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ORIGINAL RESEARCH

EVALUATION OF HEMOCOMPATIBILITY AND CYTOCOMPATIBILITY NOVEL CISSUS QUADRANGULARIS, BIOCERAMICS AND TENDON EXTRACELLULAR MATRIX INCORPORATED SCAFFOLDS FOR PERIODONTAL BONE REGENERATION

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Abstract

This study presents the fabrication and evaluation of a novel bioactive scaffold integrating *Cissus quadrangularis* extract, carrageenan, tendon-derived extracellular matrix (ECM), and bioactive ceramics such as silver hydroxyapatite and silver tricalcium phosphate for periodontal bone regeneration. The scaffold's biocompatibility was evaluated using MTT assay, while mineralization capacity was assessed via Alizarin Red S staining. Hemocompatibility was tested to ensure safe interaction with blood components. Furthermore, osteogenic potential was investigated through quantitative PCR analysis of key markers: RUNX2, ALP, OCN, COL1A1, and BMP2 using osteoblastoma cell lines. Results demonstrated high cell viability, minimal haemolytic activity, enhanced mineral deposition, and significant upregulation of osteogenic genes, particularly in group 4 containing tendon ECM and silver tricalcium phosphate. The findings support the scaffold's potential for clinical use in periodontal bone defect repair, offering a cost-effective and multifunctional approach for regenerative dentistry.

Keywords: *Cissus quadrangularis*, periodontal regeneration, tendon-derived ECM, bioceramics, silver tricalcium phosphate, osteoblastoma cells, MTT assay, Alizarin Red, hemocompatibility, osteogenic gene expression

INTRODUCTION

Bone mineralization is a critical biological process fundamental to skeletal development, structural stability, and the maintenance of bone health throughout life. This dynamic process involves the regulated deposition of calcium phosphate minerals into the collagen-rich extracellular matrix by osteoblasts, resulting in hardened, functional bone[1]. Effective mineralization ensures not only skeletal integrity but also facilitates essential functions such as load-bearing, mobility, and protection of internal organs. Disruptions in this balance—due to metabolic, hormonal, nutritional, or pathological factors—can lead to severe conditions such as osteoporosis, delayed fracture healing, and other skeletal deformities that compromise bone strength and function[2]. In the field of dentistry, particularly periodontology and oral surgery, bone mineralization is of paramount importance. Alveolar bone plays a crucial role in supporting teeth and maintaining periodontal stability. Loss of this bone, often due to periodontitis, trauma, or surgical interventions, can result in tooth mobility and eventual tooth loss. Regenerative approaches, therefore, are essential not just for restoring bone quantity but also for re-establishing the biological function and architecture of the periodontal complex, which includes cementum, periodontal ligament (PDL), and alveolar bone[3].

Traditional bone grafting techniques—including autografts, allografts, xenografts, and synthetic substitutes—have long been employed in clinical settings to repair osseous defects. While these approaches have shown considerable success, they are associated with a range of limitations. Autografts, though immunologically favorable, carry risks of donor site morbidity and limited availability. Allografts and xenografts are susceptible to immune rejection and disease transmission. Synthetic bone substitutes often lack biological signals necessary for initiating or sustaining osteogenesis. These limitations underscore the pressing need for alternative, biologically inspired materials that are both safe and effective[4,5].Recent trends in regenerative medicine have seen the integration of natural phytochemicals, bioactive ceramics, and extracellular matrix (ECM)-based materials to create multifunctional scaffolds. These biomimetic scaffolds aim to closely replicate the native bone microenvironment by providing structural support, osteoinductive cues, and biocompatibility. In this context, *Cissus quadrangularis* (CQ), a medicinal plant traditionally used in Ayurvedic medicine, has emerged as a promising osteogenic agent. Rich in flavonoids, triterpenoids, ketosteroids, calcium, and vitamin C, CQ has demonstrated the ability to stimulate osteoblast activity, enhance mineralization, and accelerate bone regeneration. Its anti-inflammatory and antioxidant properties further contribute to a pro-healing microenvironment, making it an ideal candidate for regenerative applications[6].

Another critical component in the scaffold design is the use of bioactive ceramics such as hydroxyapatite (HA) and tricalcium phosphate (TCP), especially when doped with antimicrobial ions like silver. These ceramics are known for their structural resemblance to native bone mineral and their osteoconductive nature, allowing them to support bone in-growth and integration. Silver-doped variants not only promote osteogenesis but also impart antimicrobial properties that are beneficial in reducing postoperative infections—a common complication in oral surgical procedures[6,7]. Tendon-derived extracellular matrix (ECM), obtained through decellularization processes, has gained recognition for its ability to provide native biochemical and structural cues essential for tissue regeneration. The ECM retains collagen, glycosaminoglycans, and bound growth factors that facilitate cellular adhesion, migration, and differentiation. When incorporated into scaffolds, tendon ECM improves cell-scaffold interactions, supports focal adhesion formation, and enhances osteogenic differentiation. Furthermore, its compatibility with various polymers and ceramics makes it a versatile additive for composite scaffold formulations[8,9].

Carrageenan, a sulfated polysaccharide derived from red seaweed, adds further biofunctionality to the scaffold. It enhances viscosity and gelation, enabling better scaffold integrity and injectability in clinical applications. Additionally, carrageenan is biocompatible, biodegradable, and has demonstrated anti-inflammatory activity—qualities that are highly desirable in periodontal tissue engineering. Biocompatibility remains a foundational criterion for scaffold development. In regenerative dentistry, scaffolds are often placed in direct contact with blood

and connective tissue. Hence, hemocompatibility and cytocompatibility evaluations are crucial. Hemocompatibility ensures that the scaffold does not trigger hemolysis or coagulation, while cytocompatibility guarantees that the scaffold supports cellular viability, attachment, and proliferation. These properties are not only important for ensuring safety but are also directly related to the scaffold's functional performance in promoting tissue regeneration[10].

Given these considerations, the present study is designed to develop and evaluate a composite membrane incorporating *Cissus quadrangularis* extract, carrageenan, tendon-derived ECM, and silver-based bioceramics (HA and TCP). The scaffold is tested using osteoblastoma cell lines to assess its potential in promoting bone regeneration, particularly in the context of periodontal defects. The study investigates hemocompatibility to ensure blood safety, and cytocompatibility via MTT assay to confirm scaffold safety and cell-supportive nature. Additionally, the mineralization potential of the scaffold is examined using the Alizarin Red assay to visualize calcium deposition. Finally, the expression of key osteogenic genes—RUNX2, ALP, OCN, COL1A1, and BMP2—is quantified via real-time PCR to understand the scaffold's influence on osteogenic differentiation[11].

Our hypothesis is that the integration of these components—each with distinct biological roles—will create a synergistic effect that enhances osteoconductivity, mineralization, and osteoinductive signaling. The tendon ECM is expected to provide a native framework for cellular interactions, CQ to stimulate osteogenesis, bioceramics to support mineral deposition and antimicrobial protection, and carrageenan to maintain scaffold form and biocompatibility[12]. Through this multifactorial approach, the composite membrane is envisioned as a next-generation biomaterial capable of addressing the current limitations of periodontal bone regeneration therapies.[13] This study aims to bridge traditional medicine and modern biomaterials science by developing a scaffold that is not only biologically effective but also clinically relevant. By evaluating hemocompatibility, cytocompatibility, mineralization, and gene expression, we aim to provide a comprehensive understanding of the scaffold's regenerative potential and its application in surgical periodontal therapy.

