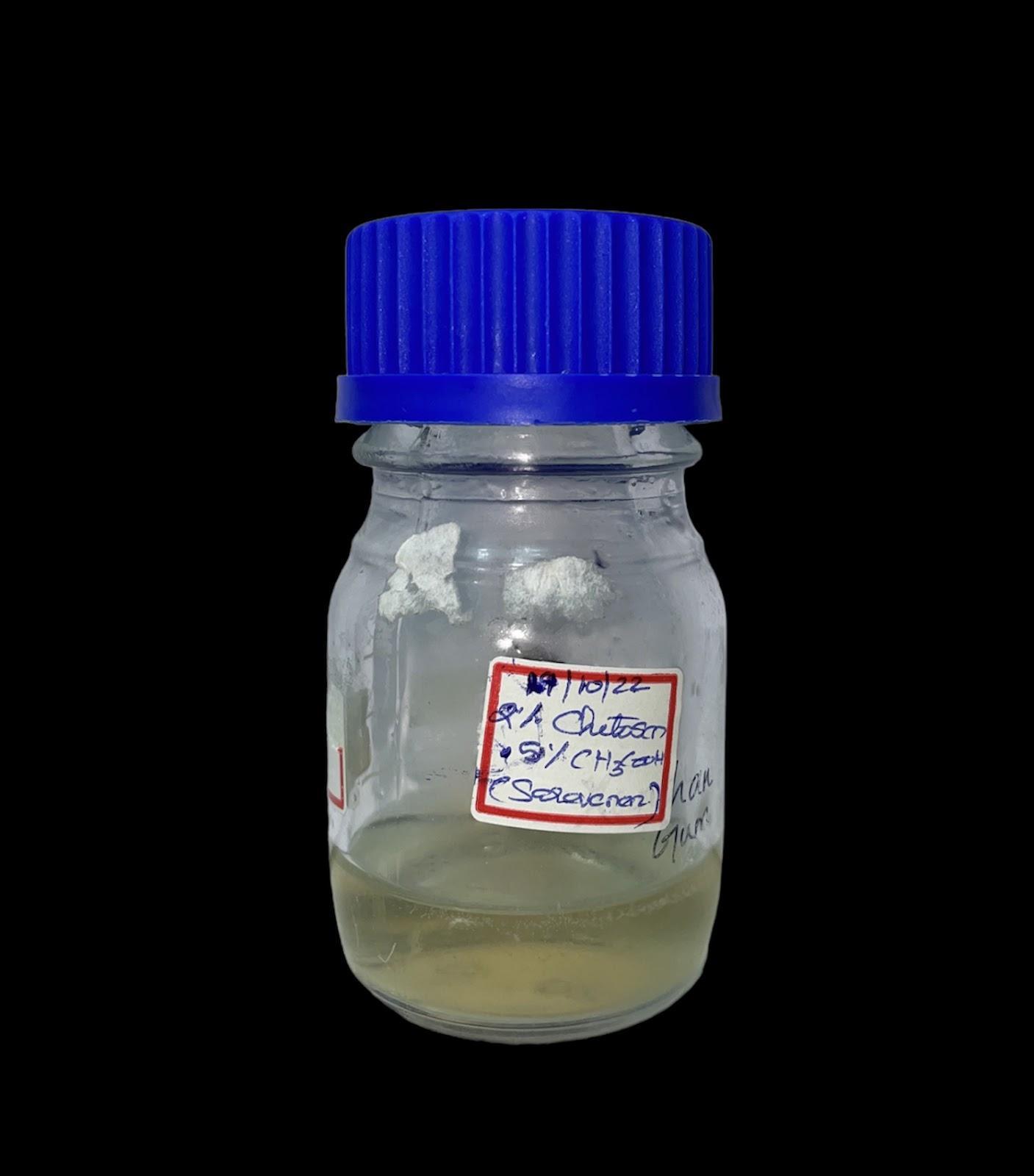


**FIGURE:1:** This Picture depicts the Fabrication of the material Test

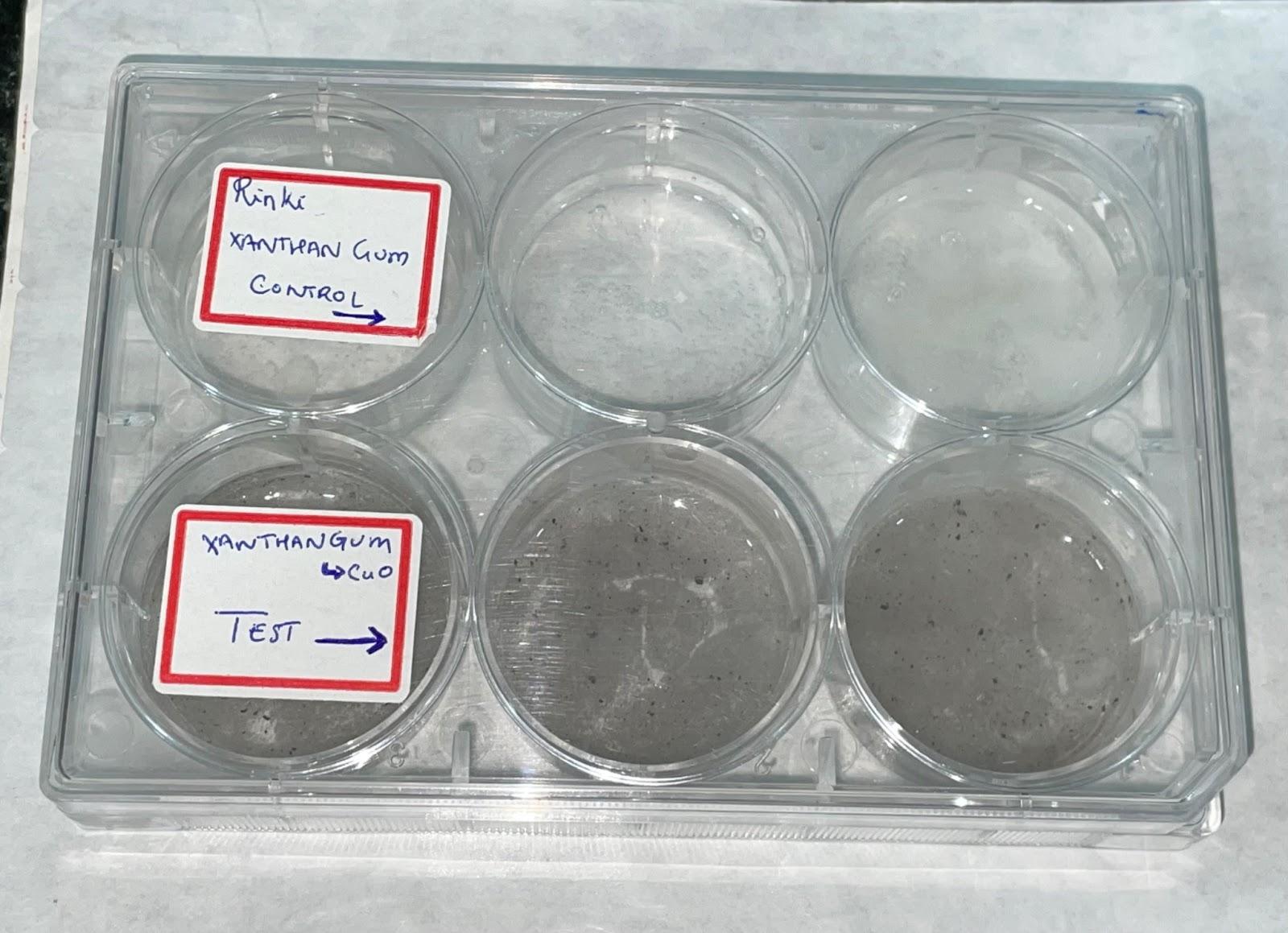
(Chondroitin Sulfate +CuO) group



**FIGURE:2:** This picture depicts the Scaffold of Control(Chondroitin sulphate) and Test(Chondroitin sulfate+CuO) group



**Figure 3**:Depicts the 2% chitosan prepared for the study

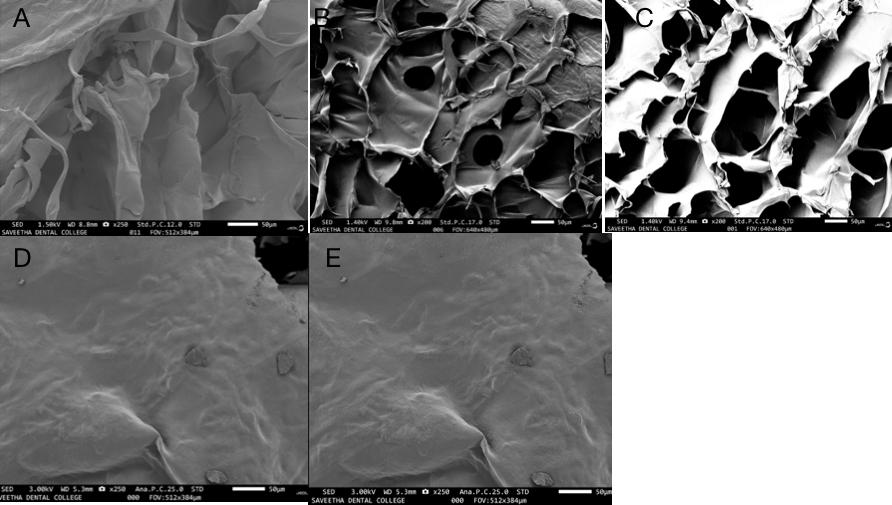


