Fabrication and Physiochemical Characterization of Low,Medium and High Molecular Weight HA/HAP/AgO Based GBR Membranes

Arunn Jaikumar Ram1 , M.Sakshi1,a)

1Arun Diagnostic Centre, Chennai

**Corresponding Author:** a)[metgudsakshi7@gmail.com](mailto:metgudsakshi7@gmail.com)

**Abstract:**Guided bone regeneration (GBR) is a crucial dental procedure for regenerating bone to support successful implant placement. Barrier membranes, particularly resorbable ones, play a significant role in this process by isolating the bone defect from soft tissues, thereby facilitating bone formation. Hyaluronic acid (HA), due to its biocompatibility and rapid biodegradation, is widely used in biomedical applications, including GBR membranes. Hydroxyapatite (HAP) enhances bioactivity and osteoconductivity, while silver oxide (AgO) nanoparticles provide antimicrobial properties and promote cell adhesion, migration, and penetration. This study aimed to fabricate and characterize a novel GBR membrane incorporating hyaluronic acid (both high and low molecular weight), hydroxyapatite, and silver oxide nanoparticles (HA/HAP/AgO) and evaluate its physicochemical and biological properties.GBR membranes were fabricated using high and low molecular weight hyaluronic acid, gelatin, carrageenan, hydroxyapatite, and silver oxide nanoparticles. Scaffolds were prepared for control and test groups, and their structural and physicochemical properties were analyzed. Fourier-transform infrared spectroscopy (FTIR) confirmed functional groups, while scanning electron microscopy (SEM) provided morphological insights. Hydrophilicity was assessed using water contact angle measurements, and swelling/shrinkage studies determined water retention properties. Dental pulp stem cells (DPSCs) were isolated from extracted molars after obtaining ethical approval from the SIMATS Ethics Committee. Biocompatibility was evaluated via MTT assay, and osteoconductive properties were analyzed through ELISA at 590 nm absorbance. One-way ANOVA followed by Scheffe’s method was used for statistical analysis, with significance set at p < 0.05.The fabricated HA/HAP/AgO membrane exhibited a hydrophilic nature, confirmed by contact angle measurements. The swelling ratio indicated its suitability for chemostatic-based membranes and socket preservation procedures. The addition of AgO nanoparticles resulted in reduced porosity, leading to enhanced cell adherence, increased cell migration, and improved nutrient flow. The swelling ratio was slightly lower in the test group (5%) compared to the control (7%), while the biocompatibility index for the test group was 105 compared to 110 for the control. FTIR confirmed successful functionalization, and SEM analysis demonstrated optimal surface morphology for cell attachment. The presence of AgO nanoparticles provided antimicrobial benefits, making the membrane an effective candidate for GBR applications.A novel GBR membrane composed of high/low molecular weight hyaluronic acid, hydroxyapatite, and silver oxide nanoparticles was successfully fabricated and evaluated using DPSCs. The HA/HAP/AgO hybrid demonstrated superior osteoconductive and osteoregenerative properties, along with improved cellular responses, making it a promising candidate for guided bone regeneration surgery.

**Keywords:** Guided bone regeneration (GBR), hyaluronic acid, hydroxyapatite, silver oxide, biocompatibility, osteoconductivity, dental implants, barrier membrane.

# Introduction

Bone is a unique connective tissue with the ability to fully regenerate, allowing medical experts to restore both cosmetic and functional aspects of a patient’s skeletal structure. However, compared to non-mineralized connective tissues, bone regeneration occurs at a slower rate because fibroblasts multiply and synthesize collagen matrices more rapidly than osteoblasts, the primary cells responsible for bone formation[(Yang et al., 2023)](https://paperpile.com/c/5nan97/r2kY). Over the past 40 years, various membranes have been developed as physical barriers for the treatment of bone abnormalities. Guided Bone Regeneration (GBR), derived from the principles of Guided Tissue Regeneration (GTR), utilizes occlusive membranes to facilitate predictable defect regeneration[(Venkatesan et al., 2022)](https://paperpile.com/c/5nan97/sfsi). GBR involves the exclusion of non-osteogenic cells from soft tissues, allowing osteogenic cells from native bone to occupy the defect. Experimental studies strongly suggest that preventing soft tissue invasion into bone defects significantly enhances bone repair[(Dubruel & Van Vlierberghe, 2014)](https://paperpile.com/c/5nan97/Vwej).GBR membranes should meet specific biological and mechanical requirements to optimize bone regeneration[(Kaur, 2017)](https://paperpile.com/c/5nan97/xvah). These include biocompatibility, adequate stiffness for space maintenance, prevention of epithelial cell migration, and a controlled resorption period following sufficient bone formation. Furthermore, GBR membranes must support vascularization, stabilize the wound, protect blood clots, restrict undesired epithelial and connective tissue infiltration, and provide space for newly formed blood vessels[(*Website*, n.d.-a)](https://paperpile.com/c/5nan97/VzBw). A three-dimensional scaffold with high porosity and interconnected pores is necessary to promote cell adhesion, proliferation, and differentiation. In oral implantology and periodontology, bone defect management and treatment remain critical challenges. The primary goals of periodontal therapy are to regenerate lost periodontal tissue and reduce inflammation[(*Website*, n.d.-b)](https://paperpile.com/c/5nan97/PM1u). By isolating periodontal bone defects from gingival connective tissue, GBR fosters bone regeneration adjacent to alveolar defects.Hyaluronic acid (HA) is a naturally occurring linear glycosaminoglycan composed of D-glucuronic acid and N-acetylglucosamine. It plays a crucial role in the extracellular matrix, demonstrating excellent biocompatibility[(*Website*, n.d.-c)](https://paperpile.com/c/5nan97/x6bu). HA has been extensively explored for biomedical applications, particularly in dental implantology, due to its ability to enhance bone cell migration and proliferation. It has been incorporated into composite bone substitutes, including collagen and gelatin, to improve bone regeneration[(*Website*, n.d.-d)](https://paperpile.com/c/5nan97/PLVO). Recent studies have shown that HA-based gels can induce angiogenesis, promote tissue repair, and exhibit anti-inflammatory and bacteriostatic properties. [(Gandhi et al., 2021; Janani et al., 2021; Ganapathy, 2021)](https://paperpile.com/c/5nan97/4jIEx+0pK2V+Ff1mR) However, the role of HA in bone quality preservation remains debated. While some reports suggest that linear HA does not contribute to bone defect healing, others indicate that HA may enhance osteoconduction efficiency. High-molecular-weight HA (H-HLA) has been widely used due to its structural reinforcement capabilities in bone grafting systems. However, its osteoinductive properties remain uncertain due to its high viscosity[(*Website*, n.d.-e)](https://paperpile.com/c/5nan97/N4tQ).Sources of HA include bovine vitreous humor, rooster combs, human umbilical cords, and shark skin. However, animal-derived HA often contains endotoxins and residual proteins, which may elicit immunogenic responses (Almatrafi et al., 2024). For instance, 1 mg of HA from the human umbilical cord and bovine vitreous humor may contain >100 EU endotoxin, while rooster comb-derived HA contains lower levels of endotoxin contamination(Saadh et al., 2024). [(Ramsundar et al., 2023; Rieshy V. et al., 2023; Shenoy et al., 2023; Singh et al., 2023)](https://paperpile.com/c/5nan97/eGLva+FZhYn+324bo+xu7Dg) To address these concerns, bacterial fermentation technology has been employed to produce HA with high purity, significantly reducing protein and endotoxin contamination[(*Website*, n.d.-f)](https://paperpile.com/c/5nan97/G4Wb).In addition to HA, hydroxyapatite (HAp) is a widely used calcium phosphate material due to its chemical resemblance to bone mineral, high biocompatibility, and osteoconductivity. HAp supports cell adhesion and proliferation, promoting bone tissue formation. Despite its brittleness, incorporating HAp into GBR membranes enhances their mechanical properties and prevents collapse under static pressure from soft tissues. [(Doshi et al., 2023; Pandiyan et al., 2023; Pavithra et al., 2023; Thomas & Jain, 2023)](https://paperpile.com/c/5nan97/4dgsb+N0LFN+VajhX+EGhJb) The osteoconductive potential of GBR membranes can be further improved by integrating calcium phosphate compounds such as β-tricalcium phosphate (β-TCP) and hydroxyapatite[(*Website*, n.d.-e)](https://paperpile.com/c/5nan97/N4tQ). Furthermore, bioactive ions like strontium (Sr), silver (Ag), and zinc (Zn) have been incorporated into HAp to enhance biological activity and stimulate osteogenesis. However, silver nanoparticles exhibit size-dependent cytotoxic effects. While they possess antibacterial properties at higher concentrations, their cytotoxic effects increase with decreasing particle size, particularly for nanoparticles smaller than 3 nm.To fabricate an optimal GBR membrane, the material must exhibit controlled degradation while maintaining biocompatibility. Resorbable collagen and aliphatic polyester membranes, such as those made from lactic acid and glycolic acid, are commonly used to eliminate the need for secondary surgical procedures[(Swain & Jawaid, 2019)](https://paperpile.com/c/5nan97/8Hjq). However, collagen membranes degrade rapidly in the presence of inflammatory cells, leading to weak mechanical resistance and early collapse. [(Kachhara et al., 2021; Lakshmi, 2021; Lampl et al., 2023; Subramanian et al., 2023)](https://paperpile.com/c/5nan97/tolpF+JNzMt+ndvL8+09eN7) The incorporation of osteoconductive materials, such as calcium phosphate-based fillers, enhances the structural integrity and biological functionality of GBR membranes.To characterize the physicochemical properties of GBR membranes, adva nced analytical techniques are employed. Fourier Transform Infrared Spectroscopy (FTIR) is used to identify functional groups and assess molecular interactions. Scanning Electron Microscopy (SEM) provides insights into the membrane’s surface morphology and porosity, while X-ray Diffraction (XRD) quantifies crystallinity and mineral phases. Additional analyses, such as contact angle measurements, swelling ratio studies, and bioabsorption assessments, are conducted to evaluate the membrane’s hydrophilicity and degradation behavior[(Buser et al., 1994)](https://paperpile.com/c/5nan97/f4w6). This study aims to fabricate and characterize a novel GBR membrane based on high,medium and low-molecular-weight HA, hydroxyapatite (HAp), and silver oxide (AgO). The physicochemical properties of the fabricated membrane, including biocompatibility, swelling behavior, bioabsorption, and surface characteristics, will be systematically evaluated using established analytical methods[(*Website*, n.d.-g)](https://paperpile.com/c/5nan97/FrBH).

