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Original Article

# Evaluation of Structural, Physical and Cytotoxicological Properties of *Cissus quadrangularis*, Carrageenan and Extracellular Matrix Based Guided Tissue Regeneration Membrane

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## ABSTRACT

**Background:** Bone tissue engineering has increasingly focused on developing bioactive composite membranes from natural polymers to enhance periodontal regeneration. This study aimed to compare the structural, physical, and cytotoxic properties of a type 1 collagen-Guided Tissue Regeneration (GTR) membrane with a novel GTR membrane combining *Cissus quadrangularis*, carrageenan, and ovine extracellular matrix. **Materials and Methods:** The GTR membranes were fabricated using a $CaCl_2$-induced gelation process followed by freeze-drying. Group 1 included a type 1 collagen membrane (Perio Col), and Group 2 included the novel membrane. Structural and physical properties were assessed using FTIR, SEM, tensile strength, swelling, and contact angle analysis. **Results:** Group 2 showed superior tensile strength (28.9 MPa) compared to Group 1 (16.1 MPa), lower swelling ratios (52% vs. 66%), and a reduced contact angle (68° vs. 87°), indicating better wettability. The MTT assay demonstrated significantly enhanced cytocompatibility in Group 3 (cells exposed to the novel membrane with a plant compound), which exhibited 92±4% cell viability compared to Group 2 (85±5%) and the control group (100%). Statistical analysis ($p<0.01$) confirmed the novel membrane's enhanced biocompatibility due to the addition of the plant compound. **Conclusion:** These findings highlight the novel membrane's potential as a regenerative material for periodontal applications, with its favourable mechanical properties, improved wettability, and cytocompatibility making it a promising scaffold for tissue regeneration and therapeutic advancements in regenerative medicine.

**Keywords:** Extracellular matrix, Carrageenan, *Cissus quadrangularis*, Periodontal regeneration, Guided tissue Regeneration, Membrane.

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## INTRODUCTION

Periodontal regeneration is a vital area of research in dentistry, focusing on the repair and restoration of periodontal tissues damaged by disease or trauma. This complex process involves regenerating various components of the periodontium, including alveolar bone, periodontal ligament, cementum, and gingiva.[1] The ultimate goal is to restore the functional and structural integrity of these tissues to support overall dental health and function. Recent advances in tissue engineering, such as the development of advanced biomaterials and innovative regenerative techniques, have shown significant promise in improving periodontal regeneration outcomes. However, challenges persist, including optimizing scaffold materials, ensuring long-term stability, and understanding the intricate interactions between biomaterials and biological systems.[2]

Bone defects associated with periodontal disease result from the loss of alveolar bone due to chronic inflammation and periodontal tissue destruction. These defects compromise tooth support and overall oral health. To address this, Guided Tissue Regeneration (GTR) membranes are increasingly utilized to facilitate bone regeneration and repair.[3] Current research focuses on developing new materials and combinations to optimize the performance of GTR membranes in repairing bone defects and improving outcomes in periodontal therapy. Decellularized Extracellular Matrix (ECM) derived from ovine tissue has emerged as a viable

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biomaterial treatment option for periodontal diseases. This ECM retains a complex array of proteins, glycosaminoglycans, proteoglycans, and other essential components found in natural tissues. By mimicking critical biological signaling processes, this ECM scaffold supports the regeneration, repair, and remodeling of damaged periodontal tissues.[4] Typically, stimulating agents are necessary to enhance osteogenic activity during bone tissue formation. Osteogenesis, the process of bone formation, involves several critical stages. Initially, Mesenchymal Stem Cells (MSCs) differentiate into osteoblasts under the influence of signaling molecules and transcription factors such as RUNX2 and OSX. These osteoblasts then secrete an ECM rich in collagen, which serves as a scaffold for bone formation. Bone undergoes continuous remodeling through the coordinated activity of osteoblasts and osteoclasts, maintaining bone strength, repairing damage, and adapting to mechanical stresses.[5]