**FIGURE 4:** This picture depicts the Scaffold preparation of Control(Xanthan gum) and Test(Xanthan gum+CuO) group

# Results and discussion

## Surface Morphology and Porosity Analysis

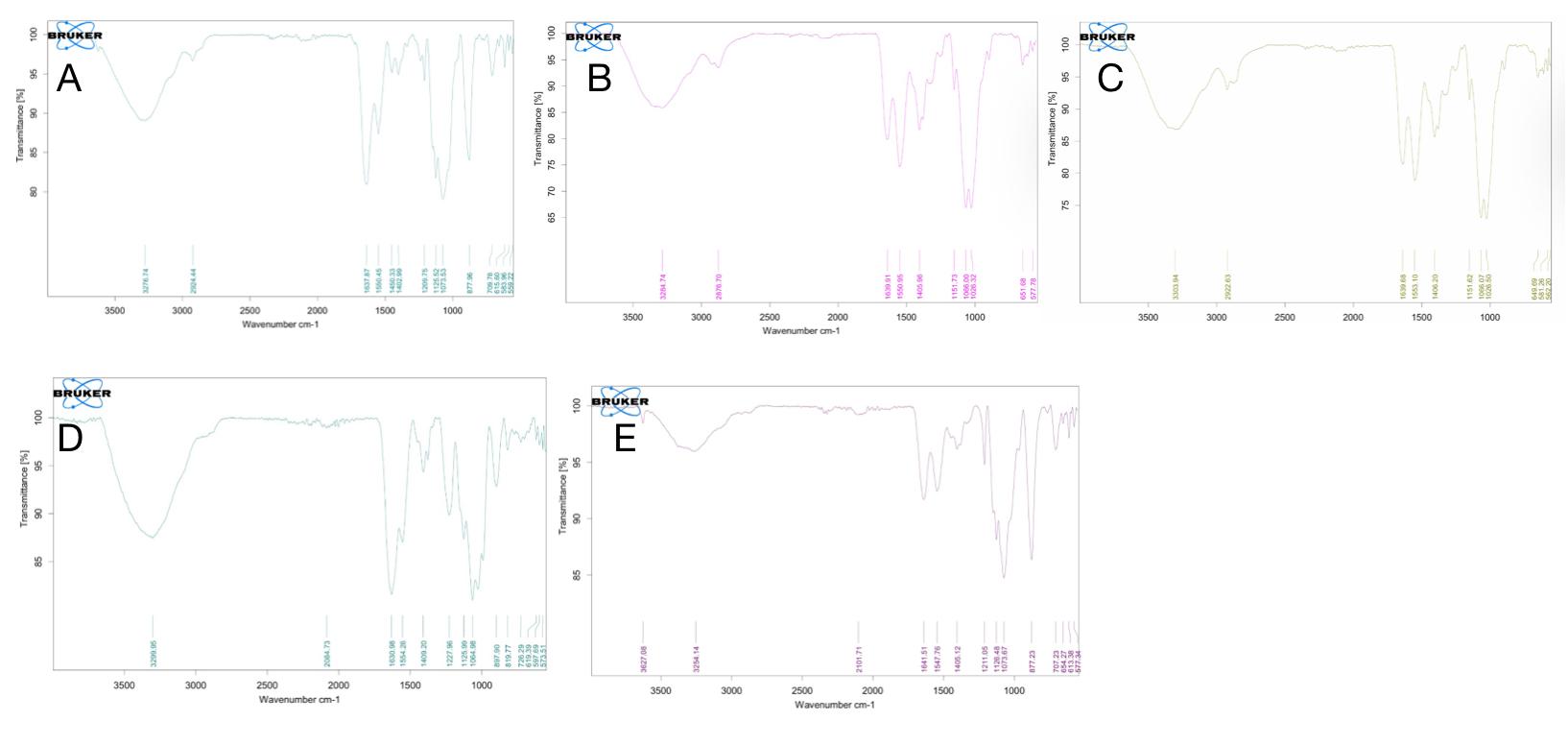
The SEM analysis of the control and test groups revealed distinct variations in porosity depending on the composition of the membranes. The CuO-based membranes exhibited reduced porosity with an even distribution, whereas chitosan, xanthan gum, and chondroitin sulfate-based membranes showed higher porosity in a scattered manner. This difference in porosity influences cell adhesion, migration, penetration efficiency, and nutrition flow, making CuO-based membranes more suitable for guided tissue regeneration (GTR) and guided bone regeneration (GBR) applications.



