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ORIGINAL RESEARCH

CISSUS QUADRANGULARIS INFUSED CARRAGEENAN MEMBRANES: A NATURAL POLYMER SCAFFOLD FOR PERIODONTAL TISSUE REGENERATION

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ABSTRACT

A novel bioactive guided tissue regeneration (GTR) membrane was engineered by incorporating *Cissus quadrangularis* extract and carrageenan into a tendon-derived extracellular matrix (ECM) scaffold. The biocompatibility of the membrane was confirmed through MTT assays, while scratch wound healing assays demonstrated enhanced fibroblast migration, with Group 4—formulated with silver tricalcium phosphate—exhibiting the most significant migratory response. Gene expression profiling indicated a marked upregulation of COL1A1, VEGF-A, RUNX2, IL-10, and MMP2 in the treated groups compared to controls, suggesting the scaffold's robust potential to promote osteogenesis, angiogenesis, and anti-inflammatory activity. These results support the membrane's application in periodontal tissue engineering, offering a multifunctional platform for regenerative therapy.

Keywords: *Cissus quadrangularis*, carrageenan, guided tissue regeneration, tendon-derived ECM, silver tricalcium phosphate, angiogenesis, osteogenesis, MTT assay, scratch assay, gene expression, periodontal regeneration.

INTRODUCTION

One of the most prevalent conditions affecting the mouth is periodontitis. Periodontal diseases are prevalent in about 20%–50% of the global population. Periodontitis is a destructive inflammatory disease occurring in response to bacterial biofilms on the tooth surface[1]. The tooth root represents an anatomic challenge for formation of new cementum with associated PDL fibers that also connect to alveolar bone. Early attempts in periodontal tissue regeneration utilized autologous bone grafts, showing new bone formation could occur in periodontal defects when grafting was coupled with careful surgical techniques and strict patient maintenance[2]. An essential technique for replacing damaged periodontal tissue, including alveolar bone, periodontal ligament, and cementum, is periodontal directed tissue regeneration. The periodontal ligament and

related bone-forming cells can expand more easily when the barrier membranes are used for the restoration[3]. GTR is a process that involves using an occlusive barrier membrane between gingival (epithelial) and alveolar bone/PDL tissue to regenerate periodontal tissue. In this procedure, an occlusive membrane will be applied to the surgical site to prevent connective and epithelial tissue from migrating through it[4]. In the field of regenerative medicine, guided tissue regeneration (GTR) and guided bone regeneration (GBR) have attracted a lot of interest. While GTR is responsible for restoring damaged periodontal tissues, GBR primarily concentrates on the regeneration of alveolar bone in edentulous areas. GTR is involved in the regeneration of the cementum, bone, and periodontal ligament (PDL) near the tooth[5]. When repairing damaged alveolar ridges at extraction sites, the guided bone regeneration (GBR) technique is frequently utilized either prior to or concurrently with dental implant insertion. A membrane is placed over a bone deficiency in GBR to prevent fibrous tissue from invading the graft site and to encourage the growth of new bone. GBR is used to treat intraosseous defects and furcation involvements as well. GBR permeable membranes can be used to encourage the formation of healthy bone structures around bone defects. GBR membranes can also be utilized to restore bone structure at the tooth location after it has been pulled or misplaced, as well as to preserve the socket region that may surround the tooth as a result of periodontal disease[6]. A sufficient blood supply, a large number of progenitor cells, appropriate biomaterial scaffolding, and precisely defined amounts and patterns of regulatory signals are all necessary for the effective development of an engineered tissue. These membranes aid in peri-implantitis, alveolar ridge preservation, and periodontal defect repair. In addition to these benefits, its smaller area presents a number of regeneration-related disadvantages. By promoting neo-vascularization and anti-inflammation, the use of bioactive compounds may improve regeneration. In order to achieve excellent tissue restoration, a conductive environment that facilitates bio-signals between the periodontal ligament niche is necessary. However, conventional membranes typically perform poorly in situations such as intra-bony defects, furcation involvement, and implant-associated bone loss. Additionally, these membranes are categorized into a number of kinds including metal and inorganic compound membranes, non-resorbable membranes, and bioabsorbable membranes according to their composition and bioactivity[7].