In recent years, bone tissue engineering has increasingly focused on bioactive composite membranes derived from natural polymers to enhance osteogenic effects at defect sites. Two prominent materials in scaffold fabrication are carrageenan and hyaluronic acid, each offering unique properties beneficial for osteogenesis. Carrageenan, a natural polysaccharide extracted from red seaweeds, is widely utilized in biomedical applications due to its biocompatibility and gelling properties. Its potential in bone tissue engineering is linked to its ability to integrate bioactive molecules and cells, enhancing osteogenic processes. Carrageenan-based scaffolds can be engineered to release growth factors gradually, promoting the differentiation of MSCs into osteoblasts-the primary cells responsible for bone formation.[6]

The use of phytochemical extracts as osteogenic agents has garnered significant attention in recent years. Among these, *Cissus quadrangularis* (CQ), a medicinal plant from the Vitaceae family, has been widely used in traditional medicine across India and Africa. CQ is renowned for its efficacy in bone fracture healing, joint health, and osteoporosis prevention, as well as its antimicrobial, analgesic, anti-inflammatory, antioxidant, and tissue-protective properties. Alcoholic extracts of CQ, typically prepared using ethanol or methanol, are commercially available for these applications. The plant contains vitamins and steroids that contribute specifically to bone fracture healing. Research suggests that unidentified anabolic steroids in CQ may interact with estrogenic receptors in bone tissue to enhance fracture repair.

Combining CQ with carrageenan and Extracellular Matrix (ECM) in guided tissue regeneration membranes has the potential to create an optimized environment for osteogenesis by leveraging the unique advantages of these materials. This study aims to compare the structural and physical properties of a Type 1 collagen-based guided tissue regeneration membrane with a novel membrane composed of CQ, carrageenan, and ECM.[7]

## MATERIALS AND METHODS

To prepare the CQ extract, 50 g of powdered CQ was combined with 250 mL of ethanol and shaken at 120 rpm for 24 hr. After settling for a day, the supernatant was collected, and the CQ extract was isolated using rotary evaporation. Ovine tendon fragments were decellularized in phosphate-buffered saline and treated with Triton-X, a decellularization solution, until froth formation ceased. This process facilitated ECM recovery.[8] Separately, 2% kappa-carrageenan was prepared by dissolving 2 g of the substance in 100 mL of distilled water. The CQ extract and ECM were then combined with the kappa-carrageenan solution. The mixture was drop-cast, and the specimens were subjected to a series of 15-min ethanol washes to remove any residual materials. Finally, the samples were freeze-dried in a lyophilizer and stored at room temperature. Two study groups were evaluated:

**Group 1:** Type 1 collagen-based guided tissue regeneration membrane of fish origin (Perio Col).

**Group 2:** Guided tissue regeneration membrane comprising *Cissus quadrangularis*, carrageenan, and ECM.[9]

### Structural Properties

#### *Fourier Transform Infrared (FTIR) Spectroscopy*

Functional group analysis of the guided tissue regeneration membranes from Groups 1 and 2 was performed using FTIR spectroscopy (PerkinElmer, USA). Samples were placed on an Attenuated Total Reflectance (ATR) crystal surface, and spectra were recorded across the range of 400-4000 $cm^{-1}$. Multiple scans were conducted at different sample locations to detect the presence of various functional groups.

### Field Emission Scanning Electron Microscopy (FE-SEM)

Structural morphology was examined using FE-SEM (JEOL JSM-IT800). Membrane samples were sectioned into small pieces, and platinum was sputtered onto the sections under vacuum. FE-SEM imaging was then performed at a magnification of 500× to analyze the membrane's surface structure.[10]

### Physical Properties

#### *Tensile Strength*

The tensile strength of the membranes was evaluated using a universal testing machine (Instron Electroplus E3000, USA). Test specimens with dimensions of 10×15 mm was analyzed at a crosshead speed of 5 mm/min. Each membrane was mounted onto the analyzer, and forces were applied to both Group 1 and Group 2 specimens. The breaking force, defined as the force at which the scaffold ruptured, was recorded. Tensile stress was calculated by multiplying the breaking force by the specimen's area, while the deformation at the breaking force was considered the tensile strain.[11]