**Figure 5: A** → Xanthan-CuO composites,  **B** → Chitosan-based composites, **C** → CuO-based group, **D** → Chondroitin sulfate-CuO composites, **E** → Chitosan-CuO composites

# Spectral Analysis and Peak Identification

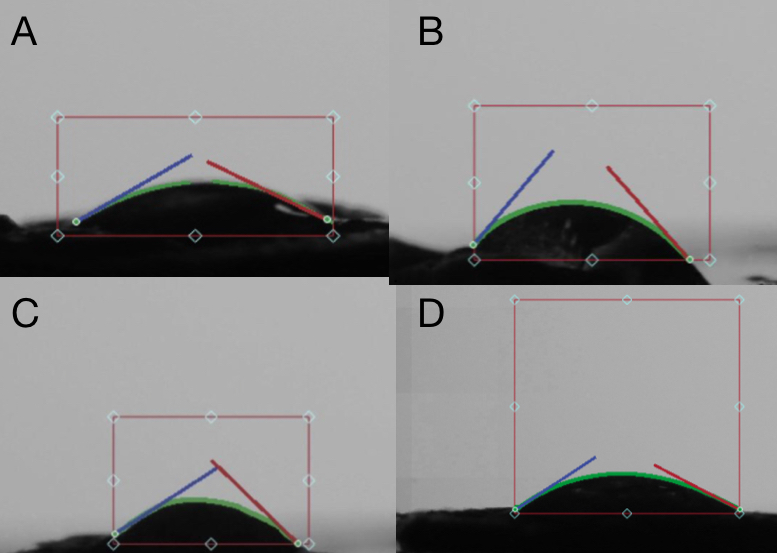
The spectral analysis of various compositions provided insights into the chemical characteristics of the membranes. The FTIR spectra confirm the successful incorporation of nanoparticles into the polymeric matrices by revealing characteristic peak shifts and intensity changes.**Figure A** (Xanthan-CuO composites): The peak at 1073.5 cm⁻¹ corresponds to C-O stretching, confirming the presence of xanthan gum. The incorporation of CuO is evident from additional bands and possible shifts in the fingerprint region.**Figure B** (Chitosan-based composites): The amine peak at 327.9 cm⁻¹ is characteristic of chitosan, while a peak at 877 cm⁻¹ suggests interactions with CuO.**Figure C** (CuO-based group): The strong absorption bands indicate CuO presence, with characteristic metal-oxygen (Cu-O) stretching vibrations.**Figure D** (Chondroitin sulfate-CuO composites): The peak at 1021 cm⁻¹ is attributed to sulfate groups in chondroitin sulfate, indicating successful composite formation.**Figure E** (Chitosan-CuO composites): A peak at 877 cm⁻¹ suggests CuO incorporation, similar to Figure B, while characteristic chitosan peaks are retained.



