Research Article

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Carrageenan, Hydroxyapatite, Tricalcium Phosphate and *Cissus quadrangularis*-Impregnated Hybrid Polymer Membrane for Periodontal Guided Tissue Regeneration

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ABSTRACT

The present investigation focused on the development and characterization of a hybrid polymer membrane incorporating hydroxyapatite (HA), tricalcium phosphate (TCP), kappa-carrageenan (CAR), and *Cissus quadrangularis* (CQ) for potential application in periodontal guided tissue regeneration. A 2% solution of kappa-carrageenan was prepared in distilled water, after which TCP synthesized by solid-state reaction was blended with HA and CQ to generate the composite. The homogenized mixture was cast into Petri dishes and lyophilized to form membranes. Two groups were fabricated: the control membrane containing CAR and HA, and the test membrane containing CAR, HA, TCP, and CQ.The membranes were evaluated using Fourier Transform Infrared Spectroscopy (FTIR), Scanning Electron Microscopy (SEM), tensile strength analysis, contact angle measurement, and antibacterial assays against *Streptococcus mutans*. The test membrane displayed a notable increase in tensile strength (4.12 MPa) compared with the control (2.65 MPa). Contact angle assessment indicated enhanced hydrophilicity (56.15°), suggesting improved potential for cell attachment. FTIR and SEM confirmed successful incorporation of TCP and CQ, with SEM images revealing a rougher surface architecture favorable for tissue interaction. Antibacterial activity was also superior in the test membrane, producing a 12 mm inhibition zone versus 8 mm in the control. Overall, the CAR+HA+TCP+CQ hybrid membrane demonstrated improved mechanical, surface, and antimicrobial properties, supporting its suitability as a candidate for periodontal regeneration.

Keywords: Tricalcium phosphate; *Cissus quadrangularis*; Hybrid polymer membrane; Periodontal tissue regeneration; Carrageenan.

1 INTRODUCTION

Tissue engineering has emerged as a key domain in regenerative medicine, focusing on the development of biomaterials that can facilitate the repair and regeneration of soft and hard tissues. In intrabony defect periodontitis cases, regeneration of soft and hard tissue could benefit the management. The field focuses on creating scaffolds or membranes that mimic the extracellular matrix and promote cellular growth, differentiation, and tissue integration. Among various approaches, guided tissue regeneration (GTR) has gained significant traction, particularly in periodontology and maxillofacial surgery, as it creates a conducive environment for tissue regrowth by acting as a physical barrier against unwanted cell migration (Venkataiah *et al.* 2025; Govindarajan *et al.* 2024). Hybrid polymer membranes are at the forefront of GTR due to their customizable properties, biocompatibility, and potential for bioactivity. However, developing a material that balances mechanical stability, biodegradability, and bioactivity remains a challenge (Kishen *et al*. 2023). In this context, incorporating bioactive compounds like tricalcium phosphate and natural plant-derived components such as *Cissus quadrangularis* offers a promising solution (Meenakshi *et al.* 2025). Tricalcium phosphate, a well-established biomaterial in bone and tissue engineering, is known for its osteoconductive and biocompatible properties. It provides a structural matrix that supports cellular attachment and proliferation, making it a crucial component for scaffolds in regenerative applications. On the other hand, *Cissus quadrangularis*, commonly referred to as the “bone setter”, has been used traditionally in Ayurveda for its potential to accelerate fracture healing and tissue regeneration. Its active constituents, including flavonoids, ascorbic acid, and phytosterols, are known to enhance collagen synthesis, reduce inflammation, and promote angiogenesis, making it a valuable addition to biomaterials aimed at soft tissue regeneration (Ganesh *et*

al. 2025; Sairaman *et al.* 2022). The integration of tricalcium phosphate and cissus quadrangularis into a hybrid polymer membrane combines the structural and bioactive advantages of both materials. This synergy is expected to create a membrane with enhanced mechanical strength, biocompatibility, and regenerative potential, addressing the limitations of conventional membranes (Henkel *et al.* 2013). Furthermore, the hybrid polymer matrix serves as a versatile platform for incorporating these bioactive agents, ensuring controlled release and sustained activity at the site of regeneration (Bottino *et al*. 2012). This study explores the fabrication and characterization of HA-TCP-CAR-CQ-impregnated hybrid polymer membrane for periodontal guided tissue regeneration. Key parameters such as, structural integrity, hydrophilicity, tensile strength, and antibacterial activity were assessed to demonstrate the membrane's potential as a next-generation material in the field of periodontal regenerative medicine.