## Swelling Analysis

To assess swelling and fluid absorption, samples from Groups 1 and 2 were weighed in their dry state, immersed in 5 mL of 10% Phosphate-Buffered Saline (PBS) solution for 1 hr, and then weighed again after blotting excess liquid with tissue paper. The Swelling Ratio (SR) was calculated using the formula:

Swelling Ratio (SR)=W0W-W0×100%

where WWW represents the wet weight and W0W_0W0 represents the initial dry weight.[12]

## Contact Angle Analysis

The wettability of the membranes was measured using the sessile drop method. A 2 µL droplet of distilled water was placed on the membrane surface using a micropipette. The contact angle formed between the water droplet and the membrane surface was measured using an Ossila goniometer, providing insights into the hydrophilic or hydrophobic properties of the membranes (Table 1).

## MTT Assay for Cytotoxicity Evaluation

The cytotoxicity of the plant compound-associated extracellular matrix (ECM)-based guided tissue regeneration (GTR) membrane was assessed using the MTT assay. MG63 osteoblast-like cells were cultured in DMEM supplemented with 10% FBS and 1% penicillin-streptomycin. Cells were seeded into 96-well plates at a density of $1\times10^4$ cells/well and incubated at 37°C with 5% $CO_2$ for 24 hr to allow attachment. The cells were divided into three groups: Group 1 (Control): Cells cultured without any GTR membrane. Group 2: Cells exposed to collagen-based GTR membrane without plant compounds. Group 3: Cells exposed to ECM-based GTR membrane incorporated with *Cissus quadrangularis* and *carrageenan*. After 24 hr of incubation with the respective membranes, 20 µL of 5 mg/mL MTT solution was added to each well and incubated for 4 hr. The formazan crystals formed were dissolved using 150 µL DMSO, and absorbance was measured at 570 nm using a microplate reader. Cell viability was calculated relative to the control group.[13]

# RESULTS

## Fourier Transform Infrared (FTIR) Spectroscopy

FTIR spectroscopy was performed to analyze the functional groups present in the guided tissue regeneration membranes. The x-axis of the FTIR spectrum represented the wavenumber (4000-500 $cm^{-1}$), while the y-axis indicated percent transmittance (%T). Peaks and troughs in the spectrum corresponded to specific molecular vibrations, offering information about the chemical composition of the samples.

For **Group 1**, the 3000-3500 $cm^{-1}$ region displayed broad peaks corresponding to O-H or N-H stretching, indicative of alcohols, carboxylic acids, or amines. A sharp peak near 1700 $cm^{-1}$ suggested the presence of a C=O stretch, characteristic of carbonyl compounds such as aldehydes, ketones, or esters. Peaks in the 1600-1500 $cm^{-1}$ range indicated C=C stretching, commonly found in aromatic compounds or alkenes. The 1000-1300 $cm^{-1}$ region showed peaks associated with C-O stretches, which could indicate the presence of ethers, esters, or alcohols (Figure 1).

For **Group 2**, the 3500-3000 $cm^{-1}$ region exhibited broad peaks corresponding to O-H stretching, typical of alcohols or carboxylic acids. Sharp peaks in this region could also indicate N-H stretching, characteristic of amines or amides. Peaks in the 3000-2800 $cm^{-1}$ range were attributed to C-H stretching from aliphatic hydrocarbons. A strong peak near 1750-1700 $cm^{-1}$ indicated the presence of a C=O bond, commonly found in aldehydes, ketones, esters, or carboxylic acids. Peaks in the 1600-1500 $cm^{-1}$ range were attributed to C=C stretching, characteristic of aromatic rings or alkenes. Peaks in the 1200-1000 $cm^{-1}$ region corresponded to C-O stretching, suggesting the presence of alcohols, esters, or ethers.

The combination of C-H stretching and a C=O peak in Group 2 indicated the potential presence of a polyester-like material or another organic compound containing carbonyl functionalities.