**Figure 6: A** → Xanthan-CuO composites,  **B** → Chitosan-based composites, **C** → CuO-based group, **D** → Chondroitin sulfate-CuO composites, **E** → Chitosan-CuO composites

## Contact Angle and Hydrophilicity

The contact angle measurements revealed a shift in hydrophilicity upon the addition of CuO nanoparticles.The contact angle measurement determines the wettability of the membranes, influencing cell attachment and protein adsorption. The results showed:Chondroitin Sulfate/CuO had the lowest contact angle (30.6°), indicating high hydrophilicity and excellent cell adhesion properties.CuO-Based GBR (32°) and Xanthan Gum/CuO (36.1°) also exhibited good hydrophilicity, promoting better cellular interactions.Chitosan-Based GBR had a contact angle of 40°, suggesting moderate wettability.Chitosan/CuO had the highest contact angle (54.4°), indicating lower hydrophilicity but better mechanical stability.Since lower contact angles correlate with better hydrophilicity, Chondroitin Sulfate/CuO and CuO-Based GBR appear to be the most favorable for cell attachment in GBR applications.



**Figure 7: A:** Chitosan+CuO,Figure B : Chitosan based GBR,Figure C:CuO based GBR,Figure D:Xanthan +CuO

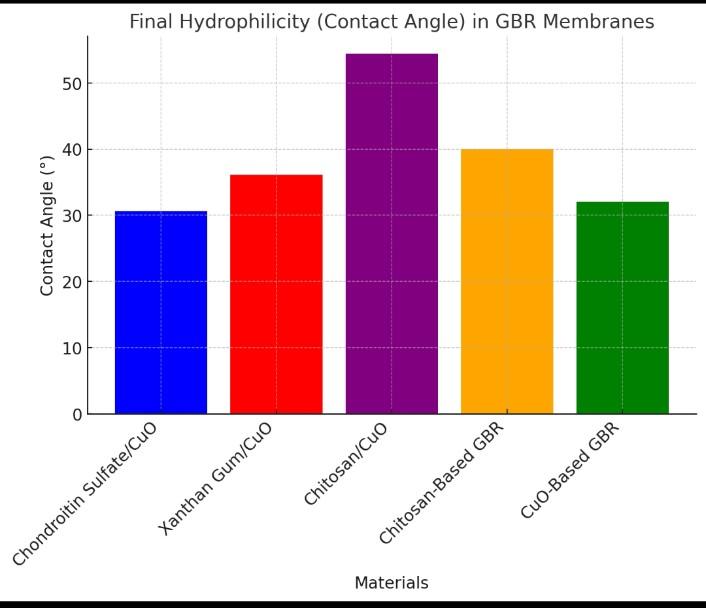


Figure 8: Swelling Percentage analysis

The swelling properties of membranes are crucial for maintaining hydration and nutrient exchange in the defect site. Swelling increased in all cases upon nanoparticle incorporation, making them ideal for chemostatic-based membranes or socket preservation procedures.Chitosan/CuO (35%) and Xanthan Gum/CuO (34%) demonstrated the highest swelling capacity, making them ideal for socket preservation, where maintaining hydration is crucial for early-stage bone healing.Chondroitin Sulfate/CuO (24%) exhibited lower swelling, ensuring better mechanical stability, making it more suitable for ridge augmentation, where maintaining membrane integrity is essential.CuO-Based GBR (28%) and Chitosan-Based GBR (30%) maintained moderate swelling properties, offering a balance between hydration and mechanical strength, making them potential candidates for general GBR applications.These findings suggest that GBR membranes should be selected based on the clinical requirement, with higher swelling membranes preferred for soft tissue healing and lower swelling membranes for load-bearing applications.