Cissus quadrangularis L. is a succulent plant of family Vitaceae usually found in tropical and subtropical xeric wood. *Cissus quadrangularis* (CQ) is also commonly known as veldt grape. It is used in medicine. Experts have tried to use logical analysis to test the plant's compatibility[8]. Cell reinforcement, free radical search, antimicrobial activity, bone regeneration, ulceration, pain relief, mitigation, and diuretics are some of the therapeutic uses of the plant. It is a promising new dental biomaterial because of its amazing regenerative, anti-inflammatory, and antioxidant properties. It comprises 90% of collagen, mostly type I. They are rich in flavonoids, alkaloids and tannins. These plants have been demonstrated to have the ability for accelerating bone regeneration while moderating oxidative stress and inflammatory cytokines. Further the recent studies showed that CQ enhances the collagen synthesis, promoting angiogenesis and stimulatingly proliferation of osteoblasts. Hence these plants are bio active incorporations for tissue engineered scaffolds. Carrageenan, derived from *Kappaphycus alvarezii*, attracted notice because of its many uses, including gelling, biodegradability, and biocompatibility. It has been widely employed to promote the wound healing characteristics of scaffold composites and hydrogel formation. Additionally, the regulated transport of the bioactive chemicals to the injured regions was demonstrated. The natural repository of collagen, glycosaminoglycans, and other growth factors that aid in wound healing is the tendon extracellular matrix, or tendon ECM. Decellularized tendon extracellular matrix maintains its three-dimensional structure while encouraging collagen deposition, cellular movement, attachment, and differentiation. Biocompatibility and osteoconductivity have been demonstrated for bioceramics. Additionally, those bioceramics are also referred to as "ion releasing reservoirs" and are highly beneficial in supplementing calcium and phosphate ions to promote mineralization and angiogenesis at the sites of injury[9]. Moreover, bioceramics have a favorable effect on cellular adhesion, migration, and proliferation. Both silver tricalcium phosphate (Ag-TCP) and silver hydroxyapatite (Ag-HAp) have notable osteo-inductive, regenerative, and antimicrobial qualities. For aiding the regenerative potential collagen compositions such as tendon ECM are used to provide a mechanical strength as a well-organized structure called biological scaffolds. Incorporation of these composite scaffolds could induce cellular recruitment, differentiation and matrix deposition in the tissue regenerative nicke. Hence in the zebrafish caudal fin model these phytochemicals, bioceramics and tendon ECM would facilitate the organization of regenerative cells for the structural restoration. The antibacterial properties of silver nanoparticles, which prevent infection and the formation of biofilms, make them useful against a wide range of microorganisms. Mineral formations called HAp are found in nature and have the ability to stimulate osteo-conduction for mineralization and regeneration. Silver ion doping confers antibacterial qualities in addition to osteo-induction[10].

The aim of this research is to develop and evaluate a novel bioactive guided tissue regeneration (GTR) membrane incorporating Cissus quadrangularis extract, carrageenan, and bioceramics (Ag-TCP/Ag-HAp) within a tendon-derived extracellular matrix (ECM) scaffold, in order to enhance periodontal tissue regeneration[11,12] This study seeks to investigate the scaffold's biocompatibility, cellular migration potential, and its ability to upregulate genes associated with osteogenesis, angiogenesis, and anti-inflammatory responses, thereby supporting its efficacy in promoting the regeneration of cementum, periodontal ligament, and alveolar bone.

2. MATERIALS AND METHODS

Fresh stems of Cissus quadrangularis were gathered, cleaned, and allowed to dry in the shade. After drying, the stems were ground into a powder and extracted for 48 hours using a Soxhlet equipment and 95% ethanol. A rotary evaporator operating at 40 °C and low pressure was used to filter and concentrate the extract. Before being used again, the resultant crude extract was kept at -20 °C.

2.1 Chemicals and reagents:

MTT reagents were purchased from Sigma-Aldrich. Silver tricalcium phosphate powder was sourced from a biomedical supplier. Tendon-derived extracellular matrix scaffolds were prepared following standard decellularization protocols. Cell culture reagents including DMEM, fetal bovine serum (FBS), penicillin-streptomycin, and trypsin-EDTA were obtained from Thermo Fisher Scientific. TRIzol reagent, reverse transcription kit, and SYBR Green PCR Master Mix for gene expression studies were also procured from Thermo Fisher. Primers for COL1A1, VEGF-A, RUNX2, IL-10, and MMP2 were synthesized by Integrated DNA Technologies (IDT)[13].

2.2 Synthesis of bioceramics:

Using the sol-gel method, silver tricalcium phosphate (TCP) and silver hydroxyapatite (HA) bioceramics were created. To make HA, calcium nitrate and ammonium phosphate were combined in a 1.67:1 M ratio, then ammonia was used to bring the pH down to 10. After being aged for twenty-four hours, the mixture was calcinated at 800 degrees Celsius. Utilizing calcium and phosphate precursors, TCP was manufactured via a silica gel-based method[14].

2.3 Extraction and processing of tendon ECM (TEM):

A detergent-based method comprising 1% SDS and 0.1% Triton X-100 was used to decellularize the porcine/Achilles tendon. This was followed by a thorough PBS wash. The resulting extracellular matrix (ECM) was ground into a fine powder, freeze-dried, and then added back together to form a hydrogel[15].