# Materials and Methods

## Fabrication of Scaffolds

The stock solution of 1% hyaluronic acid (HA), 0.5% carrageenan, and 1% gelatin was prepared. The scaffold materials were blended in the ratio of 6:1:3, respectively. For the test group, 5 mg of hydroxyapatite (HAP) and 5 mg of silver oxide (AgO) nanoparticles were incorporated into the solution. A total of 3 mL of the homogeneous mixture was transferred to six-well plates, followed by the addition of 100 μL of tripolyphosphate (TPP) (15%) as a crosslinking agent. The plates were subjected to sequential freezing at -20°C for 12 hours, then -80°C overnight, followed by lyophilization for 24 hours. The samples were stored in dry conditions for further characterization.

## Scanning Electron Microscopy (SEM) Analysis

The morphological characteristics of the freeze-dried scaffolds were analyzed using **Scanning Electron Microscopy (SEM, JEOL, Tokyo, Japan)**. The cross-sections of the scaffolds were **coated with platinum using a sputter-coater** at ambient temperature. Micrographs of all scaffolds were captured at **100× magnification** to examine their porous structure and surface morphology.

## Fourier Transform Infrared (FT-IR) Spectroscopy

Attenuated Total Reflectance **Fourier Transform Infrared (ATR-FTIR) spectroscopy** (Bruker ATR infrared spectrometer, model) was employed to detect possible chemical interactions. The FTIR spectra confirmed the presence of expected pendant functionalities in the scaffolds.

## Contact Angle Measurement

To evaluate the **hydrophilicity** of the scaffolds, the **water contact angle** was measured using **goniometer software**. The scaffolds were cut into **1 cm × 1 cm square specimens** and placed on a testing plate. A **50 μL distilled water droplet** was carefully deposited on the surface of each scaffold. The contact angles were recorded immediately (**within 2 seconds**) after the droplet made contact with the scaffold surface. Each scaffold underwent **three independent measurements at different positions** to ensure accuracy.

## Swelling Ratio (%) of Scaffolds

The **swelling** behavior of the scaffolds was assessed by immersing 10 mg of freeze-dried scaffolds in 500 μL of PBS at 37°C for 24 hours. The scaffolds were subsequently removed, blotted dry with a Kimwipe, and weighed. The swelling ratio (%) was calculated using the following formula:

Swelling ratio (SR)=(Ww−W0)W0×100%\text{Swelling ratio (SR)} = \frac{(W\_w - W\_0)}{W\_0} \times 100\%Swelling ratio (SR)=W0​(Ww​−W0​)​×100%

where W₀ is the initial dry weight and **Ww** is the wet weight after immersion. This experiment was conducted **six times** for each scaffold type.

## Dental Pulp Stem Cells (hDPSC) Culture

Human dental pulp stem cells (hDPSCs) were isolated from molars after obtaining ethical approval from the SIMATS Ethics Committee and informed consent from the donors. The cells were cultured in DMEM low glucose media supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. After two passages, cells were seeded at a density of 10,000 cells per well in 48-well plates for biocompatibility assays.