2 MATERIALS AND METHODS

The primary components to develop the tricalcium phosphate-cissus quadrangularis hybrid polymer membrane included, tricalcium phosphate, *Cissus quadrangularis* extract, and a biocompatible polymer carrageenan.

2.1 Preparation of Tricalcium phosphate-*Cissus quadrangularis* Composite

The Tricalcium phosphate–Cissus quadrangularis composite was prepared through a multi-step process. Tricalcium phosphate was synthesized *via* a solid-state reaction using a calcium phosphate precursor, followed by fine grinding of the resultant powder and characterization to confirm its composition. The *Cissus quadrangularis* extract was prepared by drying the plant material, pulverizing it into fine powder, and then mixing 50 g of the powder with 250 mL of ethanol. This mixture was agitated at 120 rpm for 24 hours and allowed to settle for an additional day. The supernatant was collected, and the extract was concentrated using rotary evaporation. For composite formation, a predetermined weight ratio of tricalcium phosphate and cissus quadrangularis extract was homogenized using a ball mill to ensure uniform distribution. The composite was then incorporated into the polymer matrix. In the control group, carrageenan and hydroxyapatite were incorporated instead of tricalcium phosphate–cissus quadrangularis composite.

2.2 Characterization of Scaffold

The hybrid membranes were characterized using multiple analytical techniques. Fourier-transform infrared spectroscopy (FTIR) was employed to identify functional groups and confirm the incorporation of tricalcium phosphate and cissus quadrangularis into the carrageenan polymer matrix. Scanning electron microscopy (SEM) was used to examine the surface morphology and porosity of the membranes. Wettability was assessed through contact angle measurements using the sessile drop method with an Ossila Goniometer. Mechanical properties, including tensile strength and elongation at break were determined using a universal testing machine (UTM). Antimicrobial activity of the cissus quadrangularis-incorporated membranes was evaluated against *Streptococcus mutans* using the broth microdilution method with 2,3,5-triphenyl tetrazolium chloride (TTC) as a growth indicator (McCullen *et al*. 2009).

2.3 Fourier-transform Infrared Spectroscopy

Fourier-transform infrared spectroscopy was performed to identify functional groups and verify the successful incorporation of and cissus quadrangularis into the carrageenan polymer matrix. The control group membrane comprised of carrageenan and hydroxyapatite, whereas the test group membrane contained carrageenan, hydroxyapatite, tricalcium phosphate, and cissus quadrangularis. The FTIR spectra were recorded using KBr pellet method, and the characteristic peaks were analyzed to confirm chemical interactions and composite formation.

2.4 Scanning Electron Microscopy

The surface morphology and porosity of the membranes were examined using SEM. Samples from both control and test groups were mounted on aluminum stubs, sputter-coated with a thin layer of gold to ensure conductivity, and visualized at varying magnifications. The micrographs were analyzed to assess the structural integrity, pore size distribution, and homogeneity of particle dispersion within the polymer matrix.

2.5 Contact Angle Analysis

The wettability of the membranes was assessed through contact angle measurements using the sessile drop method. A water droplet was carefully placed on the surface of each membrane, and the contact angle formed between the droplet and the membrane surface was measured using an Ossila Goniometer. The obtained values provided insight into the hydrophilic or hydrophobic characteristics of the membranes, which are critical for their biomedical applicability.

2.6 Mechanical Testing

The mechanical properties of the membranes were evaluated by measuring tensile strength and elongation at break using universal testing machine. Membrane strips of standardized dimensions were clamped in the universal testing machine grips, and a constant crosshead speed was applied until failure occurred. The maximum load sustained before breaking

was recorded as tensile strength, while elongation at break was calculated as the percentage increase in length before rupture.

2.7 Antimicrobial Evaluation

The antimicrobial activity of the cissus quadrangularis extract incorporated into the CAR-TCP membrane was assessed using the broth microdilution method to determine the minimum inhibitory concentration (MIC) against *Streptococcus mutans*. The test included adding 2,3,5-triphenyl tetrazolium chloride salt as a growth indicator, which changes color in the presence of bacterial metabolic activity. The lowest concentration of extract-containing membrane that prevented visible growth was recorded as the MIC. Comparative evaluation was performed between the test group (CAR+HA+TCP+CQ) and the control group (CAR+HA) to assess the antimicrobial enhancement imparted by the composite.