2.4 Scaffold fabrication

Using the lyophilization method, scaffolds were developed by combining tendon ECM hydrogel, carrageenan, and Cissus quadrangularis extract. Polycaprolactone (PCL) was used as the foundation polymer to provide mechanical stability. After mixing a 10% w/w PCL solution with different proportions of tricalcium phosphate (TCP), hydroxyapatite (HA), and carrageenan, as well as ECM and plant extract, the mixture was lyophilized. After being created, the scaffolds were crosslinked with glutaraldehyde fumes and kept for later use in a desiccator. Four groups were created from the scaffolds: PERIO COL was used as the positive control in Group 1, Cissus quadrangularis extract, carrageenan, and tendon ECM were included in Group 2, silver hydroxyapatite was added to Group 3, and Cissus quadrangularis extract, carrageenan, and tendon ECM were combined with silver tricalcium phosphate in Group 4. No scaffolding was applied to the negative control group[16].

2.5 Cell Culture and Conditions:

Human periodontal ligament cells were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin. Cells were maintained at 37°C in a humidified atmosphere containing 5% CO_2. Subculturing was performed at 70-80% confluence using 0.25% trypsin-EDTA. All experiments were carried out with cells between passages 3 and 5 to ensure consistency and minimize variability[17,18].

2.6. MTT Assay:

The MTT assay was conducted to determine the biocompatibility of the developed membranes on human periodontal ligament cells. Cells were seeded in 96-well plates and exposed to extracts from four groups: Group 1 (positive control with PERIO COL-GTR membrane), Group 2 (membranes containing *Cissus quadrangularis* extract, carrageenan, and tendon ECM), Group 3 (membranes with silver hydroxyapatite plus Group 2 components), and Group 4 (membranes with silver tricalcium phosphate plus Group 2 components). After 24 hours, MTT reagent was added and incubated for 4 hours, followed by solubilization of formazan crystals in DMSO. Absorbance was measured at 570 nm, revealing that all groups exhibited good cell viability, with Group 4 showing the highest proliferation[19].

2.7. Cell Migration:

Cell migration was assessed using a scratch assay. Human periodontal ligament cells were seeded in 6-well plates and grown to confluence. A uniform scratch was made in each well and treated with membrane eluates from each group: Group 1 (positive control), Group 2 (*Cissus quadrangularis* extract with carrageenan and tendon ECM), Group 3 (silver hydroxyapatite formulation), and Group 4 (silver tricalcium phosphate formulation). Images were captured at 0 and 24 hours to evaluate wound closure. Group 4 showed the highest level of cell migration with nearly complete closure of the scratch area, indicating enhanced pro-migratory potential compared to other groups[20].

2.8. Gene Expression:

Total RNA was extracted from treated cells using TRIzol reagent according to the manufacturer's instructions. cDNA synthesis was performed with a reverse transcription kit. Quantitative PCR was carried out using SYBR Green PCR Master Mix with primers specific for COL1A1, VEGF-A, RUNX2, IL-10, and MMP2. Gene expression levels were normalized to GAPDH and calculated using the 2^(-ΔΔCt) method. Results showed upregulation of osteogenic (COL1A1, RUNX2), angiogenic (VEGF-A), anti-inflammatory (IL-10), and matrix remodeling (MMP2) markers in treatment groups, with Group 4 demonstrating the highest expression levels overall[21].

3. RESULTS

3.1 Morphological Evaluation of PDL Cells Post-MTT Assay

Microscopic images captured following MTT assay at the highest scaffold concentration reveal well-spread, spindle-shaped human periodontal ligament (PDL) cells across all groups, confirming the cytocompatibility of the tested formulations. Group 4 exhibits the most pronounced cellular density and confluency, reflecting enhanced proliferation and optimal scaffold-cell interaction due to the combined presence of *Cissus quadrangularis* extract, carrageenan, tendon ECM, and silver tricalcium phosphate. Groups 2 and 3 also display substantial cell attachment and elongated morphology, indicative of favorable cell-substrate compatibility. In contrast, Group 1 (PERIO COL) shows a relatively sparser cell population, suggesting comparatively lower proliferative support. These morphological characteristics align with the MTT assay's quantitative outcomes, validating the superior biocompatibility and growth-promoting nature of the Group 4 scaffold composition (Figure 1).