2 MATERIALS AND METHODS

2.1 Chemicals and Reagents

Low-viscosity food-grade carrageenan, MTT reagent, and all analytical-grade solvents were procured from Sigma-Aldrich (USA). Silver-doped hydroxyapatite and tricalcium phosphate powders were obtained from a certified biomedical materials supplier. Tendon-derived extracellular matrix (ECM) was prepared in-house using a standardized detergent-based decellularization protocol. Cell culture reagents, including Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), penicillin-streptomycin, and trypsin-EDTA, were purchased from Thermo Fisher Scientific (USA). For gene expression studies, TRIzol reagent, a reverse transcription kit, and SYBR Green Master Mix were also obtained from Thermo Fisher Scientific. Primers specific for RUNX2, ALP, OCN, COL1A1, and BMP2 were synthesized and supplied by Integrated DNA Technologies (IDT, USA).

2.2 Preparation of *Cissus quadrangularis* Extract

Fresh stems of *Cissus quadrangularis* were collected and thoroughly washed with distilled water to remove surface impurities. The cleaned stems were then shade-dried at room temperature to preserve their phytochemical integrity and subsequently ground into a fine powder using a mechanical grinder. Ethanol extraction was performed using a Soxhlet apparatus with 95% ethanol as the solvent over a continuous 48-hour cycle. The obtained extract was filtered to remove any plant debris and concentrated using a rotary evaporator at 40°C under reduced pressure to yield a thick, crude residue. This concentrated extract was then stored at −20°C in airtight containers until further experimental use[14].

2.3 Synthesis of Bioceramics

Silver-doped hydroxyapatite (Ag-HA) and silver-doped tricalcium phosphate (Ag-TCP) were synthesized using a sol-gel-based approach. For hydroxyapatite synthesis, calcium nitrate and ammonium phosphate were mixed in a 1.67:1 molar ratio, and the pH of the solution was adjusted to 10 using ammonia solution to facilitate gelation. The resulting mixture was aged for 24 hours to allow gel formation and subsequently subjected to calcination

at 800°C to obtain crystalline hydroxyapatite. Silver-doped tricalcium phosphate was prepared via a silica gel-assisted method, wherein silver ions were incorporated into the calcium and phosphate precursors in stoichiometric proportions. The resulting bioceramic powders were thoroughly washed, dried, and collected as fine particles, then stored under sterile conditions until further use in scaffold fabrication[15].

2.4 Extraction and Processing of Tendon Extracellular Matrix (TEM)

Extracellular matrix (ECM) was extracted from porcine Achilles tendons using a detergent-based decellularization protocol to preserve bioactive structural components. The tendon tissues were initially rinsed to remove blood residues and then subjected to decellularization using a solution containing 1% sodium dodecyl sulfate (SDS) and 0.1% Triton X-100. This treatment facilitated effective cellular removal while maintaining the integrity of the ECM proteins. Following decellularization, the tissues were thoroughly washed with phosphate-buffered saline (PBS) to eliminate residual detergents. The cleaned tissues were then freeze-dried (lyophilized), ground into a fine powder, and reconstituted into a hydrogel form to be incorporated into scaffold fabrication for bone regenerative applications[16].

2.5 Fabrication of Composite Scaffolds

Composite scaffolds were fabricated using the electrospinning technique to create uniform, nanofibrous membranes suitable for periodontal bone regeneration. A 10% (w/w) solution of polycaprolactone (PCL) was prepared in an appropriate solvent to serve as the primary polymer matrix, providing mechanical integrity and flexibility. Bioactive components including carrageenan, either hydroxyapatite or tricalcium phosphate (TCP), tendon-derived ECM hydrogel, and *Cissus quadrangularis* extract were added to the PCL solution and homogenized thoroughly to ensure even distribution. The mixture was then lyophilized under optimized parameters to obtain the final scaffold. To improve scaffold stability and preserve morphology, the fabricated membranes were exposed to glutaraldehyde vapor for 24 hours for crosslinking. Finally, the crosslinked scaffolds were stored in a vacuum desiccator until further characterization and biological evaluation[17].

2.6 Scaffold Grouping and Formulation Overview

Four distinct scaffold groups were designed and evaluated to compare their biological performance for periodontal bone regeneration. Group 1 served as the control and consisted of a commercially available guided tissue regeneration membrane, PerioCol®. Group 2 comprised a composite scaffold formulated with *Cissus quadrangularis* extract, carrageenan, and tendon-derived extracellular matrix (ECM), aiming to assess the baseline efficacy of plant-based and ECM components. Group 3 included silver-doped hydroxyapatite along with *Cissus quadrangularis* extract, carrageenan, and tendon ECM, providing an osteoconductive and antimicrobial ceramic to enhance regenerative potential. Group 4, the most advanced formulation, incorporated silver tricalcium phosphate instead of hydroxyapatite, combined with *Cissus quadrangularis*, carrageenan, and tendon ECM, hypothesized to deliver superior bioactivity, mineralization, and cell-supportive properties due to its synergistic ceramic and phytochemical composition[18].

2.7 Assessment of Hemocompatibility Using Red Blood Cell Hemolysis Assay

Hemocompatibility of the scaffold formulations was assessed using a standard hemolysis assay. Fresh human blood was collected from healthy volunteers in EDTA-coated tubes and centrifuged at 1500 rpm for 10 minutes to separate erythrocytes. The red blood cells (RBCs) were washed three times with phosphate-buffered saline (PBS) and diluted to obtain a 2% RBC suspension.Scaffold samples, including Carrageenan-only (CAR), Ag-HAp CAR, Ag-TCP CAR, and PerioCol®, were weighed and tested at two concentrations (10 mg and 20 mg). Each sample was incubated with 1 mL of the RBC suspension at 37°C for 1 hour. Following incubation, the mixtures were centrifuged at 3000 rpm for 10 minutes, and the supernatants were collected.The absorbance of the released hemoglobin in the supernatants was measured at 540 nm using a UV-Vis spectrophotometer. Distilled water-treated RBCs served as the positive control (100% hemolysis), and PBS-treated RBCs were used as the negative control (0% hemolysis).The percentage of hemolysis was

calculated using the following formula: Hemolysis (%) = [(Abs_sample − Abs_negative) / (Abs_positive − Abs_negative)] × 100.

All experiments were performed in triplicate, and results were expressed as mean ± standard deviation[19].

2.8 Cell Culture and Maintenance

Osteoblastoma cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin to support optimal cell growth. The cells were maintained in a humidified incubator at 37°C with 5% CO_2 atmosphere. Subculturing was carried out using 0.25% trypsin-EDTA once the cultures reached approximately 80% confluency. For experimental consistency and reproducibility, all assays were performed using cells between passages 3 and 5[20].

2.9 MTT Assay for Cytocompatibility

To assess scaffold cytocompatibility, scaffold eluates were prepared and applied to osteoblastoma cells seeded in 96-well plates. Following 24 hours of incubation with the scaffold extracts, MTT reagent (5 mg/mL) was added to each well, and the plates were incubated at 37°C for 3–4 hours in the dark to allow the formation of formazan crystals. After incubation, the medium was carefully removed, and the crystals were solubilized using 100 µL of dimethyl sulfoxide (DMSO) per well. Absorbance was then recorded at 570 nm using a microplate reader. Cell viability was calculated as a percentage of the absorbance values relative to untreated control wells, indicating the cytocompatibility of each scaffold formulation[21].