3. RESULTS AND DISCUSSION

3.1 FTIR Analysis

The FTIR analysis compares the spectra of CAR + HA (green line) and CAR + HA + TCP + CQ (brown line), highlighting key differences in their chemical composition and structural interactions. Both materials exhibit characteristic peaks around 1000–1100 cm^{-1}, corresponding to phosphate (PO_4^{3-}) groups, and peaks near 3500 cm^{-1}, indicating hydroxyl (-OH) groups, characteristic of hydroxyapatite. Peaks around 1400–1500 cm^{-1} suggest the presence of carbonate (CO_3^{2-}) groups, indicating possible carbonate substitution (Fig. 1). The addition of TCP to the composite introduces shifts or additional peaks, particularly affecting phosphate-related vibrations, reflecting structural or chemical interactions between HA and TCP.

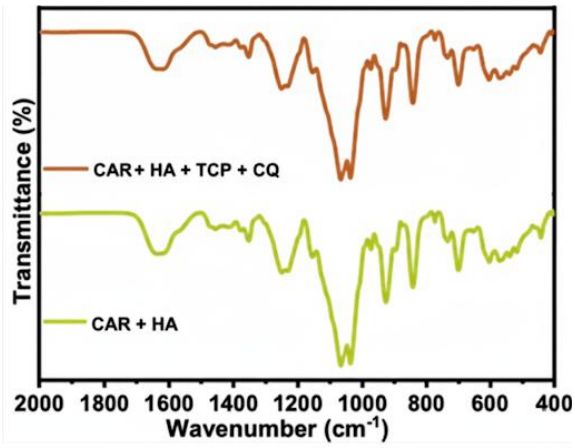


Fig. 1: FTIR spectra of control (CAR+HA) and test (CAR+HA+TCP+CQ) membranes

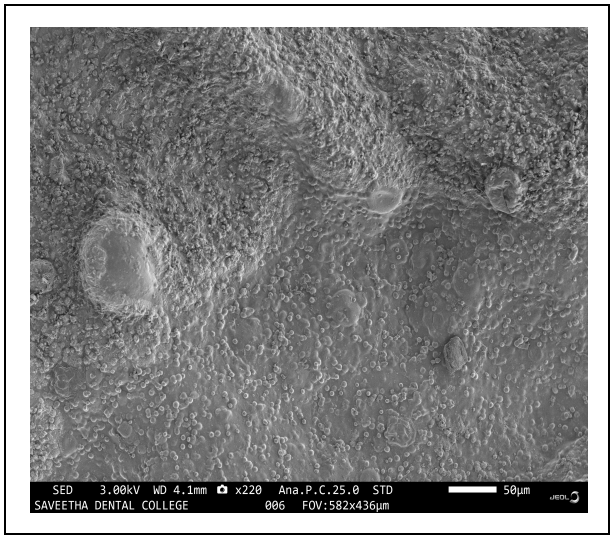


Fig. 2: SEM micrographs of control and test membranes

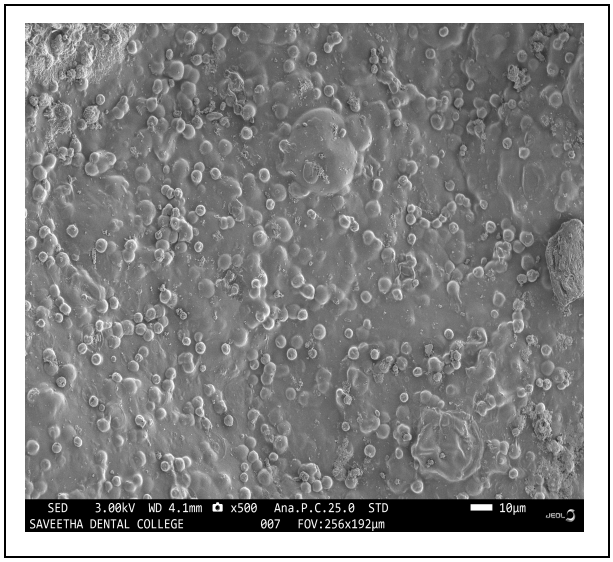


Fig. 3: SEM micrographs of control and test membranes (CAR+HA+TCP+CQ)