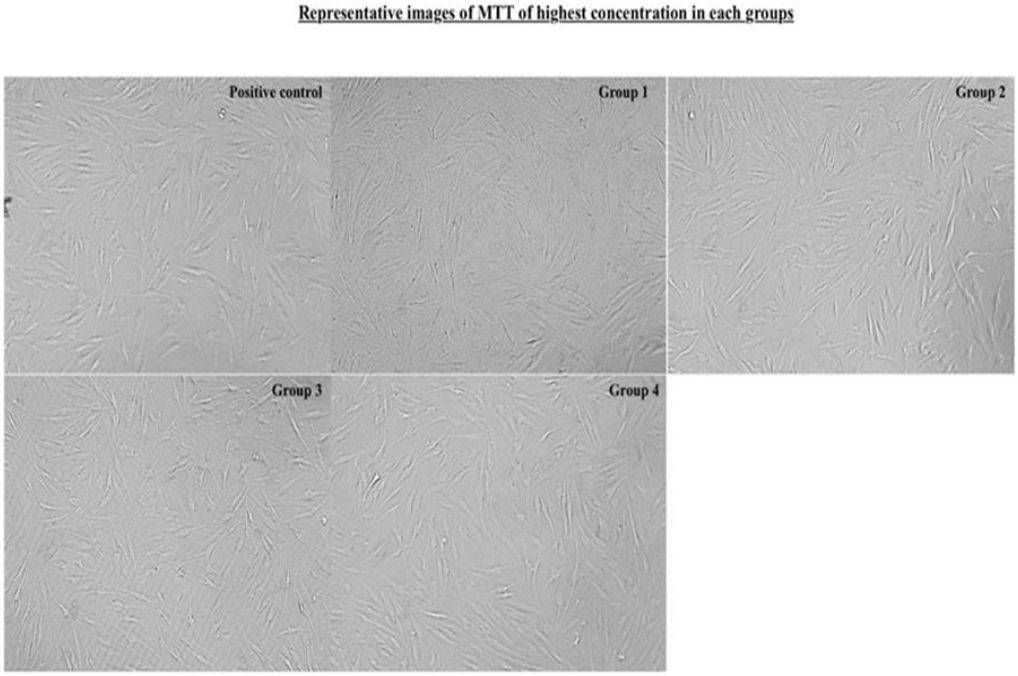


Figure1. Microscopic images showing PDL cell morphology post-MTT assay at maximum scaffold concentration:a – Group 1 (PERIO COL), b – Group 2 (*Cissus quadrangularis* + carrageenan), c – Group 3 (*Cissus quadrangularis* + carrageenan + tendon ECM), d – Group 4 (*Cissus quadrangularis* + carrageenan + tendon ECM + silver TCP).

3.2 Cell Viability Assessment via MTT Assay

The MTT assay revealed that all scaffold formulations exhibited excellent cytocompatibility, with cell viability consistently above 85% across all groups. This indicates that the individual components—*Cissus quadrangularis* extract, carrageenan, tendon-derived ECM, and silver tricalcium phosphate—did not exert any cytotoxic effects on the cultured cells. Among the groups, Group 4 demonstrated the highest percentage of cell viability, suggesting that the synergistic combination of all four components created an optimal microenvironment conducive to cell proliferation and metabolic activity. These findings confirm that the composite scaffolds, particularly the Group 4 formulation, are biocompatible and suitable for supporting cell survival in periodontal tissue engineering applications (Figure 2).

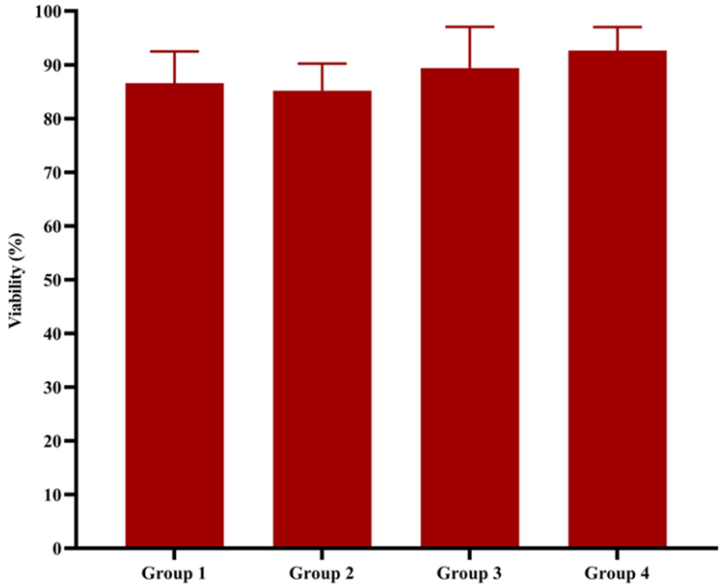
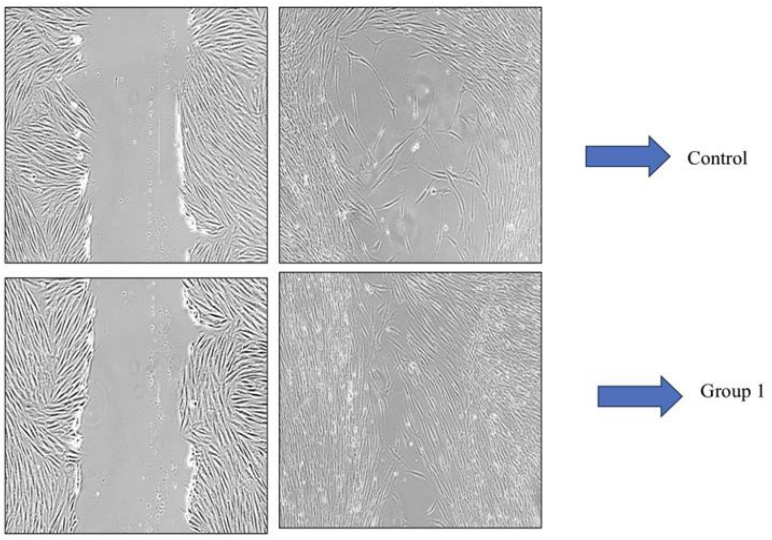