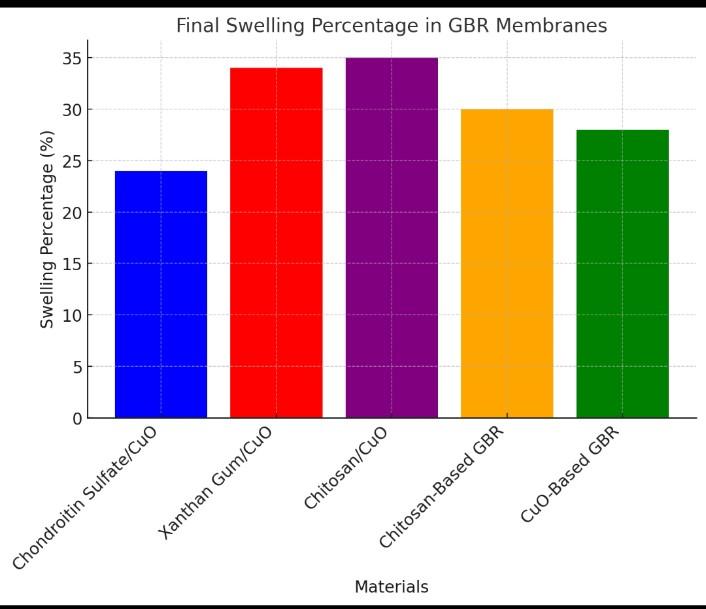
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Figure 9: Biocompatibility and Cell Interaction

Biocompatibility testing evaluates how well cells adhere to and proliferate on GBR membranes. The MTT assay results showed:Chondroitin Sulfate/CuO had the highest biocompatibility score (147%), making it the most suitable candidate for GBR applications.CuO-Based GBR showed a biocompatibility score of 110%, demonstrating strong cellular interactions.Chitosan-Based GBR had a biocompatibility score of 120%, suggesting favorable cell viability.Chitosan/CuO exhibited a score of 90%, showing good but slightly reduced biocompatibility compared to others.Xanthan Gum/CuO had the lowest biocompatibility score (56%), indicating a need for further optimization.