3.2 SEM Analysis

The SEM analysis reveals distinct differences in surface morphology between the CAR + HA and CAR + HA + TCP + CQ samples, providing insight into their potential applications in biomedical engineering. The CAR + HA sample displays a relatively smooth surface with uniformly distributed spherical particles and smaller granular features. This homogeneity indicates a well-integrated dispersion of CQ within the carrier (CAR) matrix, resulting in a microstructure that promotes mechanical stability and uniformity. The smooth morphology suggests that this composition could be advantageous for applications requiring minimal surface roughness, where low friction or consistent structural properties are critical (Fig. 2).

On the other hand, the CAR + HA+ TCP+CQ sample demonstrates a significantly rougher and more heterogeneous surface morphology. The addition of TCP leads to the formation of agglomerates and distinct raised regions on the surface, indicative of phase separation or incomplete integration within the matrix (Fig. 3).

3.3 Contact Angle Analysis of Scaffold

Contact angle was measured using an Ossila Goniometer. Control CAR+HA membrane had a contact angle of 54.02°, whereas the CQ and HA incorporated membrane with TCP had a contact angle of 56.15°. Thus, the test group membrane had a good wettability property (Fig. 4).

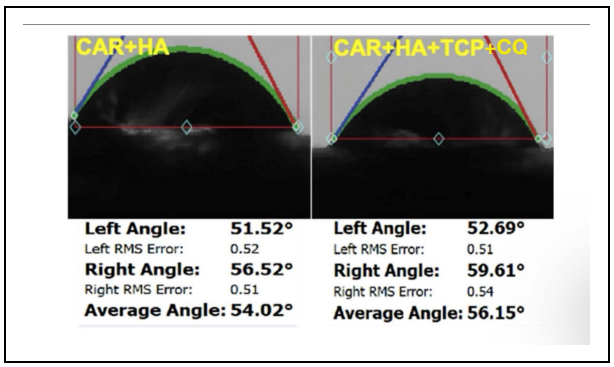
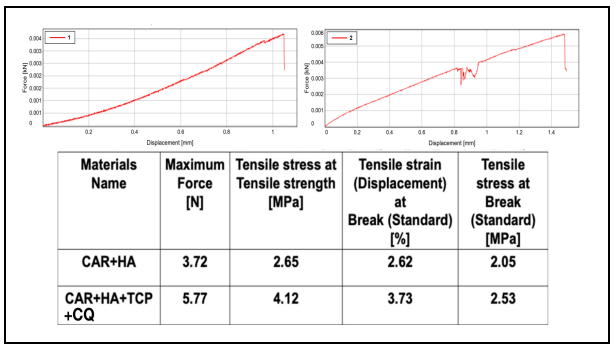


Fig. 4: Contact angle measurements of control and test membranes illustrating differences in wettability between groups

3.4 Tensile Strength Analysis of Scaffold

Tensile strength was tested using the INSTRON 3000 universal testing machine. The tensile stress for 5% GEL (control group) was 2.65 MPa, with 2.62% tensile strain at break and 2.05 MPa tensile stress at break whereas the addition of CQ and HA to TCP (test group) showed a tensile stress of 4.12 MPa with 3.73% tensile strain at break and 2.53 MPa tensile stress at break. This shows an enhanced property of tensile strength in the membrane that was incorporated with hydroxyapatite and *Cissus quadrangularis* (Fig. 5).



Materials Name	Maximum Force [N]	Tensile stress at Tensile strength [MPa]	Tensile strain (Displacement) at Break (Standard) [%]	Tensile stress at Break (Standard) [MPa]
CAR+HA	3.72	2.65	2.62	2.05
CAR+HA+TCP +CQ	5.77	4.12	3.73	2.53

Fig. 5: Tensile strength and elongation at break of control and test membranes

3.5 Antimicrobial Activity Analysis

The antimicrobial activity of the three groups was assessed by measuring the zone of inhibition against *Streptococcus mutans*. Group 1: AB - Antibiotic Control Group; 12 mm zone of inhibition was observed, Group 2: CAR + HA; a larger zone of inhibition was noted, measuring around 8 mm, suggesting effective antimicrobial properties. Group 3: CAR + HA + TCP + CQ; the zone of inhibition was also around 12 mm, indicating strong efficacy against this pathogen (Fig. 6)

