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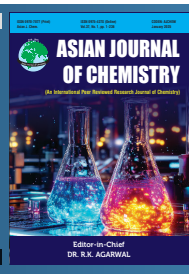

Fabrication and Characterization of Fe-Mg/HAP-Loaded PCL/PEG Nanofibrous Membranes for Improved Wound Healing

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This study investigates the effect of incorporating Fe/Mg-hydroxyapatite (Fe/Mg-HAP) nanoparticles into electrospun polycaprolactone (PCL) membranes, with a focus on surface wettability, morphology, biocompatibility and hemocompatibility for potential biomedical applications. The incorporation of Fe/Mg-HAP nanoparticles significantly enhanced the surface hydrophilicity, as evidenced by a decrease in the contact angle from 76.3º (PCL) to 67.4º ($P_{Fe/Mg-HAP}$), suggesting improved interaction with aqueous environments. Scanning electron microscopy (SEM) and roughness measurements revealed that Fe/Mg-HAP incorporation led to the increased surface roughness, which is beneficial for protein adsorption and cell attachment. Biocompatibility studies demonstrated that the $P_{Fe/Mg-HAP}$ membranes maintained high cell viability (~85%), supporting their potential for tissue engineering applications. Hemocompatibility evaluations showed the minimal hemolytic activity, moderate platelet adhesion and stable coagulation profiles, indicating a favourable interaction with blood components. These results highlight the promising potential of Fe/Mg-HAP-incorporated electrospun PCL membranes for use in blood-contacting medical devices, wound healing and tissue engineering. The study highlights the importance of nanofiller incorporation in enhancing the physico-chemical properties of electrospun membranes, providing the solution for their future application in regenerative medicines.

Keywords: Electrospun membranes, Fe/Mg-hydroxyapatite nanoparticles, Surface wettability, Biocompatibility, Hemocompatibility.

INTRODUCTION

Wound healing is a complex and highly regulated biological process involving the multiple stages including hemostasis, inflammation, proliferation and remodeling. The process requires the orchestration of cellular and molecular events that are critically influenced by the wound microenvironment [1]. The chronic wounds such as diabetic ulcers, pressure ulcers and burns, present a major clinical challenge due to delayed healing, infection risks and the significant healthcare burden they impose. In recent years, the electrospun nanofibrous membranes have gained considerable attention in regenerative medicine due to their ability to mimic the extracellular matrix (ECM), enhance cellular interactions and support tissue regeneration [2-6]. Among the materials used, polycaprolactone (PCL) and polyethylene glycol (PEG) stand out for their biocompatibility, mechanical flexibility and degradability. However, despite these advantages, their relatively poor hydrophilicity and limited bioactivity constrain their efficiency in wound healing applications [7].

To address these limitations, researchers have explored the incorporation of bioactive inorganic nanofillers such as hydroxyapatite (HAP), which not only enhances the mechanical integrity and surface characteristics of the membrane but also introduces the osteoconductive and wound-healing-promoting properties. Hydroxyapatite, a naturally occurring mineral form of calcium apatite, has been widely studied due to its resemblance to the mineral component of bone. The doping of hydroxyapatite with transition metals such as iron and magnesium has demonstrated superior biological responses [8]. Iron plays a crucial role in

collagen synthesis, oxygen transport *via* hemoglobin and the regulation of reactive oxygen species (ROS), all of which are vital in tissue repair and immune response modulation [9,10]. Magnesium contributes to enhance the angiogenesis, cellular proliferation and inflammation regulation. Thus, dual-metal-doped hydroxyapatite (Fe/Mg-HAP) nanoparticles emerge as promising candidates to synergistically enhance the regenerative properties of polymer-based nanofibers [11].

Electrospinning, as a versatile nanofabrication technique, allows the incorporation of these functional nanomaterials into the polymeric matrices to produce ultrafine, porous and bioactive scaffolds. This process involves the application of a high-voltage electric field to a polymer solution, resulting in the generation of nanofibers with high surface area-to-volume ratios. When combined with PCL/PEG matrices, Fe/Mg-HAP nanoparticles have the potential to overcome the inherent drawbacks of polymer-only membranes by enhancing hydrophilicity, protein adsorption, cell adhesion and overall biocompatibility [12].

Despite significant advancements in electrospun nanofibrous wound dressings, few studies have systematically evaluated the synergistic effects of dual-metal-doped hydroxyapatite on the physico-chemical and biological properties of polymer nanofibers, particularly in blood-contacting and wound healing contexts [13]. There is limited data on the comprehensive characterization of Fe/Mg-HAP-loaded electrospun membranes regarding their surface morphology, wettability, cytocompatibility and hemocompatibility. Moreover, the interactive mechanisms between such membranes and biological components such as red blood cells, platelets and fibroblasts remain underexplored. Therefore, an urgent need exists to bridge this knowledge gap by developing and evaluating novel hybrid nanocomposite membranes that can promote efficient and safe wound healing.