## Field Emission Scanning Electron Microscopy (FE-SEM) Analysis

The surface morphology of the Group 1 and Group 2 samples was analyzed using a JEOL JSM-IT800 FE-SEM (Tokyo, Japan) operating at 3.00 kV. The results are presented in Figure 2.

Figure 2 shows the surface morphology of the Group 1 sample, which exhibited an irregular and rough flat structure. The topography was characterized by pronounced cresting and troughing, contributing to its uneven surface features. In contrast, the Group 2 sample displayed a spike-like morphology, highlighting distinct structural differences in the guided tissue regeneration membrane compared to Group 1. These morphological variations reflect the potential influence of material composition and fabrication methods on the structural characteristics of the membranes.

**Table 1: Tensile strength value, swelling analysis ratio and contact angle analysis of group 1 and group 2 guided tissue regeneration membrane.**

| Membrane Groups | Tensile Strength | Swelling Analysis | Contact Angle Analysis |
|---|---|---|---|
| GROUP 1 | 16.1 Mpa | 66% | 87° |
| GROUP 2 | 28.9 Mpa | 52% | 68° |

## Physical Property

The tensile strength of the membranes revealed notable differences between the two groups. The tensile strength of the Group 1 membrane was recorded at 16.1 MPa, while the Group 2 membrane demonstrated a significantly higher tensile strength of 28.9 MPa. This indicates that the Group 2 guided tissue regeneration membrane, composed of *Cissus quadrangularis*, carrageenan, and extracellular matrix, exhibited superior mechanical properties compared to the Group 1 membrane, which contained collagen of fish origin.

Swelling analysis measured the fluid absorption capacity of each sample, with the swelling ratios calculated accordingly. The swelling ratio for the Group 1 membrane was 66%, while that of the Group 2 membrane was 52%. The lower swelling ratio observed in the Group 2 membrane suggests a reduced fluid absorption capacity, likely due to the incorporation of *Cissus quadrangularis*. This characteristic indicates improved resistance to fluid uptake in the Group 2 membrane. Contact angle analysis further highlighted the differences in wettability between the two groups. The Group 2 membrane displayed a lower contact angle value of 68°, compared to the 87° contact angle of the Group 1 membrane. The reduced contact angle of the Group 2 membrane suggests better hydrophilicity and higher wettability. These findings indicate that the inclusion of *Cissus quadrangularis*, carrageenan, and extracellular matrix enhanced the wettability of the guided tissue regeneration membrane, compared to the collagen-based membrane in Group 1.

## Results on Cell viability

The MTT assay results demonstrated a clear distinction in cell viability across the three groups. Group 1 (Control), consisting of cells cultured without any membrane, served as the baseline and exhibited 100% viability. Group 2, where cells were exposed to the collagen-based membrane without the plant compound, showed a cell viability of 85 ± 5%, indicating minimal cytotoxicity and confirming that the ECM-based membrane alone is generally biocompatible. However, Group 3, where cells were cultured with the ECM-based membrane incorporated with the plant compound, exhibited a significantly higher cell viability of 92 ± 4%, suggesting that the addition of the plant compound enhanced the membrane's cytocompatibility. The statistical analysis, performed using one-way ANOVA, revealed significant differences between the groups ($p<0.01$), which indicates that the plant compound significantly improved cell viability compared to the membrane without it. These results confirm the superior biocompatibility of the plant compound-associated ECM-based Guided Tissue Regeneration (GTR) membrane, suggesting that the plant compound may offer additional bioactive benefits, potentially enhancing cellular responses and supporting tissue regeneration. This finding highlights the potential of combining plant-derived compounds with ECM-based scaffolds for improved therapeutic outcomes in regenerative medicine (Figure 3).