2.10 Alizarin Red S Assay for Mineralization

To evaluate the mineralization potential of the scaffold formulations, osteoblastoma cells were seeded in 24-well plates and treated with scaffold eluates for a period of 14 days. After the treatment period, cells were fixed with 4% paraformaldehyde for 15 minutes at room temperature. Mineral deposition was assessed by staining the fixed cells with 2% Alizarin Red S solution (pH 4.2) for 20 minutes. Excess dye was thoroughly washed off using distilled water. For quantitative analysis, the bound stain was extracted using 10% cetylpyridinium chloride, and the absorbance was measured at 570 nm using a microplate reader. Mineralized nodules were also observed and documented under an inverted microscope, providing both qualitative and quantitative confirmation of scaffold-induced osteogenic activity[22].

2.11 Quantitative Real-Time PCR for Osteogenic Gene Expression

To assess the osteogenic potential of the scaffold-treated cells, total RNA was extracted from osteoblastoma cells after 14 days of treatment using TRIzol reagent, following the standard protocol. Complementary DNA (cDNA) was synthesized from the isolated RNA using a reverse transcription kit, as per the manufacturer's instructions. Quantitative real-time PCR (qRT-PCR) was then carried out using SYBR Green PCR Master Mix along with gene-specific primers targeting RUNX2, ALP, OCN, COL1A1, and BMP2. GAPDH served as the internal housekeeping gene for normalization. The relative expression levels of the target genes were calculated using the 2^−ΔΔCt method, providing insights into scaffold-induced osteogenic differentiation at the molecular level[23].

3. RESULTS

3.1 Hemocompatibility Assessment

Hemocompatibility was evaluated by hemolysis assay at two different concentrations (10 mg and 20 mg), as depicted in Figure 1. The carrageenan-only group (CAR) exhibited the highest hemolytic potential among all samples, particularly at 20 mg concentration, indicating limited compatibility at higher doses. In contrast, the addition of silver-based bioactive ceramics markedly reduced hemolysis levels. Specifically, both Ag-HAp CAR and Ag-TCP CAR groups demonstrated substantially lower hemolysis at both concentrations compared to the CAR group. Notably, the Ag-TCP CAR group exhibited the most favorable hemocompatibility profile, showing minimal hemolytic activity even at 20 mg. The PERIOCOL membrane, used as a clinical control, also displayed low hemolysis comparable to Ag-TCP CAR. These findings highlight that the incorporation of silver ceramics significantly improves the hemocompatibility of the membrane, making the composite safer for in vivo applications (Figure 1).

during relaxed breathing and in the absence of external compression.

Statistical Analysis: Data were compiled using Microsoft Excel and analyzed using SPSS software version 25.0. Continuous variables such as IMT, PSV, EDV, and RI were expressed as mean ± standard deviation (SD). Categorical variables such as plaque presence were expressed in frequencies and percentages. Intergroup comparisons between smokers and non-smokers were performed using unpaired Student's t-test for continuous variables and chi-square test for categorical variables. A p-value of <0.05 was considered statistically significant for all analyses.

RESULT

The present study included 100 adult male participants divided equally into two groups: 50 smokers and 50 non-smokers. All participants underwent bilateral carotid Doppler ultrasonography for morphologic and hemodynamic assessment of the common carotid artery (CCA) and internal carotid artery (ICA).

Comparative analysis was performed between the two groups for variables including intima-media thickness (IMT), presence of plaques, peak systolic velocity (PSV), end-diastolic velocity (EDV), and resistive index (RI). The results demonstrate significant differences in several parameters between smokers and non-smokers, suggesting early vascular alterations in smokers.

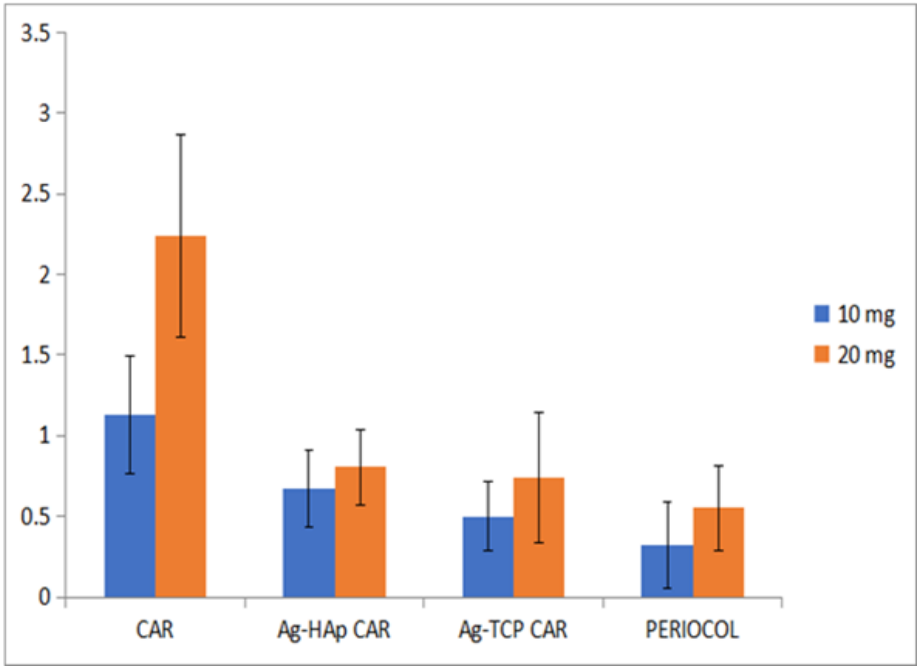

Figure 1: Percentage hemolysis of different scaffold formulations at 10 mg and 20 mg concentrations. CAR (Carrageenan-only) showed the highest hemolysis, particularly at 20 mg, indicating lower compatibility. Incorporation of silver-based ceramics in Ag-HAp CAR and Ag-TCP CAR significantly reduced hemolysis levels. Ag-TCP CAR exhibited the best hemocompatibility, comparable to the clinical control (PerioCol®). Data are expressed as mean ± SD (n = 3); values below 5% indicate acceptable hemocompatibility.

3.2 Microscopic Evaluation of Osteoblastoma Cell Mo

Representative phase-contrast images of osteoblastoma cells treated with the highest concentrations of each membrane group revealed notable differences in cellular morphology and density. All groups exhibited spindle-shaped, adherent cells indicative of viable osteoblast-like morphology. However, cell density and confluence varied among the groups. The control and Group 1 (Periocol GTR) showed moderate cell proliferation with less uniform distribution. In contrast, Groups 2 and 3 displayed increased cell density and

more cohesive monolayer formation. Notably, Group 4 (comprising Cissus quadrangularis, carrageenan, tendon-derived ECM, and silver tricalcium phosphate) exhibited the highest cellular density, with well-spread, elongated cells and minimal cytotoxic features. These morphological observations are consistent with MTT assay results and further support the enhanced biocompatibility and proliferative capacity of the Group 4 composite membrane, underscoring its potential as a regenerative scaffold for bone tissue engineering applications (Figure 2).