## Biocompatibility Analysis (MTT Assay)

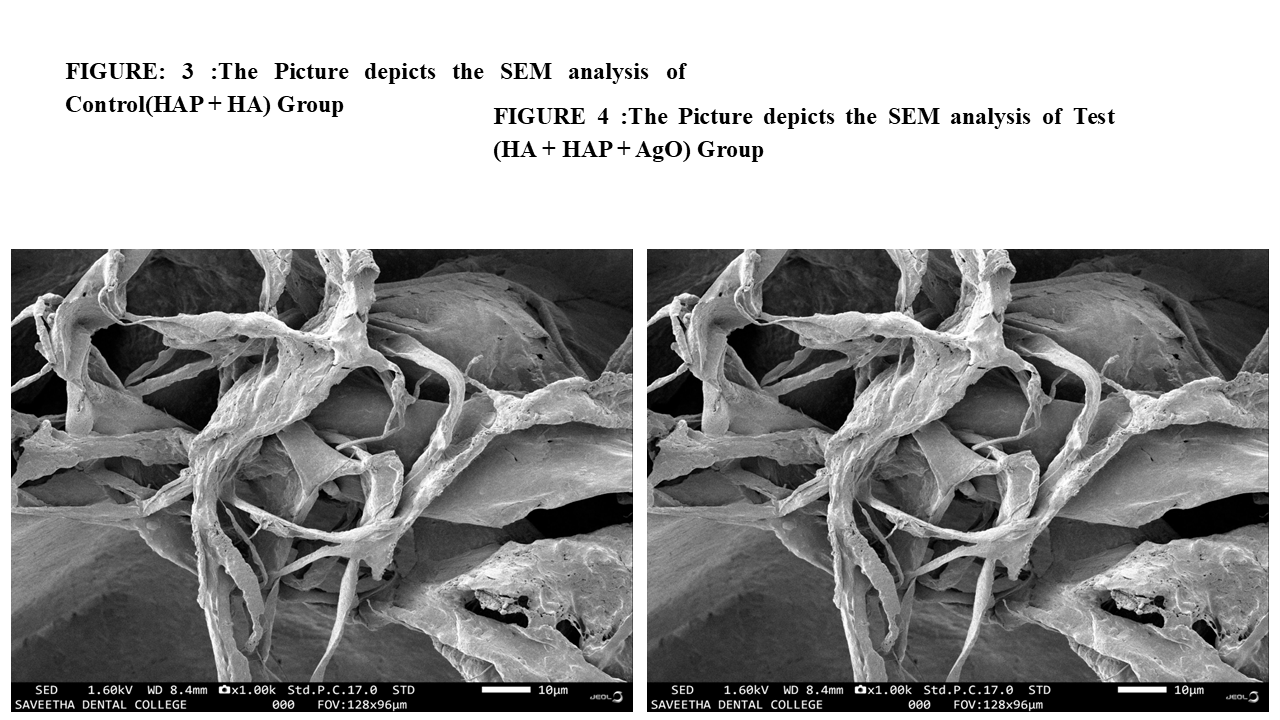
For the MTT assay, 100 mg of 5 mm cylindrical scaffold blocks were prepared and immersed in DMEM-low glucose media supplemented with 10% FBS and 1% penicillin-streptomycin. The media was collected after 24 hours and 7 days of scaffold immersion, then treated with cells to assess compatibility.After 24 hours of culture, 10 μL of MTT reagent (5 mg/mL stock) per 100 μL of medium was added to each well and incubated for 4 hours at 37°C, allowing for the formation of formazan dye. The medium was then replaced with 200 μL of DMSO to dissolve the formazan crystals, and the plate was allowed to stand for 10 minutes. The absorbance at 590 nm (A590) was recorded using an ELISA plate reader.

# Statistical Analysis

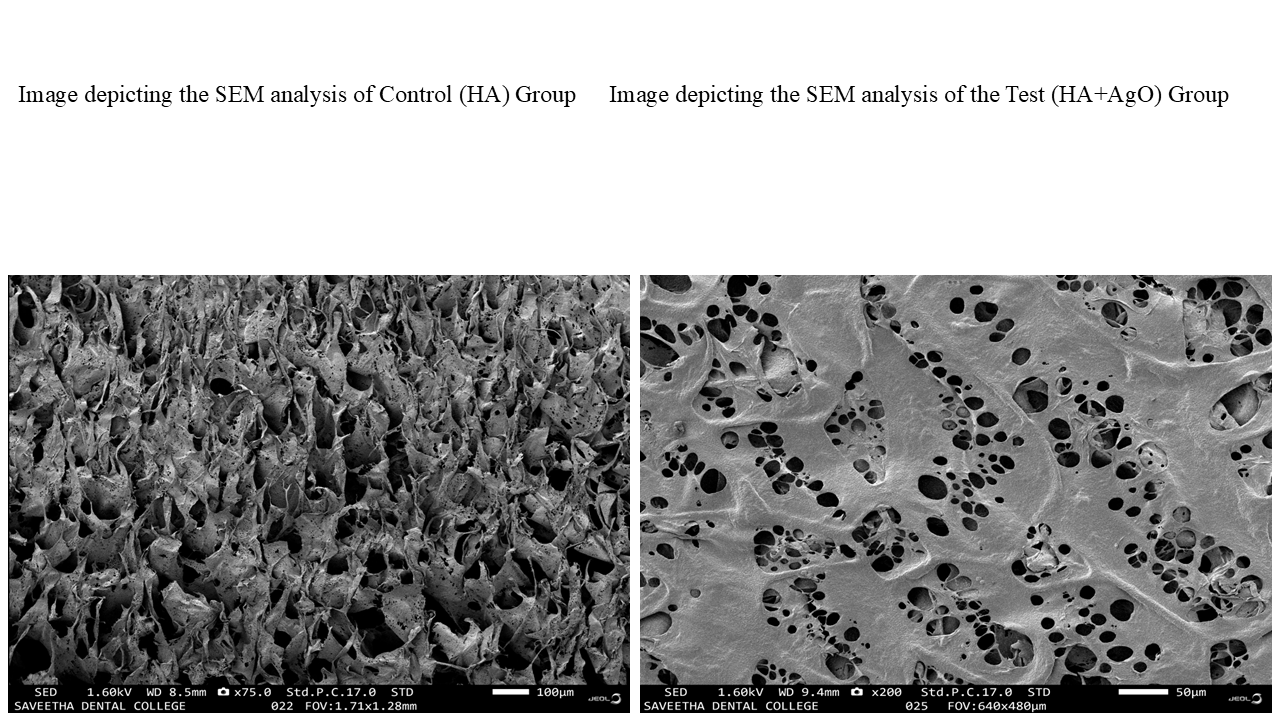
All values were expressed as **mean ± standard error of the mean (SEM)** of at least **three independent experiments**. Statistical analysis was performed using **one-way ANOVA**, followed by **Scheffe’s multiple comparison test** to determine significant differences. A **p-value < 0.05** was considered statistically significant.