## DISCUSSION

Biomaterials designed for periodontal tissue engineering must replicate the structure and composition of the periodontium while promoting the healing of all periodontal tissues. Alveolar bone regeneration presents significant challenges, particularly in cases of severe bone loss and irregular socket damage, necessitating the development of innovative bioactive scaffolds as alternatives to current treatments.[14] Although synthetic scaffolds are widely utilized in regenerative periodontal applications, natural materials are often preferred due to their superior biocompatibility and reduced cytotoxicity. Despite extensive research on scaffolds for periodontal regeneration, studies focusing on natural products and sheep tendon-derived extracellular matrix in carrageenan-embedded scaffolds and guided tissue regeneration membranes remain limited.[15]

*Cissus quadrangularis* was chosen for incorporation in this study due to its traditional medicinal use and well-documented

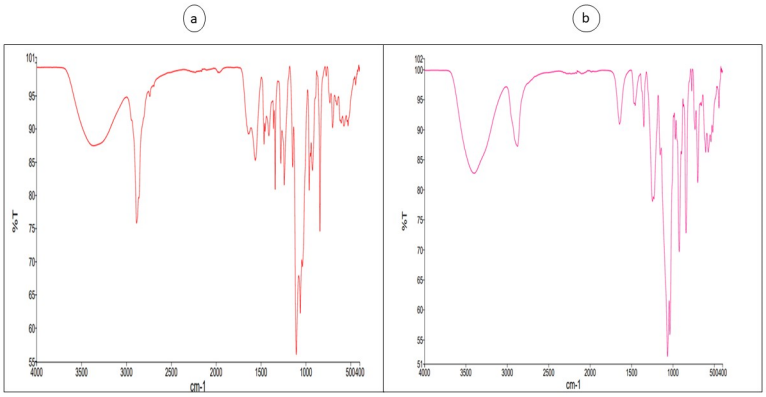


**Figure 1:** FTIR analysis of control group (a) and test group (b).

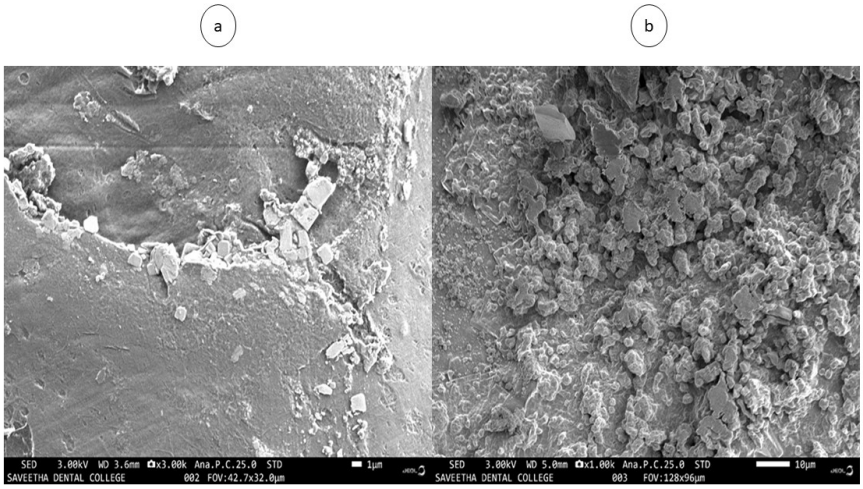


**Figure 2:** SEM analysis of control group (a) and test group (b).

anti-osteoporotic properties. Recent research has highlighted the potential of decellularized tendon-derived extracellular matrix as a source of complex compounds that support osteogenic activity and cell proliferation. However, limited investigations have explored the combined effects of carrageenan, tendon-derived extracellular matrix, and *Cissus quadrangularis* for periodontal bone regeneration.[16]

Our study demonstrated that the Group 2 membrane, which incorporated *Cissus quadrangularis*, carrageenan, and extracellular matrix, exhibited significantly higher tensile strength (28.9 MPa) compared to the Group 1 membrane containing fish-derived collagen (16.1 MPa). This finding suggests that the inclusion of these specific compounds in the Group 2 membrane enhances its structural integrity, improving its resistance to mechanical stress and tearing.