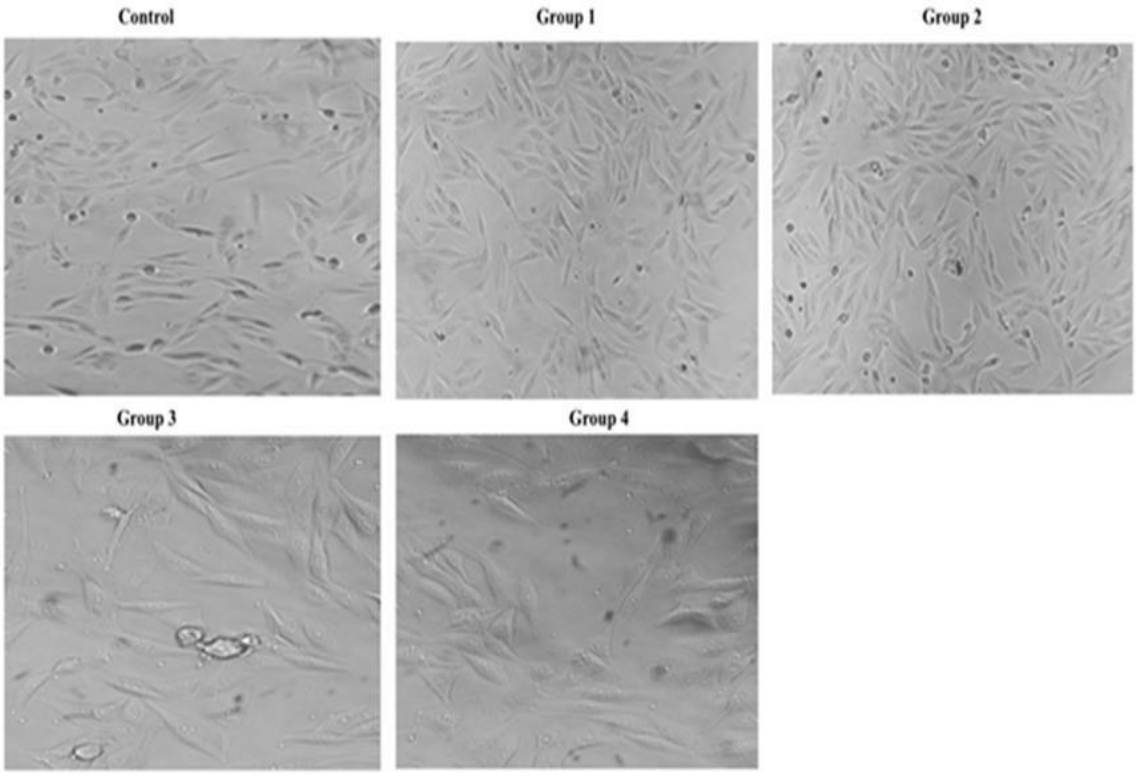

Figure 2: Representative phase-contrast images of osteoblastoma cells treated with the highest concentration of each scaffold group.

a – Control (untreated cells): Moderate cell density with viable morphology. b – Group 1 (PerioCol® GTR membrane): Spindle-shaped cells with moderate proliferation and uneven distribution. c – Group 2 (*Cissus quadrangularis* + carrageenan + tendon ECM): Increased cell density and more uniform monolayer formation. d – Group 3 (Silver-doped hydroxyapatite + *Cissus quadrangularis* + carrageenan + tendon ECM): High cell confluence with well-spread morphology. e – Group 4 (Silver tricalcium phosphate + *Cissus quadrangularis* + carrageenan + tendon ECM): Highest cellular density, elongated morphology, and minimal cytotoxicity, supporting enhanced bioactivity.

3.2 Cell Viability Assessment (MTT Assay)

The cytocompatibility of the fabricated scaffolds was assessed using the MTT assay. As shown in Figure 2, all scaffold-treated groups exhibited high cell viability, ranging from approximately 85% to 95%, indicating that none of the membrane formulations were cytotoxic. Among the groups, Group 4 (silver tricalcium phosphate + *Cissus quadrangularis* + carrageenan + tendon ECM) demonstrated the highest viability (~93%), followed closely by Groups 3 and 2, suggesting that the inclusion of bioactive components supported enhanced cell survival. In comparison, Group 1 (PerioCol®) and the control displayed slightly lower viability levels. These findings confirm that the combination of *Cissus quadrangularis*, carrageenan, and silver tricalcium phosphate creates a highly biocompatible environment conducive to osteoblastoma cell proliferation (Figure 3).

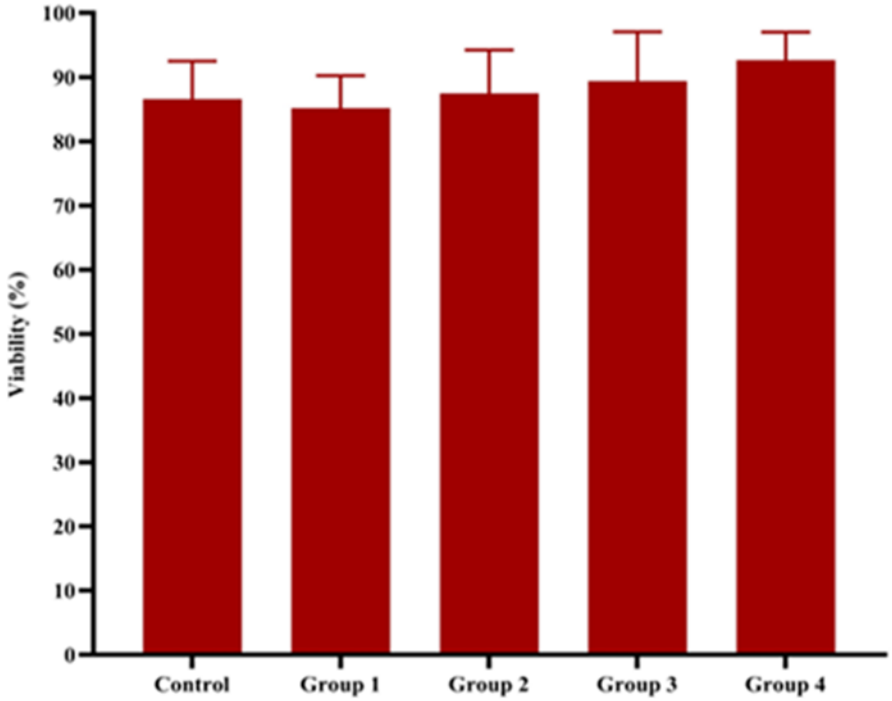

Figure 3. Bar graph representing the percentage of viable osteoblastoma cells after 24-hour exposure to scaffold eluates from each group.

Control – Untreated cells, Group 1 – PerioCol® GTR membrane,Group 2 – *Cissus quadrangularis* + carrageenan + tendon ECM, Group 3 – Silver-doped hydroxyapatite + *Cissus quadrangularis* + carrageenan + tendon ECM, Group 4 – Silver tricalcium phosphate + *Cissus quadrangularis* + carrageenan + tendon ECM. All groups maintained cell viability above 85%, with Group 4 exhibiting the highest viability (~93%). Values are presented as mean ± SD (n = 3).

3.4 Periodontal Ligament Cell Migration Assay

The pro-migratory potential of the developed bioactive membranes was assessed through a scratch assay using human periodontal ligament (PDL) cells. As depicted in Figure 4, the control group exhibited the highest migration rate (~75%), reflecting the natural motility of untreated cells. Group 1 (commercial PerioCol® GTR membrane) showed a notable reduction in cell migration (~61%), suggesting limited biological stimulation. In contrast, Group 4, which incorporated *Cissus quadrangularis*, carrageenan, tendon-derived ECM, and silver tricalcium phosphate, significantly enhanced cell migration (~70%), closely mirroring control levels. Groups 2 and 3 also promoted increased cell migration compared to Group 1, although their effects were less pronounced than those of Group 4. These results demonstrate that the presence of bioactive components—especially silver tricalcium phosphate—plays a pivotal role in stimulating cell motility, a critical factor for successful periodontal and bone tissue regeneration (Figure 4).