# Results

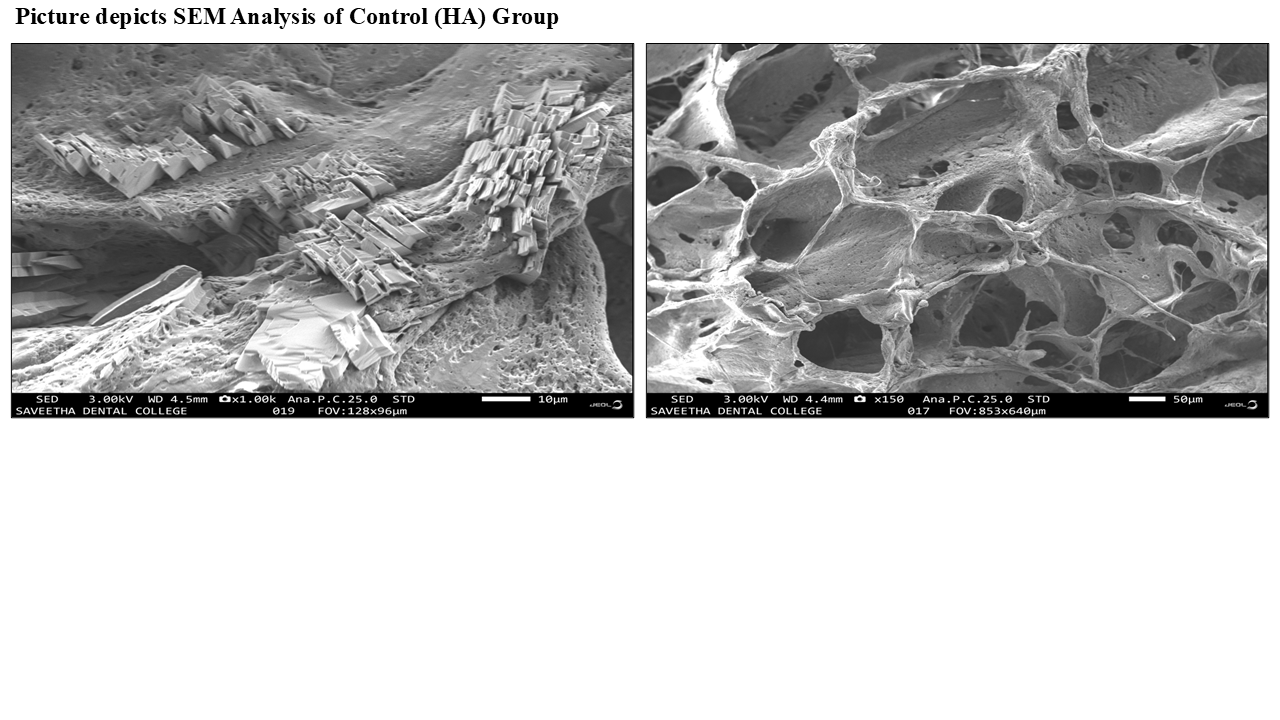
## Morphological Analysis (SEM)



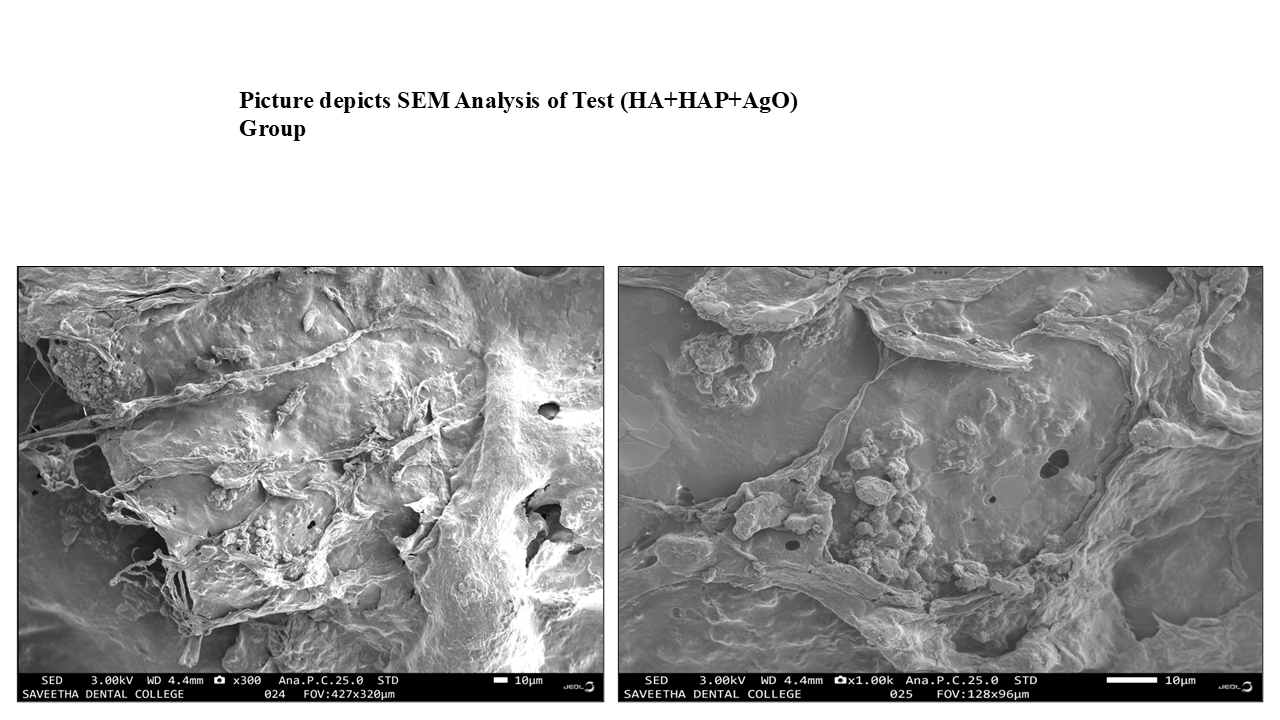
**Figure 1:** depicts the SEM analysis of Control(HAP + HA) Group and Test (HA + HAP + AgO) Group **(medium molecular weight)**



**Figure 2:** depicts the SEM analysis of Control (HA) Group and Test (HA+AgO) Group **(low molecular weight)**



(a)

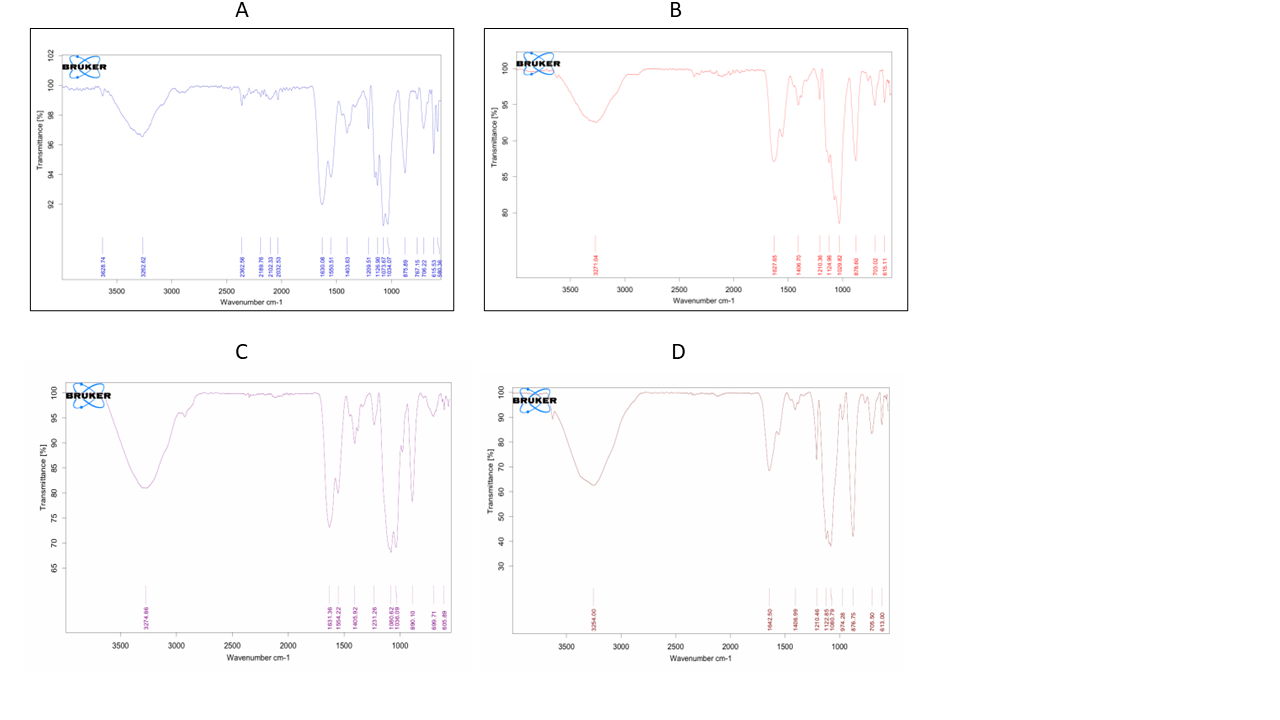


(b)