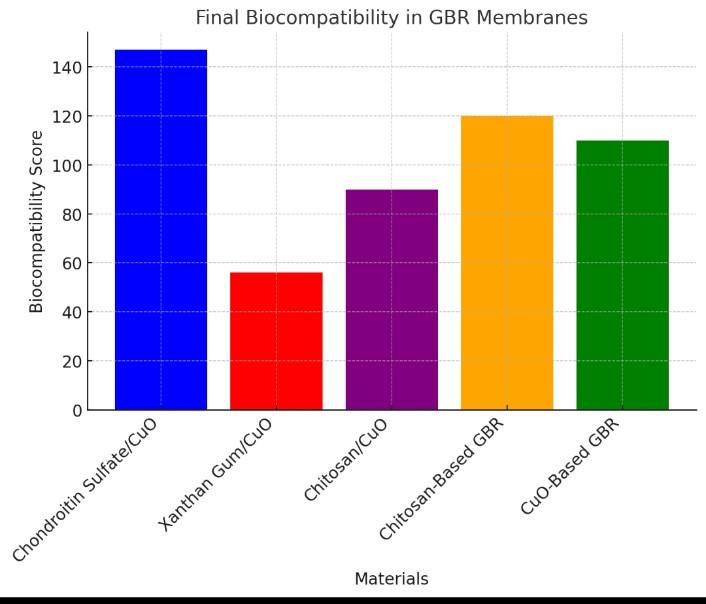


Figure 10: Statistical analysis

To determine the statistical significance of the observed variations among different GBR membrane formulations, a One-Way ANOVA test was conducted for swelling ratio, contact angle (hydrophilicity), and biocompatibility (MTT assay).Swelling Ratio: The swelling ratio analysis revealed significant differences among the GBR membranes (p = 1.03 × 10⁻¹²). Chitosan/CuO exhibited the highest swelling percentage (35%), followed closely by Xanthan Gum/CuO (34%), both of which demonstrated high water absorption capacity. In contrast, Chondroitin Sulfate/CuO had the lowest swelling (24%), ensuring a more controlled hydration profile and better structural stability. These results indicate that swelling properties should be optimized depending on clinical needs, with highly absorbent membranes being useful for socket preservation and controlled-swelling membranes being ideal for ridge augmentation.Contact Angle Measurement (Hydrophilicity): The One-Way ANOVA test confirmed statistically significant differences in surface wettability among the GBR membranes (p = 1.26 × 10⁻²²). Chondroitin Sulfate/CuO recorded the lowest contact angle (30.6°), indicating superior hydrophilicity and excellent cell adhesion potential. CuO-Based GBR (32°) and Xanthan Gum/CuO (36.1°) also exhibited good hydrophilicity, promoting better tissue integration. In contrast, Chitosan/CuO exhibited the highest contact angle (54.4°), suggesting lower hydrophilicity but improved mechanical stability. Since hydrophilicity plays a crucial role in protein adsorption and cell attachment, materials with lower contact angles are generally preferred for enhanced biocompatibility.Biocompatibility (MTT Assay): The biocompatibility assessment, measured using the MTT assay, also showed a statistically significant difference among the GBR membranes (p = 2.24 × 10⁻³⁶). Chondroitin Sulfate/CuO had the highest biocompatibility score (147%), confirming its superior compatibility with osteogenic cells. CuO-Based GBR (110%) and Chitosan-Based GBR (120%) also demonstrated high biocompatibility, making them suitable candidates for GBR applications. In contrast, Xanthan Gum/CuO (56%) had the lowest biocompatibility, indicating a need for further modifications to improve cellular interactions and osteogenic potential.Overall, the statistical analysis confirms that the variations in swelling ratio, contact angle, and biocompatibility among different GBR membrane formulations are highly significant (p < 0.05). Chondroitin Sulfate/CuO emerges as the most promising candidate for GBR applications, owing to its high biocompatibility and superior hydrophilicity, making it ideal for bone regeneration and long-term clinical applications. Meanwhile, Chitosan/CuO demonstrates excellent swelling properties, making it well-suited for applications requiring enhanced moisture retention and hydration stability.

# DISCUSSION

The findings of this study emphasize the importance of material selection in determining the performance of Guided Bone Regeneration (GBR) membranes. The swelling analysis revealed that Chitosan/CuO (35%) and Xanthan Gum/CuO (34%) exhibited the highest water absorption capacity, making them suitable for applications where moisture retention is critical, such as socket preservation[(Khoury, 2021)](https://paperpile.com/c/owM7Tl/hVCJ). However, excessive swelling can compromise mechanical integrity, making controlled swelling materials like Chondroitin Sulfate/CuO (24%) preferable for load-bearing applications such as ridge augmentation[(Froum, 2015)](https://paperpile.com/c/owM7Tl/QnbR). Similar observations have been reported in previous studies, where hydrophilic modifications in GBR membranes significantly influenced their clinical efficacy[(Jain et al., 2023; Schoenbaum, 2018)](https://paperpile.com/c/owM7Tl/cHB2+L6Ro).Hydrophilicity plays a crucial role in GBR membrane performance, as it directly affects protein adsorption, cell adhesion, and subsequent bone regeneration. The results demonstrated that Chondroitin Sulfate/CuO (30.6°) exhibited the highest hydrophilicity, followed by CuO-Based GBR (32°) and Xanthan Gum/CuO (36.1°). These findings align with prior research indicating that CuO-based membranes exhibit superior hydrophilic properties, facilitating better osteoblast attachment and proliferation[(Dharman et al., 2023; Gao et al., 2017)](https://paperpile.com/c/owM7Tl/6wNk+Nt34). In contrast, Chitosan/CuO showed the highest contact angle (54.4°), suggesting reduced wettability but enhanced mechanical strength. This balance between hydrophilicity and mechanical stability should be considered when selecting GBR membranes, as some clinical applications require stronger membranes despite lower wettability[(Lakshmi, 2021; Website, n.d.)](https://paperpile.com/c/owM7Tl/PT0d+krsR).The biocompatibility analysis further supports the potential of Chondroitin Sulfate/CuO as an ideal GBR membrane, with the highest cell viability (147%). This suggests that chondroitin sulfate enhances osteoblast activity, making it a strong candidate for bone regeneration applications. CuO-Based GBR (110%) and Chitosan-Based GBR (120%) also demonstrated high biocompatibility, validating their clinical potential. However, Xanthan Gum/CuO showed the lowest biocompatibility (56%), suggesting the need for further optimization to improve cellular interactions. Similar trends have been observed in other studies, where CuO-based GBR membranes have shown varying levels of biocompatibility depending on their composition and nanoparticle distribution[(Govindaraj et al., 2023; Meyer et al., 2009)](https://paperpile.com/c/owM7Tl/OoLT+y5oc). Future research should explore additional surface modifications and bioactive factor incorporation to optimize GBR membranes for enhanced osteogenic potential[(Akshayaa & Ganesh, 2025; Kabilamurthi et al., 2021; Neeharika et al., 2023)](https://paperpile.com/c/owM7Tl/JbAP+olDr+C2OW).