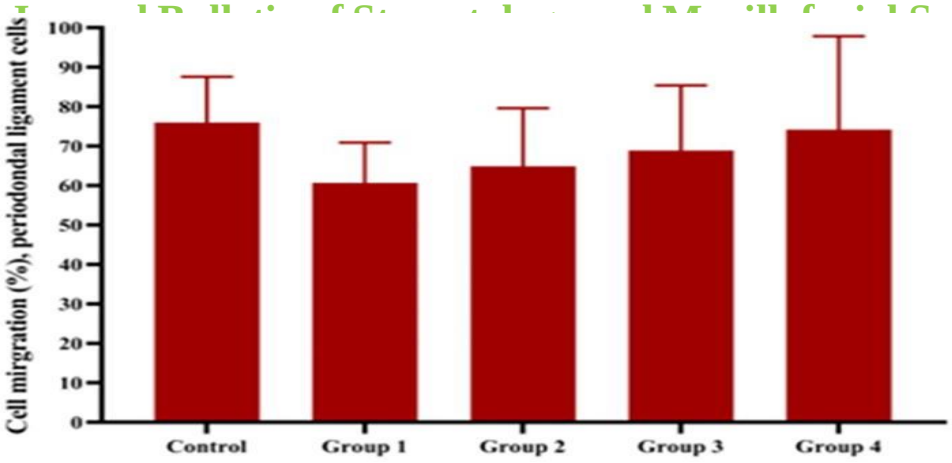

Figure 4. Bar graph representing the percentage of wound closure by human periodontal ligament (PDL) cells after 24 hours in the scratch assay for each scaffold group. Control – Untreated cells (baseline migration), **Group 1** – PerioCol® GTR membrane, Group 2 – *Cissus quadrangularis* + carrageenan + tendon ECM, Group 3 – Silver-doped hydroxyapatite + *Cissus quadrangularis* + carrageenan + tendon ECM, Group 4 – Silver tricalcium phosphate + *Cissus quadrangularis* + carrageenan + tendon ECM. Group 4 showed the highest migration among the scaffold-treated groups (~70%), closely approaching control values (~75%), while Group 1 exhibited the lowest migration (~61%). Values are presented as mean ± SD (n = 3).

3.5 Alkaline Phosphatase (ALP) Activity

Alkaline phosphatase (ALP) activity was measured on Days 7 and 14 to assess early-stage osteogenic differentiation in response to the scaffold formulations. As shown in Figure 1, ALP levels were comparable across all groups at Day 7, indicating a similar baseline osteogenic response (Figure 5). However, by Day 14, there was a significant increase in ALP activity in Groups 3 and 4, with Group 4 exhibiting the highest enzymatic activity among all treatments (Figure 6). This time-dependent elevation suggests progressive osteoblastic differentiation, and highlights the role of the composite formulation in Group 4—containing *Cissus quadrangularis*, carrageenan, tendon-derived ECM, and silver tricalcium phosphate—in effectively enhancing early osteogenesis. These results reinforce the scaffold's potential to support periodontal bone regeneration by promoting early markers of bone formation (Figure 7)

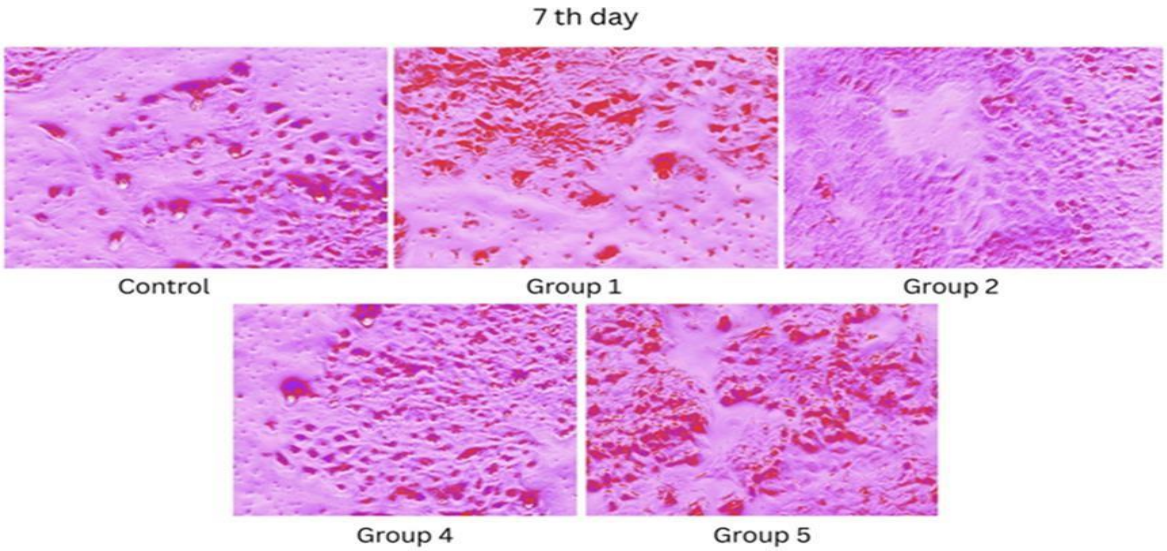

Figure 5. Representative images and quantitative data showing calcium deposition by osteoblastoma cells after 7 days of treatment with scaffold eluates.

Control – Untreated cells showing minimal mineralization, Group 1 – PerioCol® GTR membrane with limited calcium deposition, Group 2 – *Cissus quadrangularis* + carrageenan + tendon ECM, showing moderate mineralized nodule formation, Group 3 – Silver-doped hydroxyapatite + *Cissus quadrangularis* + carrageenan + tendon ECM, showing enhanced mineralization, Group 4 – Silver tricalcium phosphate + *Cissus quadrangularis* + carrageenan + tendon ECM, exhibiting the highest calcium deposition and intense Alizarin Red staining. Images correlate with absorbance values measured at 570 nm. Values expressed as mean ± SD (n = 3).

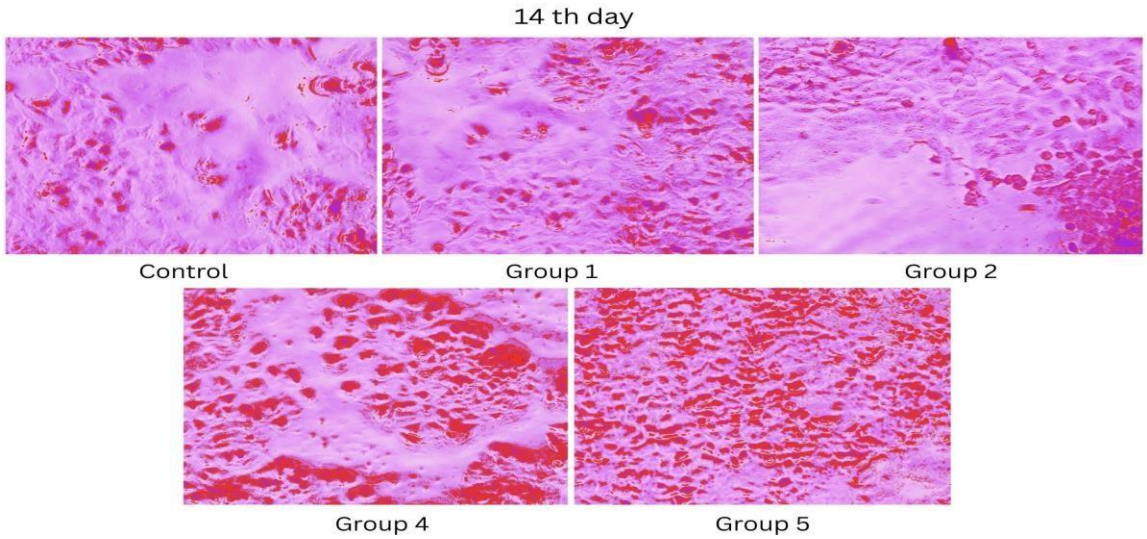

Figure 6. Representative images and quantitative analysis of calcium deposition by osteoblastoma cells after 14 days of treatment with scaffold eluates.