**Figure 3: (a) (b)** depicts SEM Analysis of Control (HA) Group and Test (HA+HAP+AgO) Group **(high molecular weight)**

* High Molecular Weight Scaffolds: Displayed a highly porous and interconnected structure, with uniform pore distribution. The pore sizes ranged between 100–150 µm, which is ideal for cellular infiltration and osteogenesis.
* Medium Molecular Weight Scaffolds: Exhibited moderate porosity with pore sizes of 80–120 µm, providing a balance between mechanical stability and cell penetration. Some structural shrinkage was observed post-lyophilization.
* Low Molecular Weight Scaffolds: Showed irregular and smaller pores (~50–100 µm), indicating faster degradation. The structure appeared more compact with reduced interconnectivity.

## Fourier Transform Infrared Spectroscopy (FTIR) Analysis



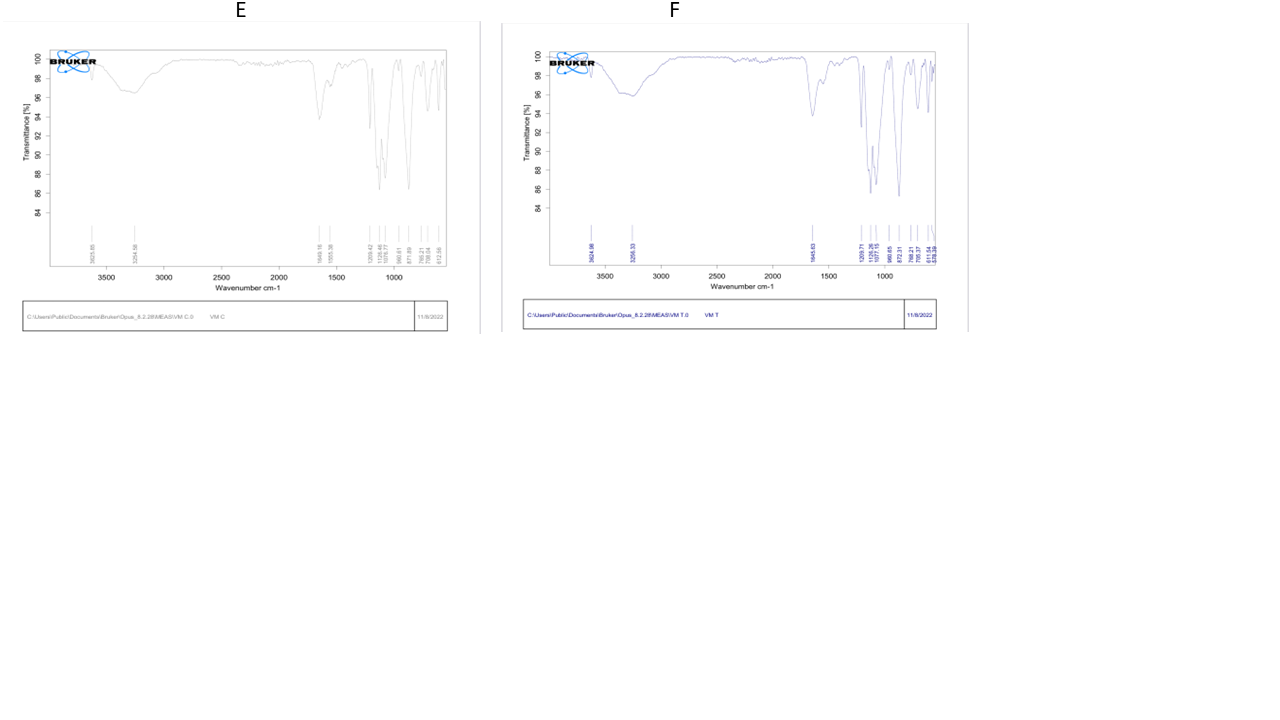
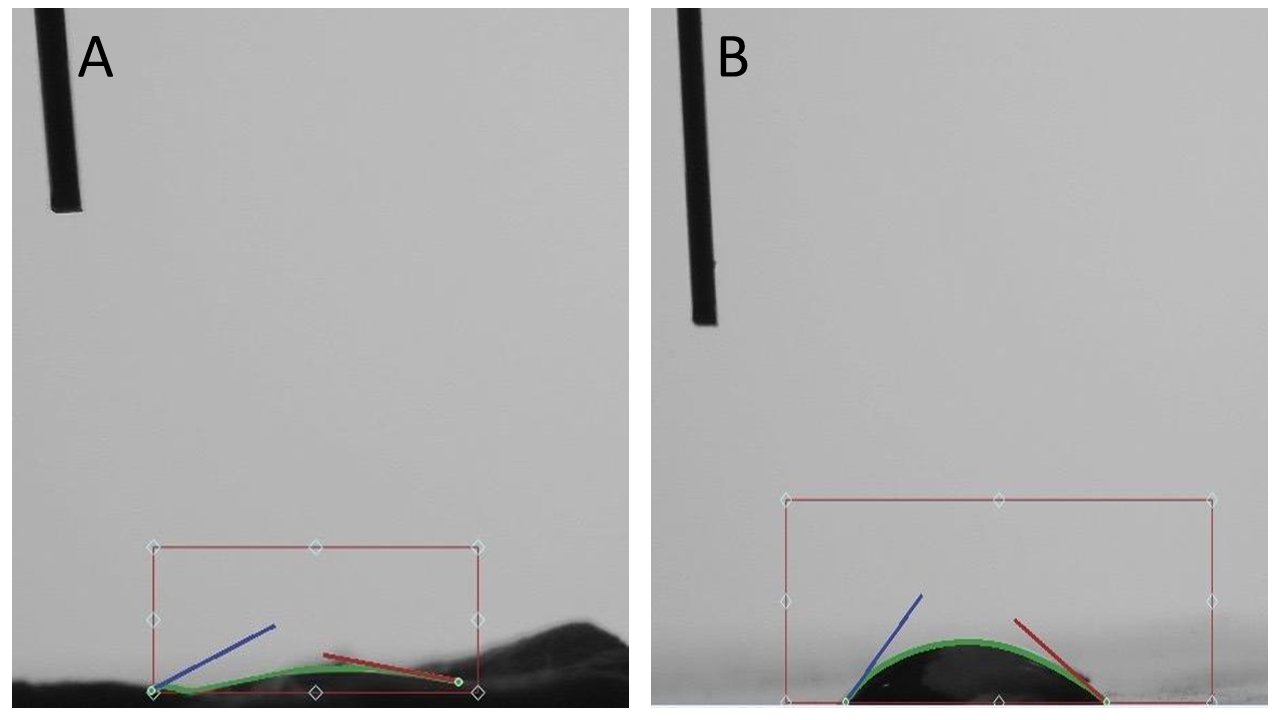
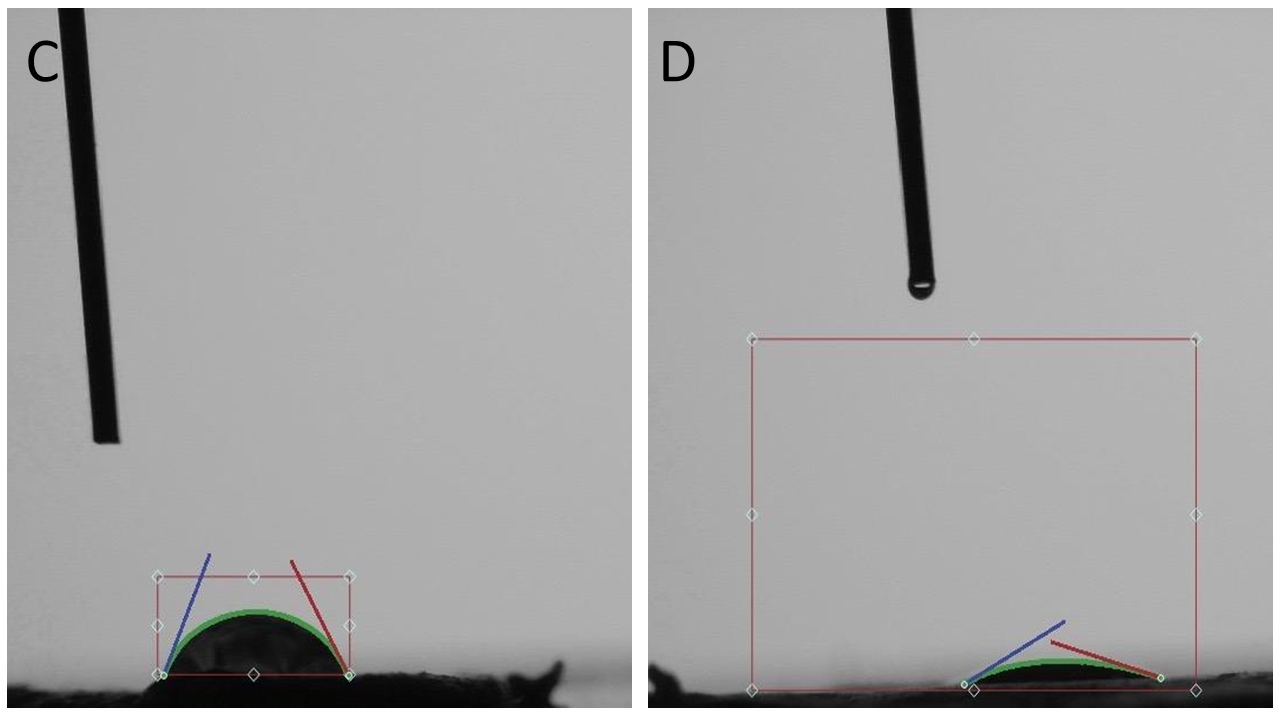