## Future Implications and Research Directions

With continuous advancements in biomaterials and nanotechnology, CuO-based membranes present significant potential for bone regeneration, implantology, and maxillofacial surgery. Future research should focus on:Long-Term Clinical Trials – Studying the degradation rate of CuO-infused membranes and their effects on bone regeneration over extended periods. Patient-Specific GBR Membranes – Developing customizable membranes based on individual patient needs using 3D printing and bioengineering techniques.Combination Therapies – Exploring the synergy between CuO-based membranes and growth factors like BMP-2 and VEGF for enhanced osteogenesis.Enhanced Structural Integrity – Investigating novel crosslinking agents to improve the mechanical stability of resorbable CuO-based GBR membranes and further tests with in vivo testing and clinical trials are required for long term biocompatibility and bone integration.

# Conclusion

This study analyzed five different GBR membrane formulations by incorporating Chondroitin Sulfate, Chitosan, and Xanthan Gum with CuO nanoparticles to evaluate their swelling behavior, hydrophilicity, and biocompatibility. The SEM analysis confirmed that Chitosan/CuO and CuO-Based GBR exhibited highly porous structures, which are crucial for facilitating cell migration and nutrient diffusion. The FTIR analysis validated the successful integration of CuO nanoparticles, highlighting their role in improving the osteogenic potential of the membranes. Our findings demonstrate that Chondroitin Sulfate/CuO had the highest biocompatibility (147%), while Chitosan/CuO exhibited superior swelling properties (35%), making them highly suitable for clinical GBR applications.The hydrophilicity analysis revealed that Chondroitin Sulfate/CuO (30.6°) and CuO-Based GBR (32°) were the most hydrophilic, ensuring optimal cell adhesion and protein adsorption(Almatrafi et al., 2024). In contrast, Chitosan/CuO (54.4°) showed moderate hydrophilicity, balancing mechanical integrity and cellular interaction(Saadh et al., 2024). The biocompatibility results further supported Chondroitin Sulfate/CuO and CuO-Based GBR as the most favorable candidates for bone regeneration, as they exhibited higher cell viability and proliferation rates compared to Xanthan Gum-based membranes. Our swelling analysis showed that Xanthan Gum/CuO (34%) and Chitosan/CuO (35%) retained more moisture, making them beneficial for socket preservation and early-stage bone healing.Overall, this comparative study highlights that CuO-based GBR membranes provide enhanced mechanical strength, bioactivity, and osteoconductivity, making them promising candidates for clinical bone regeneration applications. Future studies should explore long-term in vivo degradation patterns, the incorporation of bioactive growth factors, and the development of customized GBR membranes using 3D bioprinting techniques. By refining CuO-based membranes further, their potential for maxillofacial surgery, implantology, and orthopedic applications can be fully realized.

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