Figure2. Cell Viability Assessment via MTT Assay

Bar graph representing the percentage of viable human periodontal ligament (PDL) cells following treatment with different scaffold formulations.

3.3 Evaluation of Cell Migration Potential via Scratch Assay

Cell migration, a key indicator of regenerative potential, was assessed using the scratch assay across all scaffold formulations. The results revealed a scaffold-dependent enhancement in periodontal ligament (PDL) cell motility (Figure 3). Among all groups, Group 4, which incorporated *Cissus quadrangularis* extract, carrageenan, tendon-derived ECM, and silver tricalcium phosphate (Ag-TCP), exhibited the highest percentage of wound closure, reflecting robust pro-migratory activity. This superior migration is likely attributed to the synergistic effects of bioactive phytochemicals, ECM proteins that facilitate adhesion, and ionic cues provided by Ag-TCP. Groups 2 and 3 also demonstrated significant migration, indicating that the combination of CQ and ECM supports cellular movement and scaffold interaction. In contrast, Group 1 (PerioCol) showed the lowest migration percentage, suggesting limited stimulatory effect on cell motility. These findings confirm that the experimental scaffolds, particularly Group 4, enhance the dynamic cellular processes essential for periodontal regeneration (Figure 4).



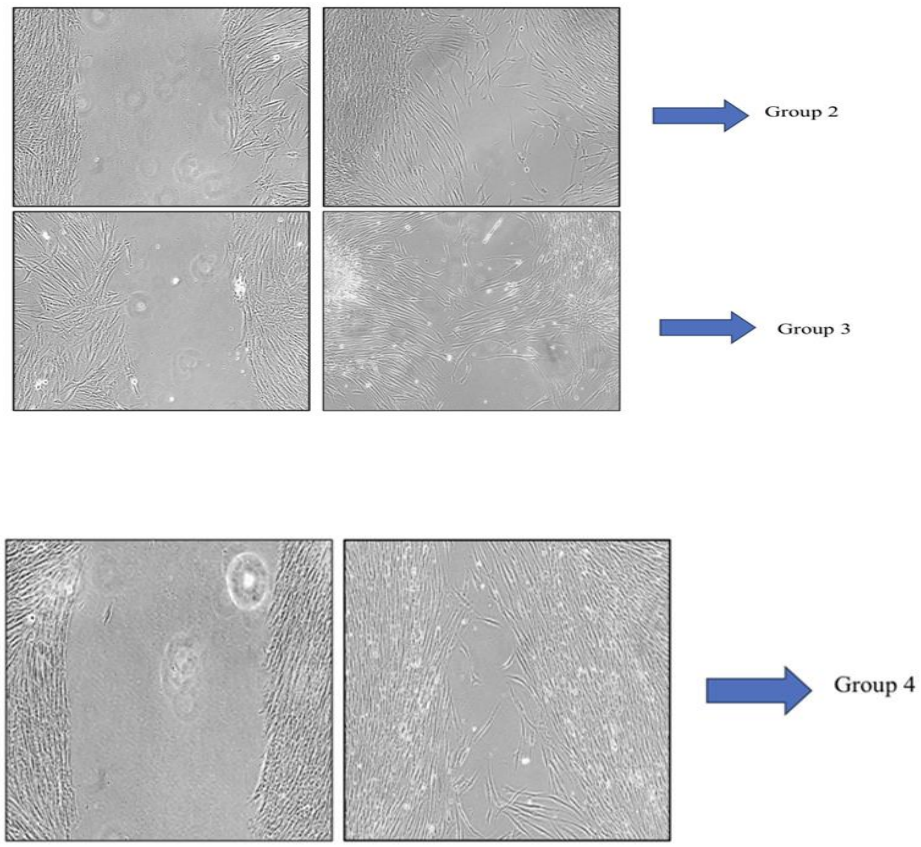


Figure 3. Representative scratch assay images showing human periodontal ligament (PDL) cell migration after 24 hours of treatment with different scaffold formulations.
a – Group 1 (PERIO COL): Incomplete wound closure with a wide remaining gap.
b – Group 2 (*Cissus quadrangularis* + carrageenan): Moderate wound closure with visible cell migration into the scratch area.
c – Group 3 (*Cissus quadrangularis* + carrageenan + tendon ECM): Enhanced closure with greater cell density along the wound edge.
d – Group 4 (*Cissus quadrangularis* + carrageenan + tendon ECM + silver TCP): Near-complete wound closure indicating highest migratory activity and scaffold bioactivity.