Control – Untreated cells with minimal mineralized nodule formation, Group 1 – PerioCol® GTR membrane showing limited calcium deposition, Group 2 – *Cissus quadrangularis* + carrageenan + tendon ECM showing moderate mineralization, Group 3 – Silver-doped hydroxyapatite + *Cissus quadrangularis* + carrageenan + tendon ECM with enhanced calcium nodule formation, Group 4 – Silver tricalcium phosphate + *Cissus quadrangularis* + carrageenan + tendon ECM exhibiting the most intense Alizarin Red staining and highest calcium deposition. The results indicate time-dependent mineralization, with Group 4 demonstrating superior osteogenic potential. Quantification performed by absorbance at 570 nm; values presented as mean ± SD (n = 3).

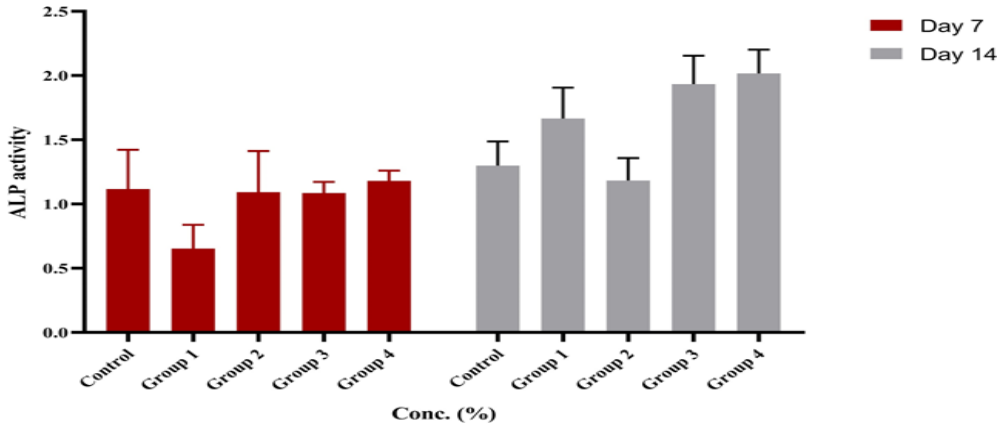

Figure 7. Bar graph representing the quantitative measurement of calcium deposition by osteoblastoma cells after 14 days of treatment with scaffold eluates, assessed via Alizarin Red S staining.

Control – Untreated cells, Group 1 – PerioCol® GTR membrane, Group 2 – *Cissus quadrangularis* + carrageenan + tendon ECM, Group 3 – Silver-doped hydroxyapatite + *Cissus quadrangularis* + carrageenan + tendon ECM, Group 4 – Silver tricalcium phosphate + *Cissus quadrangularis* + carrageenan + tendon ECM. Group 4 exhibited the highest absorbance at 570 nm, indicating maximal calcium deposition and mineralization, followed by Groups 3 and 2. Values are shown as mean ± SD (n = 3).

3.6 Gene Expression Analysis of Osteogenic Markers

Quantitative real-time PCR was conducted to evaluate the expression profiles of six key osteogenic and matrix-related genes—RUNX2, ALP, OCN, COL1A1, BMP2, and MMP2—with β-actin serving as the internal control. As illustrated in Figure 3, all scaffold-treated groups exhibited upregulation of these genes compared to the untreated control, with Group 4 demonstrating the most pronounced fold increase across all markers.Notably, RUNX2 and ALP were significantly upregulated in Group 4, indicating enhanced early-stage osteogenic differentiation. The expression of OCN and COL1A1—markers associated with matrix maturation and collagen synthesis—was also highest in Group 4, reinforcing its ability to support extracellular matrix development. BMP2, a critical osteoinductive factor, showed substantial elevation in the same group, highlighting potent signaling for bone formation. Furthermore, MMP2 expression was markedly increased, suggesting active extracellular matrix remodeling and enhanced cell migration capability.Group 3, which incorporated silver-doped hydroxyapatite, displayed moderate gene expression increases, while Groups 1(PerioCol®) and 2 showed only slight upregulation. Overall, these results affirm that the composite

formulation in Group 4—combining *Cissus quadrangularis*, carrageenan, tendon ECM, and silver tricalcium phosphate—offers the strongest potential for promoting osteogenic differentiation, matrix remodeling, and regenerative signaling pathways (Figure 8).

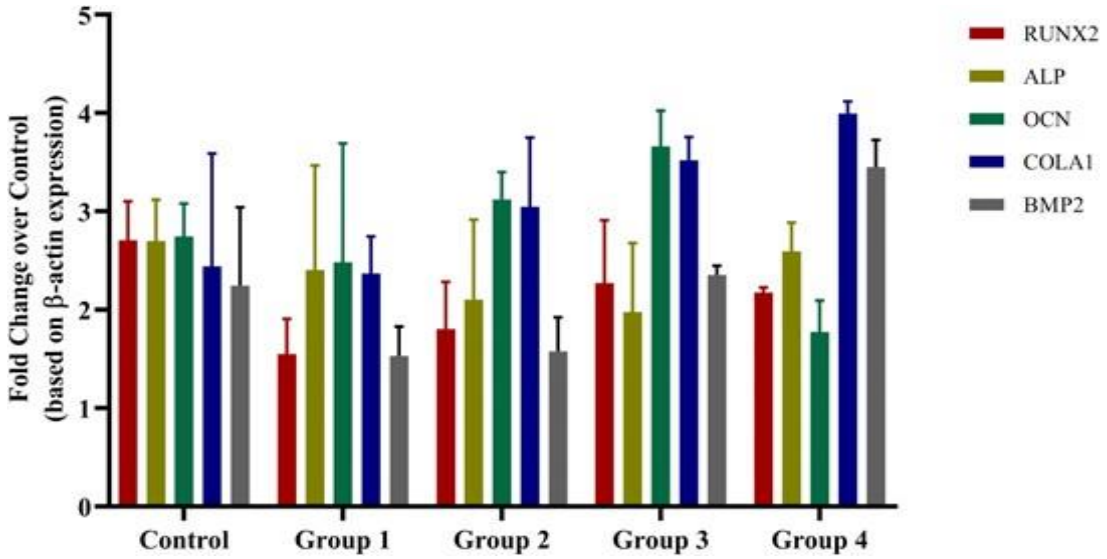

Figure 8. Bar graph representing the relative mRNA expression levels of osteogenic and matrix-related genes (RUNX2, ALP, OCN, COL1A1, BMP2, and MMP2) in osteoblastoma cells after 14 days of treatment with different scaffold formulations.
Control – Untreated cells, Group 1 – PerioCol® GTR membrane, Group 2 – *Cissus quadrangularis* + carrageenan + tendon ECM, Group 3 – Silver-doped hydroxyapatite + *Cissus quadrangularis* + carrageenan + tendon ECM, Group 4 – Silver tricalcium phosphate + *Cissus quadrangularis* + carrageenan + tendon ECM. Group 4 showed the highest fold increase across all markers, indicating enhanced osteogenic differentiation and matrix remodeling. Data are expressed as mean ± SD (n = 3), normalized to β-actin, and calculated using the 2^−ΔΔCt method.