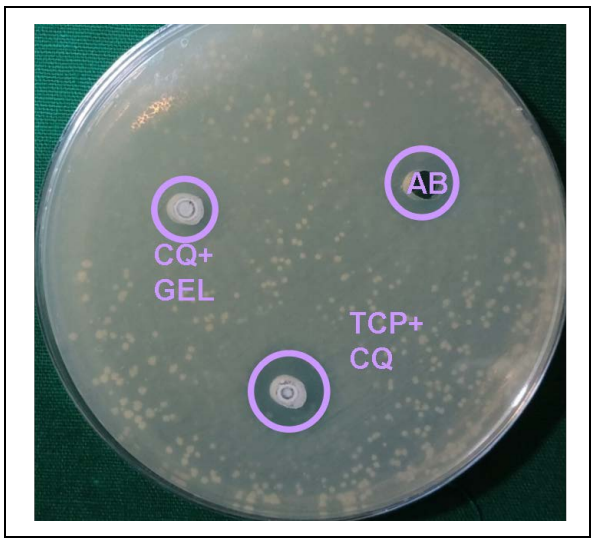


Fig. 6: Antimicrobial activity of control and test membranes against *Streptococcus mutans* (MIC results based on TTC colorimetric assay)

The results demonstrate that the CA + TCP + CQ hybrid polymer membrane exhibited the highest antimicrobial activity against *S. mutans*, with significantly larger zones of inhibition compared to the control group membrane. This suggests that the incorporation of TCP into the CQ membrane enhances its antimicrobial properties, making it a promising candidate for applications in periodontal tissue regeneration.

This study comprehensively evaluated the physicochemical, mechanical, and antimicrobial properties of hybrid polymer membranes incorporating tricalcium phosphate, hydroxyapatite, and *Cissus quadrangularis*, aiming to explore their potential for periodontal regenerative applications. The findings provide valuable insights into the impact of these components on the material's performance, offering a better understanding of how different compositions can influence the functionality and suitability of these materials for clinical use. FTIR analysis revealed significant differences in the chemical composition and structural interactions between the CAR + HA and CAR + HA + TCP membranes. Both compositions exhibited characteristic peaks for PO_4^{3-}, -OH, and CO_3^{2-} indicative of HA and suggesting possible carbonate substitution.

However, the incorporation of tricalcium phosphate into the carrageenan, hydroxyapatite and tricalcium phosphate system induced shifts in the phosphate-related vibrations and introduced additional peaks, implying structural interactions between tricalcium phosphate and hydroxyapatite. These results align with the conclusions of authors like (Xidaki *et al*. 2018), who found similar modifications in FTIR spectra when tricalcium phosphate was incorporated into hydroxyapatite-based scaffolds, attributing such shifts to the formation of new bonding interactions between the two compounds. Furthermore, the sharpness of the peaks in the carrageenan + hydroxyapatite + tricalcium phosphate sample suggested an increase in crystallinity, which could enhance both the mechanical stability and bioactivity of the material. This is consistent with findings by (Takemoto *et al*. 2005), who reported that increased crystallinity in bioactive ceramics led to improved mechanical properties and osteoconductivity. The incorporation of tricalcium phosphate, therefore, appears to modify the material's composition and structural features, potentially enhancing its properties through changes in vibrational modes and crystallinity.

SEM analysis highlighted distinct differences in surface morphology between the carrageenan + hydroxyapatite and carrageenan + hydroxyapatite + tricalcium phosphate + cissus quadrangularis samples. The carrageenan + hydroxyapatite sample exhibited a smooth, uniform surface with evenly distributed spherical particles, indicating the efficient integration of cissus quadrangularis within the carrageenan matrix. This morphology suggests potential applications where minimal surface roughness is desired, such as in tissue-contacting implants, where low friction or consistent structural properties are important. In contrast, the carrageenan + hydroxyapatite + tricalcium phosphate + cissus quadrangularis sample displayed a rough, heterogeneous surface with agglomerates, reflecting the impact of tricalcium phosphate addition. The increased surface roughness, which enhances bioactivity and osteoconductivity, makes this composition ideal for bone tissue engineering. The rough surface promotes cellular attachment, proliferation, and bone formation, aligning with earlier findings (Deligianni *et al*. 2000) that emphasized the importance of surface roughness in enhancing osteoblast attachment and promoting bone regeneration. These findings underline the transformative impact of tricalcium phosphate incorporation, shifting the material from a smooth to a more bioactive surface, highlighting its potential for applications in periodontal bone regeneration and cell-interactive environments.