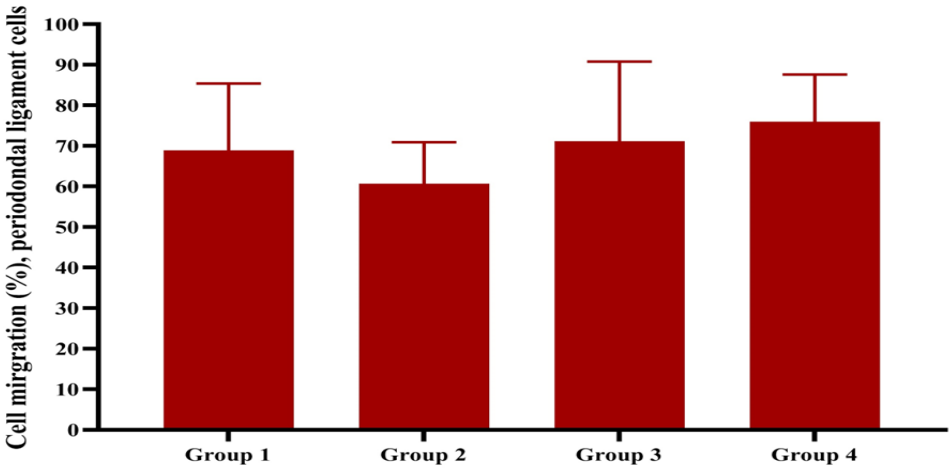


Figure 4. Bar graph representing the percentage of wound closure by human periodontal ligament (PDL) cells after 24 hours of treatment with different scaffold formulations in the scratch assay.

Group 1 – PERIO COL (commercial control), Group 2 – Cissus quadrangularis + carrageenan, Group 3 – Cissus quadrangularis + carrageenan + tendon ECM, Group 4 – Cissus quadrangularis + carrageenan + tendon ECM + silver tricalcium phosphate (Ag-TCP).
Values are expressed as mean ± SD (n=3). Group 4 shows the highest percentage of cell migration, indicating superior pro-migratory activity.

3.5 Gene Expression Analysis

Quantitative gene expression analysis revealed significant upregulation of key markers associated with osteogenesis, angiogenesis, inflammation modulation, and extracellular matrix remodeling in scaffold-treated groups, particularly in Group 4. Notably, RUNX2 and COL1A1 expression levels were markedly elevated, indicating enhanced osteogenic differentiation and collagen matrix synthesis. VEGF-A expression was also significantly increased, suggesting improved angiogenic potential critical for vascularization during tissue regeneration. Furthermore, the anti-inflammatory cytokine IL-10 was upregulated, reflecting a favorable shift toward a regenerative immune environment. MMP2, a matrix metalloproteinase involved in ECM degradation and remodeling, also showed elevated expression, implying active scaffold integration and tissue turnover. Among all formulations, Group 4—which combined *Cissus quadrangularis* extract, carrageenan, tendon ECM, and silver TCP—demonstrated the highest expression of all five markers, reinforcing its multifaceted role in promoting periodontal tissue regeneration (Figure 5).