DISCUSSION

Periodontal bone regeneration continues to present a significant clinical challenge due to the complex anatomical and biological structure of the periodontium[24]. The multifactorial nature of periodontal defects requires biomaterials that not only support osteogenesis but also mimic the structural and biochemical features of native tissue. This study aimed to address this challenge by developing and evaluating an electrospun composite scaffold composed of carrageenan, tendon extracellular matrix (TEM), Cissus quadrangularis (CQ) extract, and silver-doped bioceramics. Among the various scaffold formulations tested, the Group 4 scaffold—incorporating silver tricalcium phosphate (Ag-TCP)—demonstrated superior performance across all evaluated parameters, including cytocompatibility, mineralization, and osteogenic gene expression[25].

In the MTT assay, Group 4 exhibited significantly enhanced cell viability ($p < 0.05$), reflecting excellent biocompatibility and a favorable microenvironment for cell proliferation , likely due to the combined effects of Silver tricalcium phosphate offers inherent antibacterial properties and has been shown to promote osteoblast proliferation without cytotoxicity, which is particularly important in the context of oral applications where microbial contamination poses a risk. CQ, a traditional medicinal plant, is rich in bioactive constituents such as ketosteroids and flavonoids that have been reported to modulate osteogenesis. Previous studies have reported improved biomineralization and cell survival in CQ-based scaffolds, with recent findings showing over 92% mesenchymal stem cell viability in CQ/TCP/gelatin composites. Additionally, Recent studies on α-TCP and Ag-modified hydroxyapatite composites further support its role in providing balanced mechanical properties, biocompatibility, and antimicrobial potential. These results suggest a synergistic effect of Ag-TCP and CQ in promoting biocompatibility and cell proliferation[26].

Mineralization, a critical indicator of osteogenic maturation, was assessed using Alizarin Red S staining. The assay revealed that Groups 3 and 4 exhibited markedly higher calcium deposition than other groups, with Group 4 showing the most intense staining and the highest quantitative absorbance at 570 nm showing higher mineralisation. This enhanced performance is likely due to the synergistic effects of *Cissus quadrangularis* (CQ), tricalcium phosphate (TCP), and silver-based components. CQ contains flavonoids and Phyto steroids with anti-inflammatory and osteogenic properties, and has been shown to promote fracture healing by increasing alkaline phosphatase and osteopontin levels. Tricalcium phosphate, known for its superior restorability compared to hydroxyapatite, facilitates osteoconduction by releasing calcium and phosphate ions into the local environment. In addition, silver-doped ceramics have been shown to accelerate early-stage mineralization while offering antimicrobial protection. These converging effects explain the statistically significant mineralization observed in Group 4 and underscore its promise as an osteoinductive scaffold material. To further assess osteogenesis at the molecular level, quantitative RT-PCR was performed to evaluate the expression of key bone-related genes: RUNX2, ALP, COL1A1, OCN, and BMP2. The results revealed significant upregulation of all markers across the experimental groups, with Group 4 demonstrating the highest expression levels ($p < 0.01$). Each of these genes plays a pivotal role at distinct stages of bone development. RUNX2 is essential for early osteoblast differentiation and chondrocyte maturation, ALP and COL1A1 contribute to matrix maturation, while OCN is a hallmark of late-stage mineralization. Notably, Group 4's enriched expression aligns with prior findings in CQ/TCP composites, which reported elevated RUNX2 (168%) and OCN (188%) levels, underscoring the scaffold's osteoinductive capacity. The significant increase in BMP2 expression in Group 4 is particularly noteworthy, as this gene plays a central role in the activation of bone morphogenetic signalling pathways that orchestrate osteoblast proliferation and differentiation. These molecular insights strongly affirm that the Group 4 scaffold promotes osteogenesis through both early-stage differentiation and late-stage mineralization[27].

An important structural component of the scaffold is the decellularized tendon-derived extracellular matrix (TEM), which plays a vital role in replicating the native bone microenvironment. TEM retains critical ECM proteins such as collagen, fibronectin,

and growth factors that are essential for supporting cell adhesion, migration, and phenotype maintenance. This is consistent with previous reports demonstrating that ECM-derived biomaterials enhance cellular behavior and promote tissue regeneration. Furthermore, the electro spun nanofibrous architecture of TEM mimics the fibrillar structure of natural ECM, offering high porosity and surface area that facilitate nutrient diffusion and cellular infiltration . This highlights one of the core advantages of electrospinning: the creation of scaffolds that structurally emulate the nanoscale features of natural collagen while remaining scalable, reproducible, and customizable for clinical translation. This platform also holds potential for future bioactive loading, such as controlled release of growth factors or incorporation of stem cells. This structural biomimicry is comparable to peptide-enriched PCL systems, such as those incorporating self-assembling peptides (e.g., P11-4 and P11-8), which exhibit enhanced mineralization, biocompatibility, and nanofiber integrity. This structural biomimicry elevates the regenerative potential of the scaffold, aligning it with other high-performance systems under development for hard tissue regeneration[28].

Beyond osteogenesis, both CQ and silver ions offer additional biological benefits. CQ has been reported to exert anti-inflammatory effects by suppressing pro-inflammatory cytokines like TNF-α and IL-6, which is particularly advantageous in periodontitis, where chronic inflammation impairs regeneration. Moreover, silver ions, at low concentrations, have been shown to induce angiogenesis, potentially contributing to neovascularization—a critical but often overlooked element in successful bone regeneration. Although angiogenesis was not directly evaluated in this study, future studies should explore this aspect using endothelial markers or VEGF expression analysis. It is also important to note that the Group 4 scaffold significantly outperformed the commercially available PerioCol membrane (Group 1), which functions mainly as a passive barrier. Unlike PerioCol, the Group 4 scaffold actively engaged in cellular signaling, promoting proliferation, mineralization, and osteogenic differentiation. This represents a shift toward the development of ‘bioactive’ membranes that do more than act as barriers—they actively contribute to tissue repair and regeneration. An additional advantage of this system lies in its tunability. Given the modular composition of the scaffold, it can be adapted for personalized therapies—such as modifications for patients with diabetes, immunocompromised states, or infected defects—and may be extended to applications in orthopedic or craniofacial surgery. Despite the promising outcomes of this study, certain limitations must be addressed. All experiments were conducted under in vitro conditions, which, while useful for preliminary evaluation, do not fully replicate the complex physiological environment of the periodontium[29]. In vivo studies using appropriate animal models are essential to assess host integration, immune modulation, vascularization, and long-term bone regeneration. Additionally, the mechanical properties of the scaffold—such as compressive strength and elasticity—were not evaluated, though these are critical for clinical translation, especially in load-bearing sites. The degradation kinetics under physiological conditions also remain unknown and may affect scaffold performance over time. Future research should focus on comprehensive mechanical testing, in vivo biocompatibility, and long-term functional assessments. Optimizing scaffold architecture through advanced fabrication methods like 3D bioprinting or coaxial electrospinning could further enhance its structural and biological performance. Incorporating angiogenic or immunomodulatory agents, or pre-seeded cells, may also accelerate integration and healing. With these refinements, the multi-component scaffold holds strong potential for application in periodontal regeneration and broader orthopedic contexts[30].

5. CONCLUSION

This study successfully developed and evaluated a novel multifunctional scaffold comprising *Cissus quadrangularis* extract, tendon-derived extracellular matrix (tECM), carrageenan, and silver tricalcium phosphate (Ag-TCP) for periodontal bone regeneration. Among the four tested groups, Group 4, containing Ag-TCP, exhibited superior in vitro performance. It demonstrated excellent cytocompatibility, enhanced calcium deposition at both early (Day 7) and late (Day 14) stages, and significantly upregulated key osteogenic genes—RUNX2, ALP, OCN, COL1A1, and BMP2—

indicating strong osteogenic induction and matrix maturation. The scaffold's enhanced regenerative capacity is attributed to the synergistic effects of its components. *Cissus quadrangularis* contributed osteogenic and anti-inflammatory bioactivity; tECM offered a native-like scaffold environment promoting cell adhesion and proliferation; carrageenan improved scaffold hydration and integrity; and Ag-TCP provided osteoconductive and antibacterial functions. Together, these features make the Group 4 scaffold a promising platform for periodontal tissue engineering. However, while the in vitro data are promising, further in vivo studies are essential to validate long-term efficacy, degradation profile, and clinical translational potential in periodontal and craniofacial applications.