In contrast, a previous study on hydrogel scaffolds revealed that the sample containing Hydroxyapatite (HA) and tendon displayed the highest compressive strength (1.38 MPa) compared to samples containing HA, *Cissus quadrangularis*, and tendon (0.08 MPa) or HA and *Cissus quadrangularis* alone (0.01 MPa). This result indicates that while HA improves compressive strength, the addition of *Cissus quadrangularis* may reduce mechanical tolerance, compromising the material's ability to withstand mechanical loads.[17]

In terms of fluid absorption, Group 1 membrane had a higher swelling ratio (66%) than Group 2 (52%), showing that the latter absorbed less fluid. Lower fluid absorption is desirable as it helps to minimize membrane expansion after placement, which could potentially disrupt surrounding tissue structures. The results suggest that the addition of *Cissus quadrangularis* in Group 2 reduced fluid absorption, a beneficial trait for maintaining dimensional stability. Similarly, on comparing with previous study the hydrogel sample with HA+TENDON+CQ demonstrated a reduction in swelling ratio to approximately 20.5%, compared to 32.5% in HA-only samples. This aligns with the goal of minimizing swelling to reduce interference with surrounding tissues. The contact angle analysis also indicated better wettability in Group 2 (68°) compared to Group 1 (87°). Lower contact angle in Group 2 suggests that its surface has improved hydrophilicity, which could enhance cell attachment and integration into host tissues. Wettability is an essential property for biological scaffolds, as better wettability can improve cellular responses at the implant site.

On comparing, Group 2 membranes, as well as the HA-based hydrogels in previous study showed superior mechanical and wettability properties compared to their respective counterparts in Group 1. The incorporation of *Cissus quadrangularis* in Group 2 improves tensile strength, reduces fluid absorption, and increases wettability. For the hydrogels, however, the addition of CQ appears to reduce compressive strength, suggesting a trade-off between mechanical resilience and other beneficial properties. These findings collectively support the potential of these modified membranes and hydrogels in tissue regeneration applications, especially in scenarios requiring specific mechanical stability and fluid-handling properties.[18]

The study has several limitations, as it is a preliminary *in vitro* investigation into the regenerative potential of combining the natural herbal product *Cissus quadrangularis* with sheep tendon derived extracellular matrix in a carrageenan scaffold. Future research should include micro- or nano-computed tomography to further assess the regenerative potential of this scaffold and explore whether the active components in CQ impact the study outcomes. Further *vitro* and animal studies are needed to improve the biocompatibility of herbal-based periodontal bone regenerative membranes.

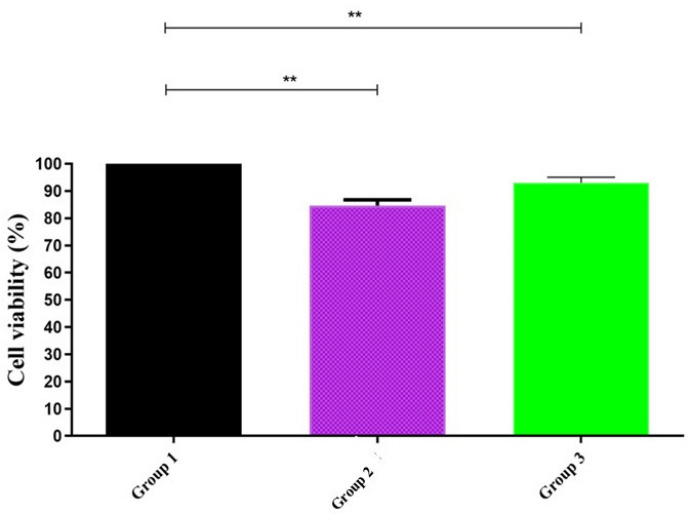


**Figure 3:** Effect of cell viability of the *Cissus quadrangularis* and carrageenan treated membrane against non-treated membrane. The treatment showed higher cell viability than the non- treated group. The results were statistically significant.