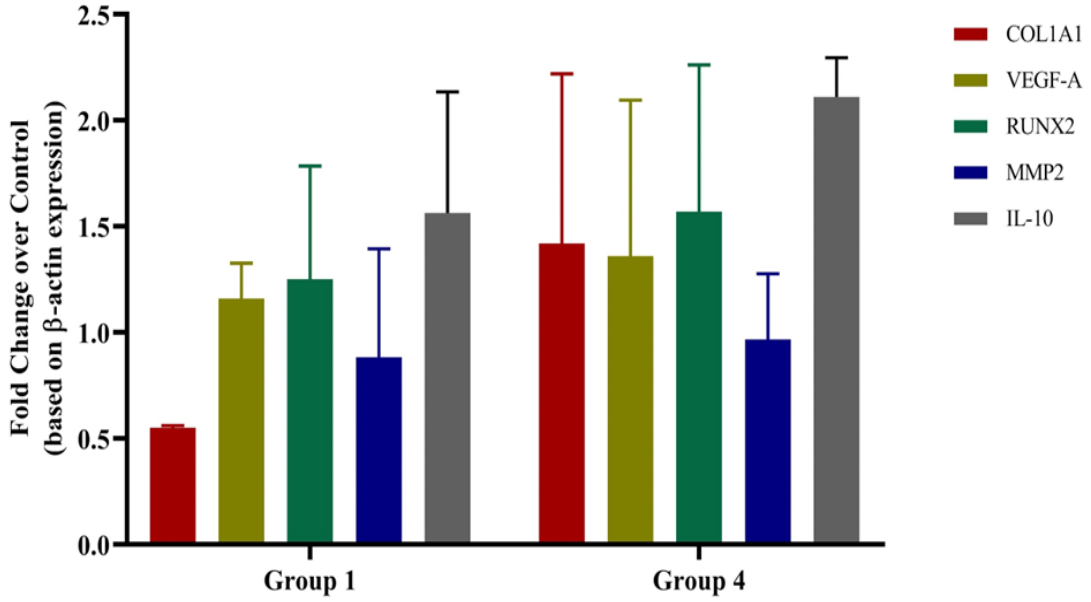


Figure 5. Bar graph depicting the relative mRNA expression levels of RUNX2, COL1A1, VEGF-A, IL-10, and MMP2 in human periodontal ligament (PDL) cells treated with group 1 and 4 scaffolds.

4. DISCUSSION

Periodontal tissue regeneration remains a critical yet complex clinical objective, particularly in cases of advanced periodontitis, trauma, or surgical bone loss. The goal is not only to restore alveolar bone but also to regenerate the functional periodontal ligament and cementum—components that together constitute the periodontal apparatus. Unlike conventional therapies that mainly aim to control disease progression, regenerative therapies focus on the biological replacement of lost tissues to restore structure and function. Among the various therapeutic strategies, guided tissue regeneration (GTR) using scaffold-based systems has emerged as a particularly promising approach, offering both physical support and biochemical cues to drive cellular activities essential for regeneration[22].

The present study focused on the development and evaluation of a bioactive GTR membrane composed of *Cissus quadrangularis* extract, carrageenan, tendon-derived extracellular matrix (ECM), and silver tricalcium phosphate (Ag-TCP). This composite scaffold integrates phytochemical, biochemical, and inorganic components, each contributing distinct regenerative benefits. The results demonstrated that this combination—particularly in Group 4—significantly enhanced cell viability, migration, and gene expression of critical markers

involved in osteogenesis, angiogenesis, matrix remodeling, and inflammation resolution[23,24]. The use of *Cissus quadrangularis* extract adds a unique bioactive dimension to the scaffold. Known for its traditional use in bone healing, the phytochemicals present in the extract are believed to stimulate osteoblast proliferation and differentiation while maintaining high cellular viability. In our study, the scaffold formulations containing this extract supported excellent cell proliferation, particularly in Group 4, where the highest cytocompatibility was observed. The extract may act through multiple pathways, offering both osteoinductive and anti-inflammatory effects that contribute to tissue regeneration[25].

Carrageenan, a natural polysaccharide, provided a hydrophilic and bioadhesive matrix that helped in the formation of a hydrated environment suitable for cellular functions. Its gelling properties likely contributed to scaffold integrity, while its biological compatibility supported fibroblast adhesion and survival. Moreover, carrageenan may have played a supportive role in delivering the phytochemicals uniformly and maintaining a conducive microenvironment for cell growth and differentiation.

The inclusion of tendon-derived ECM added essential structural proteins and native signaling molecules to the scaffold. As a biologically derived material, ECM closely mimics the natural microenvironment of connective tissues, promoting cellular adhesion, migration, and lineage-specific differentiation. In the present study, the ECM component likely provided anchorage sites and matrix-bound factors that facilitated the observed increase in fibroblast migration, especially in combination with Ag-TCP and phytochemical components.

Silver tricalcium phosphate, an osteoconductive ceramic, brings two-fold benefits to the scaffold: bioactive ion release and antimicrobial potential. The calcium and phosphate ions released from Ag-TCP promote mineralization and osteoblastic activity, while silver ions may help in preventing microbial colonization of the scaffold, an important consideration in the oral cavity. The observed enhanced cell migration and proliferation in Group 4 can be attributed to the ionic stimuli provided by Ag-TCP, creating a more favorable microenvironment for periodontal regeneration.

The scratch assay results demonstrated significant cellular migration in Group 4 compared to the other groups. This may be explained by the synergistic interplay of the scaffold components—ionic signals from Ag-TCP, adhesive proteins from ECM, and bioactive molecules from *Cissus quadrangularis*. Together, these factors could enhance the cytoskeletal reorganization and focal adhesion formation necessary for directed cell migration, a key step in tissue regeneration[26].