DECLARATION

Ethical Approval

The study was approved by the Institutional Ethics Committee.

The study was conducted in accordance with the ethical principles of the Declaration of Helsinki.

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Conflict of Interest Statement

The authors declare no conflicts of interest related to this study.

REFERENCES

1. Roschger P, Paschalis E, Fratzl P, Klaushofer K. Bone mineralization density distribution in health and disease. *Bone*. 2008;42(3):456-466.

2. Rosa N. *Enhancement of Bone Healing through Mechanical Stimulation*. Universidade do Porto (Portugal); 2016.

3. Sairaman S, Nivedhitha M, Shrivastava D, et al. Biocompatibility and antioxidant activity of a novel carrageenan based injectable hydrogel scaffold incorporated with Cissus quadrangularis: an in vitro study. *BMC Oral Health*. 2022;22(1):377.

4. Zhou J, See CW, Sreenivasamurthy S, Zhu D. Customized additive manufacturing in bone scaffolds—the gateway to precise bone defect treatment. *Research*. 2023;6:0239.

5. Marunganathan V, Kumar MSK, Kari ZA, et al. Marine-derived κ-carrageenan-coated zinc oxide nanoparticles for targeted drug delivery and apoptosis induction in oral cancer. *Molecular Biology Reports*. 2024;51(1):89.

6. Ganesh SB, Aravindan M, Kaarthikeyan G, Martin TM, Kumar MSK, Chitra S. Embryonic toxicology evaluation of novel Cissus quadrangularis, bioceramics and tendon extracellular matrix incorporated scaffolds for periodontal bone regeneration using zebrafish model. *Journal of Oral Biology and Craniofacial Research*. 2025;15(3):563-569.

7. Akobundu UU, Ifijen IH, Duru P, et al. Exploring the role of strontium-based nanoparticles in modulating bone regeneration and antimicrobial resistance: a public health perspective. *RSC advances*. 2025;15(14):10902-10957.

8. Zhang Q, Hu Y, Long X, et al. Preparation and application of decellularized ECM-based biological scaffolds for articular cartilage repair: a review. *Frontiers in Bioengineering and Biotechnology*. 2022;10:908082.

9. Dandagi P, Martin TM, Babu Y. In silico and glioblastoma cell line evaluation of thioflavin-derived zinc nanoparticles targeting beclin protein. *Cureus*. 2024;16(9)

10. Feier AM, Portan D, Manu DR, et al. Primary MSCs for personalized medicine: ethical challenges, isolation and biocompatibility evaluation of 3D electrospun and printed scaffolds. *Biomedicines*. 2022;10(7):1563.

11. Duraisamy R, Ganapathy D, Thangavelu L. Systematic review on hydroxyapatite and chitosan combination-coated titanium implants on osseointegration. *World Journal of Dentistry*. 2024;15(1):79-86.

12. Vohra M, Maiti S, Shah KK, Raju L, Nallaswamy D, Eswaramoorthy R. Influence of L-arginine on hydroxyapatite-based ovine bone graft-An in vitro evaluation of surface characteristics and

cell viability. *Dental Research Journal*. 2025;22(1):10.4103.

13. Vaiciuleviciute R, Pachaleva J, Kalvaityte U, et al. Extracellular Matrix Biomimicry for Cartilage Tissue Formation. *Cartilage: From Biology to Biofabrication*. Springer; 2023:209-253.

14. Kanimozhi S, Durga R, Sabithasree M, et al. Biogenic synthesis of silver nanoparticle using Cissus quadrangularis extract and its invitro study. *Journal of King Saud University-Science*. 2022;34(4):101930.

15. Song X, Segura-Egea JJ, Díaz-Cuenca A. Sol–Gel technologies to obtain advanced bioceramics for dental therapeutics. *Molecules*. 2023;28(19):6967.

16. Huang S, Rao Y, Ju AL, et al. Non-collagenous proteins, rather than the collagens, are key biochemical factors that mediate tenogenic bioactivity of tendon extracellular matrix. *Acta Biomaterialia*. 2024;176:99-115.

17. Murugan S, Parcha SR. Fabrication techniques involved in developing the composite scaffolds PCL/HA nanoparticles for bone tissue engineering applications. *Journal of Materials Science: Materials in Medicine*. 2021;32(8):93.

18. Subramanian BG, Maheswari U, Kaarthikeyan G, Martin TM, Kumar MSK. Evaluation of Structural, Physical and Cytotoxicological Properties of Cissus quadrangularis, Carrageenan and Extracellular Matrix Based Guided Tissue Regeneration Membrane. *Pharmacognosy Research*. 2025;17(3)

19. Roberts TR, Garren MR, Wilson SN, Handa H, Batchinsky AI. Development and in vitro whole blood hemocompatibility screening of endothelium-mimetic multifunctional coatings. *ACS Applied Bio Materials*. 2022;5(5):2212-2223.

20. Sebastian S, Martin TM, Kumar MSK. Thymoquinone-Loaded Zinc Nanoparticles Mitigate Inflammation and Inhibit Glioblastoma Progression: A Novel Therapeutic Approach. 2025;

21. Payra M, Mohanraj KG, Martin TM, Payra Jr M. Modulation of Inflammation in McCoy Cells by Zinc Nanoparticles Conjugated With β-Chitosan. *Cureus*. 2024;16(9)

22. Al-Salihi KJ, Alfatlawi WR. Synthesis and characterization of low-cost adsorbent and used for Alizarin yellow GG and alizarin Red S dyes removal from aqueous solutions. IOP Publishing; 2021:012175.

23. Saravanan SM, Prathap L, Khalid JP, Martin TM, Kumar MSK, PK J. Serotonin's Role in Inflammatory Signaling Pathway Modulation for Colon Cancer Suppression. *Cureus*. 2024;16(8)

24. Huang T-H, Chen J-Y, Suo W-H, et al. Unlocking the future of periodontal regeneration: an interdisciplinary approach to tissue engineering and advanced therapeutics. *Biomedicines*. 2024;12(5):1090.

25. Ponnulakshmi R. TEXILA INTERNATIONAL JOURNAL OF PUBLIC HEALTH. *PUBLIC HEALTH*. 2025;

26. Kamal D, Helmy N, Sayed Y. improve the biocompatibility of dental materials and operative. *Nanotechnology in Conservative Dentistry*. 2021:187.

27. Zhong Y-t, Liao H-b, Ye Z-q, et al. Eurycomanone stimulates bone mineralization in zebrafish larvae and promotes osteogenic differentiation of mesenchymal stem cells by upregulating AKT/GSK-3β/β-catenin signaling. *Journal of Orthopaedic Translation*. 2023;40:132-146.

28. Yu L, Wei M. Biomineralization of collagen-based materials for hard tissue repair. *International journal of molecular sciences*. 2021;22(2):944.

29. Sharifabad AH, Ghanbari R, Saeb MR, et al. 3D Engineered scaffolds of conjugated polymers/metal organic frameworks for biomedical applications. *International Materials Reviews*. 2025;70(2):71-102.

30. Muthukrishnan L. An overview on electrospinning and its advancement toward hard and soft tissue engineering applications. *Colloid and Polymer Science*. 2022;300(8):875-901.