Contact angle measurements revealed improved wettability in the carrageenan + hydroxyapatite + tricalcium phosphate + cissus quadrangularis group (56.15°) compared to the control group (54.02°), indicating enhanced hydrophilicity. Wettability is a critical factor for biomaterials used in tissue integration and regeneration, as it influences protein adsorption, cell attachment, and tissue healing. These results are in line with those reported by (Kumar *et al*. 2020), who demonstrated that increased wettability in composite membranes enhanced cellular interactions, improving tissue regeneration potential. The enhanced hydrophilicity of the test group membrane suggests that it is more favorable for promoting the adsorption of proteins and the attachment of cells, which is crucial for biomaterials intended for regenerative medicine.

Tensile strength testing showed a significant improvement in the mechanical properties of the carrageenan + hydroxyapatite + tricalcium phosphate + cissus quadrangularis membrane, with tensile stress increasing from 2.65 MPa in the control group to 4.12 MPa in the test group. Additionally, tensile strain at break improved, further demonstrating the reinforcing effect of tricalcium phosphate and cissus quadrangularis. These results align with previous studies (Kamel *et al*. 2022). The increased tensile strength and strain at break suggest that tricalcium phosphate and cissus quadrangularis contribute to the structural integrity of the membrane, making it suitable for applications where mechanical strength is critical, such as in bone tissue engineering and load-bearing implants.

The antimicrobial results showed a significantly higher zone of inhibition for the carrageenan + hydroxyapatite + tricalcium phosphate + cissus quadrangularis group (12 mm) against *Streptococcus mutans*, compared to the control group (8 mm). This indicates the synergistic effect of tricalcium phosphate and cissus quadrangularis in enhancing the antimicrobial properties of the membrane. The results are consistent with literature findings (Antunes *et al*. 2021). The enhanced antimicrobial performance of the carrageenan + hydroxyapatite + tricalcium phosphate + cissus quadrangularis membrane makes it a promising candidate for preventing infections in clinical applications, particularly in soft tissue regeneration and oral healthcare.

This study highlights significant advancements and potential in developing hybrid polymer membranes with tailored properties for biomedical applications, particularly in periodontal bone tissue engineering. The incorporation of tricalcium phosphate, hydroxyapatite, and cissus quadrangularis enhances the material's bioactivity, mechanical strength, and antimicrobial efficacy, with the carrageenan + tricalcium phosphate + cissus quadrangularis composition showing superior tensile strength, wettability, and strong antibacterial properties against *S. mutans*. The versatile surface morphologies observed, ranging from smooth and uniform to rough and bioactive, make these membranes suitable for a variety of applications, including implants and tissue scaffolds. However, limitations such as the need for better control over crystallinity, potential phase

separation in carrageenan + tricalcium phosphate + cissus quadrangularis, limited *in vivo* testing, and scalability challenges must be addressed. Future work can focus on optimizing material composition, conducting long-term biocompatibility studies, exploring 3D printing integration, expanding antimicrobial testing, and functionalizing surfaces with biomolecules for enhanced performance. Despite these challenges, the material demonstrates significant promise, offering a versatile, customizable solution for clinical applications in regenerative medicine and tissue engineering.

5. CONCLUSION

The findings of this study demonstrate that the incorporation of tricalcium phosphate and cissus quadrangularis into hydroxyapatite-based polymer membranes significantly enhances their chemical, mechanical, and biological properties. The Carrageenan + Hydroxyapatite + Tricalcium phosphate + Cissus quadrangularis composition, with its rough surface, improved tensile strength, superior antimicrobial activity, and good wettability, is particularly suited for applications in periodontal tissue engineering and cell-interactive environments. These results underline the potential to fabricate hybrid polymer membranes for specific periodontal tissue engineering applications by modifying their composition.

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CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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