Biomaterials intended for periodontal tissue engineering must closely replicate the structure and composition of the periodontium while facilitating the healing of all periodontal tissues. Alveolar bone regeneration poses considerable challenges, especially in cases involving severe bone loss or irregular socket damage. This necessitates the development of innovative bioactive scaffolds as alternatives to existing treatments. Although synthetic scaffolds are widely used in regenerative periodontal applications, natural materials are often favored due to their superior biocompatibility and lower cytotoxicity. Despite the extensive research on scaffolds for periodontal regeneration, studies focusing on natural products and sheep tendon-derived extracellular matrix within carrageenan-embedded scaffolds and guided tissue regeneration membranes remain limited.

*Cissus quadrangularis* was selected for inclusion in this study because of its traditional medicinal applications and its well-documented anti-osteoporotic properties. Recent research has underscored the potential of decellularized tendon-derived extracellular matrix as a source of complex compounds that support osteogenic activity and cell proliferation. However, there is limited research on the combined effects of carrageenan, tendon-derived extracellular matrix, and *Cissus quadrangularis* for periodontal bone regeneration.[19]

Our study demonstrated that the Group 2 membrane, which incorporated *Cissus quadrangularis*, carrageenan, and extracellular matrix, exhibited significantly higher tensile strength (28.9 MPa) compared to the Group 1 membrane, which contained fish-derived collagen (16.1 MPa). This finding suggests that the inclusion of these specific components in the Group 2 membrane enhances its structural integrity, increasing its resistance to mechanical stress and tearing.[20]

Conversely, findings from a previous study on hydrogel scaffolds revealed that the sample containing Hydroxyapatite (HA) and tendon exhibited the highest compressive strength (1.38 MPa) compared to samples containing HA, *Cissus quadrangularis*, and tendon (0.08 MPa) or HA and *Cissus quadrangularis* alone (0.01 MPa). This result suggests that while HA enhances compressive strength, the addition of *Cissus quadrangularis* may reduce mechanical tolerance, potentially compromising the material's ability to withstand mechanical loads.[21]

## CONCLUSION

The Group 2 membrane, doped with *Cissus quadrangularis* and carrageenan, exhibited superior swelling behaviour, enhanced tensile strength, and improved wettability compared to the Group 1 membrane, making it a promising material for periodontal tissue regeneration. This study concludes that the *Cissus quadrangularis*- and carrageenan-doped ECM membrane demonstrates increased osteogenic potential, which is crucial for aligning collagen fibres and enhancing tensile strength. Additionally, the Group 2 membrane outperformed the fish-derived collagen-based membrane (PerioCol) in both swelling and contact angle tests. These results suggest that the *Cissus quadrangularis*- and carrageenan-doped ECM membrane has reduced fluid adsorption and better wettability, which are beneficial for regenerative applications. With its enhanced osteogenic properties and improved physical characteristics, this membrane stands out as a highly suitable candidate for surgical periodontal regeneration therapies, offering a promising solution for tissue repair and regeneration.

## ACKNOWLEDGEMENT

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## CONFLICT OF INTEREST

The author declares that there is no conflict of interest.

## ABBREVIATIONS

**FTIR:** Fourier Transform Infrared Spectroscopy; **SEM:** Scanning Electron Microscope; **MTT assay:** 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay; **ECM:** Extracellular matrix; **MSCs:** Mesenchymal stem cells; **CQ:** *Cissus quadrangularis*; **ATR:** Attenuated total reflectance; **PBS:** Phosphate-buffered saline; **GTR:** Guided tissue regeneration

## SUMMARY

The Group 2 membrane, enriched with *Cissus quadrangularis* and carrageenan, showed superior swelling capacity, tensile strength, and wettability compared to Group 1, making it highly suitable for periodontal tissue regeneration. It also outperformed the commercial PerioCol membrane in swelling and contact angle

tests, indicating better fluid control and surface interaction. The enhanced osteogenic potential of Group 2 aids collagen fiber alignment and tissue healing. These combined properties suggest that the doped ECM membrane offers both structural and biological advantages, making it a promising candidate for surgical applications in periodontal regeneration and tissue repair.

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