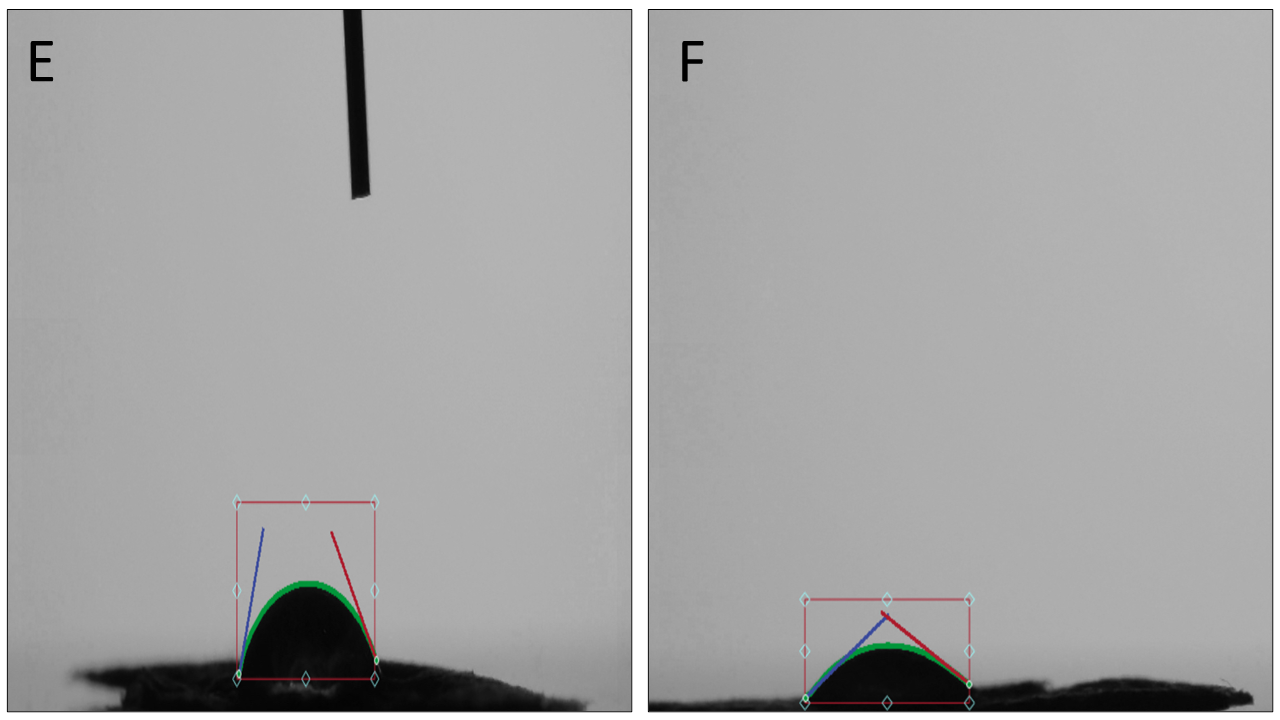
Figure 4: GRAPH A represents FTIR analysis of Control(HA) group (high molecular weight), GRAPH B represents FTIR analysis of Test (HA+HAP+AgO) group (high molecular weight), GRAPH C shows the FTIR parameter of the test group (HA(L) + HAP+AgO) group (low molecular weight), GRAPH D shows the FTIR parameter of the control group (HA) group (low molecular weight), GRAPH E represents the FTIR of CONTROL ( HA + HAP) Group (medium molecular weight), GRAPH F represents the FTIR of Test ( HA + HAP + AgO) Group (medium molecular weight)

* **High Molecular Weight Scaffolds**: FTIR spectra revealed strong absorption bands corresponding to **hydroxyl (-OH), phosphate (PO₄³⁻), and amide (C=O) groups**, confirming successful incorporation of HAP and AgO nanoparticles.
* **Medium Molecular Weight Scaffolds**: Showed characteristic peaks for **carrageenan, gelatin, and hydroxyapatite**, with minor shifts indicating moderate interactions between the polymeric matrix and the nanoparticles.
* **Low Molecular Weight Scaffolds**: Displayed slightly weaker absorption peaks, suggesting **higher degradation and possible leaching of incorporated nanoparticles** over time.