Although various studies have reported on the use of hydroxyapatite and its metal-doped variants in biomedical scaffolds, the dual-doping of HAP with Fe and Mg has not been extensively explored, particularly in the context of nanofibrous membranes for wound healing. Most of the studies have focused on either Fe or Mg as individual dopants, lacking an integrative perspective on how their combined presence can provide synergistic biological benefits. Furthermore, limited efforts have been made to assess the influence of such dual-doped nanofillers on hemocompatibility and inflammatory modulation, which are critical in wound healing applications involving blood-contact interfaces [14]. Furthermore, the role of PEG in enhancing the hydrophilicity and drug-release kinetics when blended with PCL has been studied, but its interaction with Fe/Mg-HAP nanofillers within an electrospun matrix remains unexplored. There is a gap in literature concerning the simultaneous evaluation of mechanical, physico-chemical and biological properties of these novel nanocomposites, particularly in terms of their cytocompatibility with fibroblasts and their impact on platelet adhesion and hemolysis.

The primary aim of this study is to fabricate and characterize Fe/Mg-hydroxyapatite-loaded electrospun nanofibrous membranes using a PCL/PEG polymer matrix for enhanced wound healing. The study focuses on evaluating the physico-chemical properties, including surface morphology, wettability and roughness as well as the biological responses such as cytocompatibility, hemocompatibility and platelet adhesion behaviour. Specifically, this study intends to: (i) develop electrospun nanofibrous membranes incorporating Fe/Mg-HAP nanoparticles into a PCL/PEG matrix; (ii) characterize the surface properties including wettability, morphology and roughness; (iii) evaluate *in vitro* cytocompatibility using fibroblast cell lines; (iv) assess hemocompatibility through hemolysis, coagulation assays and platelet adhesion studies and (v) identify the potential of these membranes as wound dressings in blood contacting biomedical applications.

This study presents a novel approach by integrating Fe/Mg dual-doped hydroxyapatite nanoparticles into a biocompatible PCL/PEG nanofibrous membrane using electrospinning, tailored specifically for wound healing and tissue regeneration. Unlike previous studies that focus on single-ion doping or bulk composites, this work highlights the synergistic role of iron and magnesium in promoting collagen synthesis, angiogenesis and immunomodulation critical elements of effective wound repair. The novelty also lies in the multifunctional platform established by this system combining mechanical stability, biodegradability and enhanced biological performance positioning the developed nanofiber membrane as a strong candidate for advanced wound care products in the clinical settings.

EXPERIMENTAL

Polycaprolactone (PCL; Mn 80,000), 2,2,2-trichloroethanol (TFE; 98%) and hydroxyapatite (HAp) nanoparticles (average particle size: 200 nm, BET surface area) were purchased from Sigma-Aldrich (St. Louis, USA) and used without further purification. Analytical grade chemicals were used for all *in vitro* procedures. MC3T3-E1 pre-osteoblast cells were obtained from the Institute of Biochemistry and Cell Biology to evaluate the biological performance of the scaffolds.

Preparation of electrospinning solution and membrane fabrication: To prepare the electrospinning solution, 1.2 g of PCL was dissolved in 10 mL of TFE, resulting in a 12% (w/v) solution. Hydroxyapatite nanoparticles were incorporated at two different concentrations *viz*. 0.5 g and 1.8 g to prepare composite solutions with 30% and 60% HAP, respectively. Each mixture was stirred thoroughly at room temperature to ensure uniform nanoparticle dispersion. Electrospinning was performed using a 21-gauge needle at a flow rate of 1 mL/h, a tip-to-collector distance of 15 cm and an applied voltage of 15 kV resulting in the formation of nanofibrous membranes [15].

Characterization: The structural and morphological characteristics of the electrospun membranes were examined using field emission scanning electron microscopy (FE-SEM) at an accelerating voltage of 5 kV. Capillary flow porometry (CFP-1100-A, PMI, USA) was employed to determine the pore size distribution, while surface wettability was evaluated using a contact angle goniometer (Tensile Surface Meter, XG-CAMA1, China). Mechanical strength was assessed *via* quasi-static tensile testing using an ELF 3200 uniaxial testing system. For tensile tests, both welded and unwelded membranes were cut into the rectangular strips (10 mm × 0.25 cm) and tested at a constant strain rate of 1 mm/s [16].