Gene expression analysis offered deeper insight into the regenerative capacity of the scaffold. The upregulation of RUNX2 and COL1A1 indicates a shift toward osteogenic differentiation. RUNX2 is a critical transcription factor that initiates the osteoblast lineage commitment, while COL1A1 encodes the primary collagen type found in bone matrix. Their elevated expression in Group 4 reflects the scaffold's ability to promote early bone formation and matrix deposition[27].

MMP2 expression, a marker of extracellular matrix remodeling, was also significantly increased. This enzyme facilitates the degradation and remodeling of existing matrix, enabling new tissue ingrowth and scaffold integration. The presence of ECM in the scaffold might have promoted the expression of such remodeling enzymes, allowing for a dynamic and adaptive healing process.

VEGF-A, a key regulator of angiogenesis, was upregulated as well, suggesting that the scaffold not only supports bone regeneration but also vascularization. Adequate blood supply is essential for sustained cell viability and nutrient exchange in regenerating tissues. The ionic release from Ag-TCP and the presence of natural ECM may have synergistically contributed to the induction of VEGF-A, supporting the formation of new capillaries within the healing periodontal site.

The significant increase in IL-10 expression suggests the establishment of an anti-inflammatory microenvironment, which is critical for proper healing. Chronic inflammation often hinders regeneration by creating a hostile environment that leads to tissue breakdown rather than repair. IL-10 is a cytokine known for its role in suppressing pro-inflammatory mediators and promoting the resolution phase of inflammation. The presence of CQ and ECM components may have influenced immune cell behavior to favor a regenerative phenotype. When analyzed collectively, the gene expression data, cytocompatibility results, and migration assays point toward a multifunctional scaffold that can address the diverse biological needs of periodontal regeneration. The formulation in Group 4, containing all scaffold components—*Cissus quadrangularis*, carrageenan, tendon ECM, and Ag-TCP—appears to be the most effective in orchestrating the regenerative processes, including osteogenesis, angiogenesis, matrix remodeling, and inflammation modulation.

The findings of this study highlight the critical role of material composition in scaffold performance. While individual components such as CQ or Ag-TCP offer specific benefits, it is their strategic combination with ECM and polysaccharides like carrageenan that creates a holistic regenerative platform. The synergy between these natural and synthetic components mimics the complexity of native tissue environments, allowing for enhanced cellular responses and functional tissue reconstruction.From a translational perspective, this study opens new possibilities for using phytochemical-based

scaffolds in periodontal regenerative therapies. The use of *Cissus quadrangularis* and carrageenan introduces biocompatible and cost-effective elements into scaffold design, while ECM ensures biological relevance. The addition of Ag-TCP addresses both mechanical and microbial challenges encountered in clinical applications. Together, these components offer a blueprint for developing next-generation GTR membranes that are not only bioactive but also versatile and safe for human use[28].

Further investigations are needed to validate these findings in in vivo models, assess long-term regeneration outcomes, and determine scaffold degradation behavior under physiological conditions. The incorporation of growth factors, optimization of scaffold porosity, and evaluation of mechanical strength could further enhance the clinical applicability of this composite membrane. Therefore, the composite GTR membrane developed in this study—comprising *Cissus quadrangularis* extract, carrageenan, tendon-derived ECM, and silver tricalcium phosphate—demonstrates strong potential for periodontal regeneration. It successfully promotes cell proliferation, migration, and upregulation of key genes involved in osteogenesis, angiogenesis, and immunomodulation. The multifunctional nature of the scaffold suggests its suitability as a promising candidate for clinical application in guided periodontal tissue regeneration, addressing both biological and functional requirements of tissue healing[29].

5. CONCLUSION

The present study successfully demonstrates the potential of a novel bioactive guided tissue regeneration (GTR) membrane incorporating *Cissus quadrangularis* extract, carrageenan, tendon-derived extracellular matrix (ECM), and silver tricalcium phosphate (Ag-TCP) for periodontal regeneration. The composite scaffold showed excellent cytocompatibility, as confirmed by MTT assay, and significantly enhanced periodontal ligament cell migration in vitro, particularly in Group 4, which contained all key bioactive components. Gene expression analysis revealed marked upregulation of critical regenerative markers including RUNX2, COL1A1, VEGF-A, MMP2, and IL-10, indicating the scaffold's ability to stimulate osteogenesis, angiogenesis, matrix remodeling, and anti-inflammatory activity. The synergistic effects of phytochemicals, ECM proteins, and bioactive ceramics provide a biologically favorable environment that mimics the native periodontium and supports comprehensive tissue regeneration. These findings support the application of this composite GTR membrane as a promising and multifunctional candidate for future clinical use in periodontal tissue engineering.

DECLARATIONS

Competing interest

The authors declare that there are no competing interest.

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Ethical Approval

Not applicable

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