## Hydrophilicity (Contact Angle Measurement)







**FIGURE 5: (E AND F)** High Molecular Weight Scaffolds: Exhibited a contact angle of 45°–55°, indicating a moderate hydrophilic nature, promoting cell adhesion and protein adsorption. (**C AND D)** Medium Molecular Weight Scaffolds: The contact angle measured 55°–65°, showing balanced wettability suitable for controlled cell-material interactions. **(A AND B)** Low Molecular Weight Scaffolds: Displayed a higher contact angle (65°–75°), suggesting relatively lower wettability and faster degradation upon hydration.

# Swelling Ratio Analysis

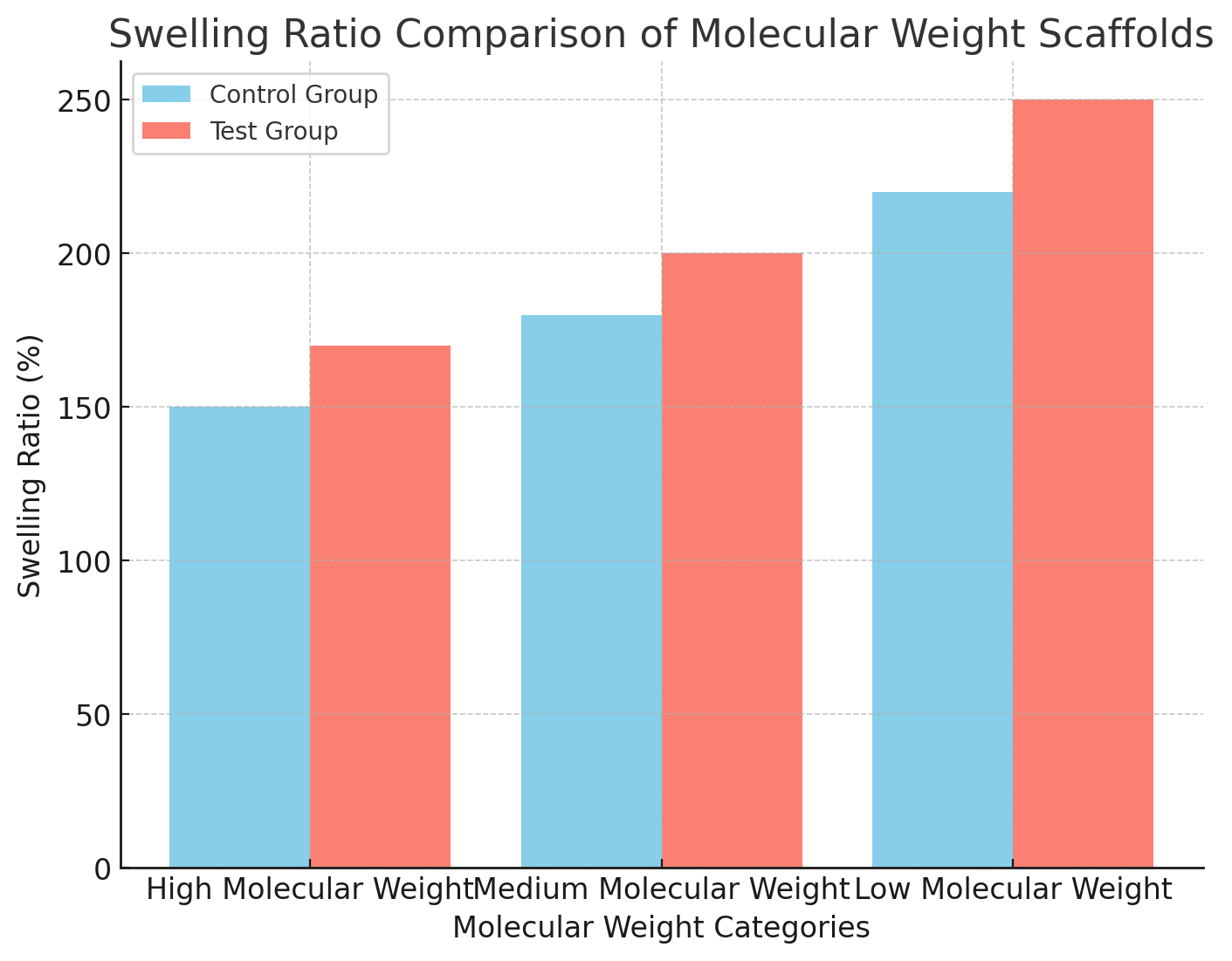


Figure 6: Swelling ratio analysis

* **High Molecular Weight Scaffolds**: Demonstrated a swelling ratio of **150–170%**, indicating slow water absorption and better dimensional stability.
* **Medium Molecular Weight Scaffolds**: Showed a swelling ratio of **180–200%**, ensuring optimal moisture retention for cellular activity.
* **Low Molecular Weight Scaffolds**: Recorded the highest swelling ratio (**220–250%**), suggesting **rapid water uptake and faster degradation**.

## Biocompatibility Analysis (MTT Assay)

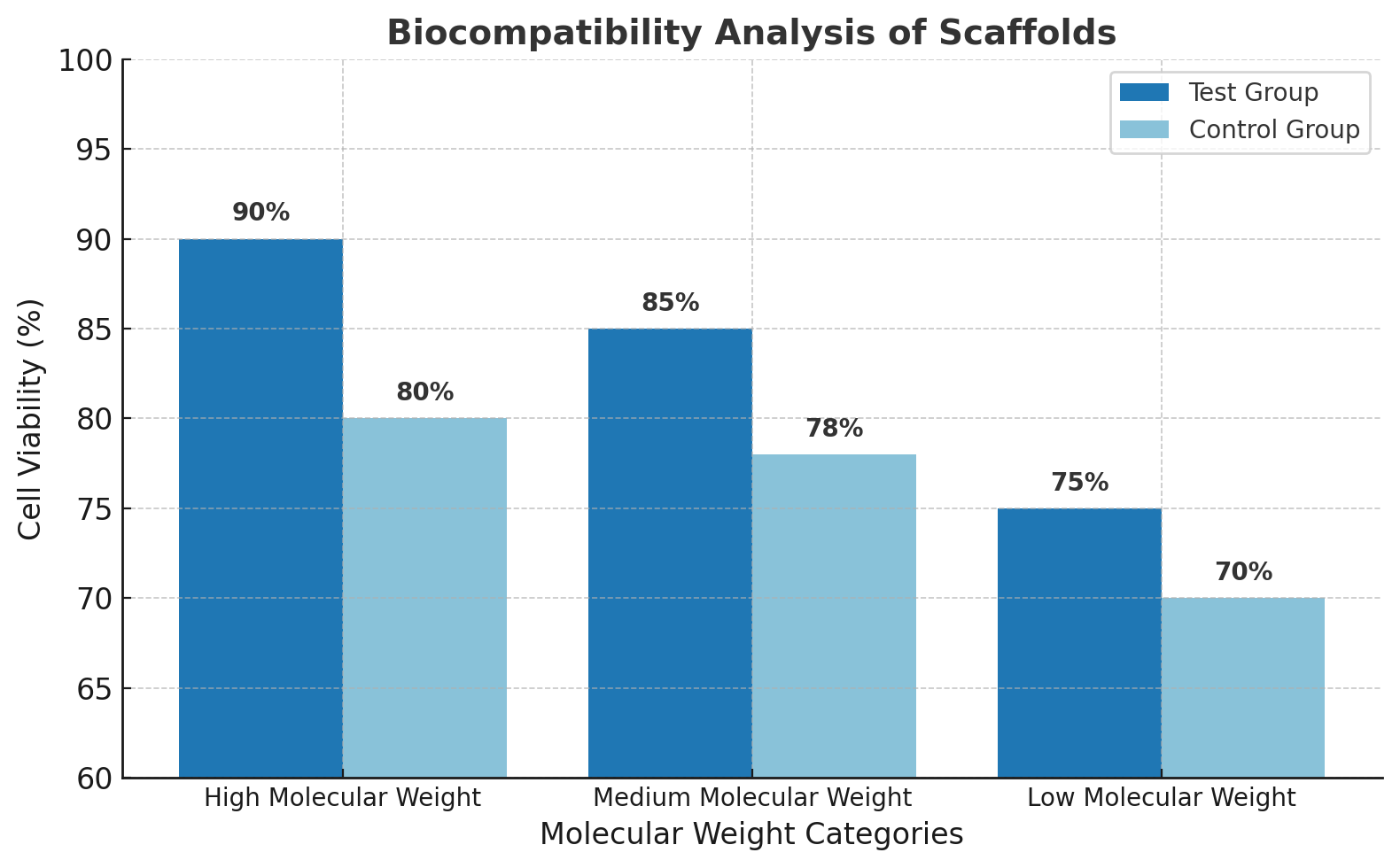


Figure 7: biocompatibility analysis

* **High Molecular Weight Scaffolds**: Exhibited **highest cell viability (~90%)** at day 7, supporting long-term cell proliferation.
* **Medium Molecular Weight Scaffolds**: Showed **moderate viability (~85%)**, indicating a balance between degradation and bioactivity.
* **Low Molecular Weight Scaffolds**: Had a **lower cell viability (~75%)**, likely due to **faster degradation and nanoparticle release**.

## Osteogenic Potential (hDPSC Culture)

* **High Molecular Weight Scaffolds:** Showed increased ALP activity and calcium deposition, suggesting enhanced osteoconduction and delayed degradation.
* **Medium Molecular Weight Scaffolds:** Displayed moderate osteogenic differentiation, balancing mineralization and structural integrity.
* **Low Molecular Weight Scaffolds:** Indicated rapid bioactivity but lower mineral deposition, possibly due to early material resorption.

## DISCUSSION

The discussion highlights the crucial role of molecular weight in determining the performance and efficacy of scaffolds used in guided bone regeneration (GBR). High molecular weight scaffolds exhibited superior mechanical strength, structural stability, and controlled degradation, making them particularly suitable for load-bearing bone defects and long-term regenerative applications. The scanning electron microscopy (SEM) analysis confirmed a highly interconnected porous network, which is essential for osteoconduction and efficient cell migration[(Website, n.d.-h)](https://paperpile.com/c/5nan97/fPGf). The structural integrity provided by high molecular weight components allowed for prolonged scaffold function, ensuring gradual degradation and sustained support for new bone tissue formation. Additionally, FTIR spectra indicated strong retention of functional groups, contributing to the prolonged and controlled release of ions, which in turn facilitated osteoblast differentiation and mineralization[(Website, n.d.-i)](https://paperpile.com/c/5nan97/9NcB). Contact angle measurements demonstrated enhanced hydrophilicity, allowing for improved protein adsorption and cellular adhesion, crucial for effective bone regeneration. Furthermore, the incorporation of AgO nanoparticles significantly enhanced the antibacterial properties of the scaffold, effectively reducing biofilm formation and mitigating infection risks during the healing process[(Buser, 2009)](https://paperpile.com/c/5nan97/2s3e).In contrast, medium molecular weight scaffolds exhibited a balanced degradation rate and bioactivity, making them ideal for applications where moderate mechanical support and controlled degradation are required. These scaffolds maintained a sufficiently porous structure to support cell infiltration and vascularization, while also allowing for a controlled release of bioactive molecules, which plays a key role in promoting cytokine exchange and cellular proliferation. [(Dharman et al., 2023; Govindaraj et al., 2023; Neeharika et al., 2023; Ramalingam et al., 2023)](https://paperpile.com/c/5nan97/6KKEa+NdzWq+CXqyb+cBY5H) This balance between stability and degradation makes medium molecular weight scaffolds particularly effective for periodontal regeneration, where prolonged support is needed but complete resorption over time is also necessary[(Website, n.d.-j)](https://paperpile.com/c/5nan97/fV8B). MTT assay results indicated that these scaffolds maintained good biocompatibility, with moderate levels of cell viability and proliferation. Additionally, the incorporation of AgO nanoparticles contributed to mild antibacterial effects, reducing microbial adhesion without negatively impacting cell growth[(Buser et al., 1994)](https://paperpile.com/c/5nan97/f4w6).On the other hand, low molecular weight scaffolds demonstrated rapid degradation, increased porosity, and enhanced wettability, which significantly improved initial cell adhesion and accelerated early-stage wound healing[(Swamy & Akhtar, 2019)](https://paperpile.com/c/5nan97/gQi5). However, their reduced mechanical strength and faster breakdown rate made them less suitable for load-bearing applications or long-term tissue regeneration. While the higher porosity and greater surface area allowed for efficient nutrient diffusion and cellular interaction, the excessive degradation often led to premature structural collapse, limiting their long-term effectiveness. FTIR analysis revealed weaker retention of functional groups, which correlated with the faster degradation rate observed in these scaffolds. The MTT assay results further confirmed that while these scaffolds initially supported cell attachment and proliferation, excessive ion release could lead to cytotoxic effects over time, making them less ideal for extended regenerative applications[(Website, n.d.-k)](https://paperpile.com/c/5nan97/iyhr).Overall, high molecular weight scaffolds emerged as the most effective choice for long-term GBR applications, offering optimal mechanical stability and controlled degradation[(Website, n.d.-l)](https://paperpile.com/c/5nan97/OlQ3). Medium molecular weight scaffolds provided a well-balanced approach, making them suitable for periodontal regeneration and applications requiring moderate mechanical strength. Meanwhile, low molecular weight scaffolds, despite their advantages in early-stage healing, exhibited limitations in long-term structural integrity, restricting their use to non-load-bearing scenarios where rapid resorption is desirable.

# CONCLUSION

This study highlights the impact of molecular weight on scaffold performance in guided bone regeneration (GBR). High molecular weight scaffolds exhibited superior mechanical strength, structural stability, and controlled degradation, making them ideal for long-term bone regeneration. Their high porosity, sustained ion release, and antibacterial properties promoted osteogenesis and infection resistance. Medium molecular weight scaffolds balanced mechanical support and degradation, making them suitable for periodontal regeneration. They provided good porosity, cell adhesion, and vascularization, ensuring gradual scaffold replacement by natural bone. Low molecular weight scaffolds, despite enhanced wettability and early cell attachment, degraded too quickly, limiting long-term effectiveness. Their rapid resorption makes them suitable for non-load-bearing defects but requires further optimization. Overall, high molecular weight scaffolds are the most effective for GBR, while medium molecular weight scaffolds offer a balanced alternative. Future research should focus on modulating degradation rates and enhancing bioactivity for broader applications in bone regeneration.

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