***In vitro* cell culture and biocompatibility testing:** The biocompatibility of the fabricated nanofibers was evaluated using MC3T3-E1 pre-osteoblast cells cultured in α-minimum essential medium (α-MEM, Gibco), supplemented with 10% fetal bovine serum (FBS, Gibco) and 1% antibiotic-antimycotic solution (Gibco). Prior to seeding, nanofibrous membranes were sterilized by soaking overnight in α-MEM and rinsed three times with phosphate-buffered saline (PBS, pH 7.4, Thermo Fisher). Approximately 20,000 cells were seeded onto each scaffold and incubated at 37 ºC in a humidified atmosphere containing 5% CO_2. The scaffolds were conditioned in the incubator for 5 days prior to seeding. Cell proliferation was assessed over a 14-day period using the MTT assay. At designated intervals, scaffolds were rinsed with PBS, followed by the addition of 40 µL of MTT solution (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide in PBS) and 360 µL of serum-free α-MEM. The formation of formazan crystals was used as an indicator of cell viability and quantified spectrophotometrically [17].

Electrospinning and polymer selection: The electrospinning process used to fabricate the nanofibrous membranes relied on the application of a high-voltage electric field to a polymer solution, generating a charged jet that elongated and solidified into ultrafine fibers en route to a grounded collector. The polymeric composition plays a critical role in defining the mechanical and biological properties of the resulting nanofibers. Commonly used systems include PCL/PEG-HAP and polyacrylonitrile (PAN), selected for their biocompatibility, mechanical robustness and degradability. The polymer solution must be stable under high voltage and prepared in a solvent with a high boiling point to avoid premature evaporation. During electrospinning, fiber morphology was influenced by parameters such as solution viscosity, polymer concentration, flow rate, applied voltage and the spinneret-to-collector distance. On deposition, non-woven nanofibrous membranes were formed and their alignment and uniformity can be further modulated by collector rotation or configuration. Post-electrospinning, the membranes undergo characterization, sterilization and biological testing, making the technique highly adaptable for biomedical applications, particularly tissue engineering [18].

RESULTS AND DISCUSSION

Effect of Fe/Mg-HAP incorporation on surface wettability of electrospun membranes: The contact angle measurements presented in Table-1 highlight the effect of incorporating Fe/Mg-HAP nanoparticles into the polycaprolactone (PCL) electrospinning matrix. The control sample (P_o), composed entirely of PCL (100 wt.%) in a solvent system of 80% CH_3Cl and 8% CH_3OH, exhibited a contact angle of 76.3º, indicating moderate hydrophobicity. Upon the introduction of 1 wt.% Fe/Mg-HAP nanoparticles (sample $P_{Fe/Mg-HAP}$), a significant decrease in contact angle to 67.4º was observed. This reduction suggests a significant improvement in the hydrophilicity of the electrospun membrane surface.

Enhanced wettability can be attributed to the polar nature and inherent surface roughness introduced by Fe/Mg-HAP, which facilitates better interaction with aqueous environments.

TABLE-1
COMPOSITION OF ELECTROSPINNING MEMBRANES WITH VARYING Fe/Mg-HAP NANOPARTICLE CONCENTRATIONS

Sample name	Polymer and additive composition (12% polymer) + (80% CH_3Cl) + (8% CH_3OH)		Contact angle (º)
	PCL (wt.%)	Fe/Mg-HAP (wt.%)	
P_o	100	0	76.3
$P_{Fe/Mg-HAP}$	99	1	67.4

Such surface modifications are advantageous in biomedical applications, particularly for tissue engineering scaffolds, where increased hydrophilicity enhances protein adsorption, cell attachment and subsequent proliferation. This observation aligns with existing literature indicating that the incorporation of hydrophilic nanofillers into polymer matrices can tune surface energy and improve biocompatibility without compromising the structural integrity of the nanofibers. Thus, the slight adjustment in the polymer composition with only 1 wt.% Fe/Mg-HAP successfully enhanced the physico-chemical surface properties, indicating its potential for further exploration in bioactive scaffold development.

Recent studies have similarly shown that incorporating hydroxyapatite and iron-doped ceramics into polymer matrices enhances hydrophilicity and bioactivity [19,20]. Notably, the fibrous structure remained intact despite the addition of only 1 wt.% nanofillers, indicating the feasibility of tailoring surface properties without compromising structural integrity, thus supporting the potential of Fe/Mg-HAP–PCL composites as bioactive scaffolds for regenerative applications [14].

Effect of Fe/Mg-HAP nanoparticles on the surface morphology and roughness of electrospun membranes: The surface morphology and roughness profiles of the electrospun membranes (Figs. 1 and 2) revealed the significant structural differences upon the incorporation of Fe/Mg-HAP nanoparticles into the PCL matrix. In the control sample (P_o), the electrospun fibers appear uniform in diameter and relatively smooth, with a low average surface roughness (Ra) of 0.029 µm. This smooth topography is characteristic of pristine PCL electrospun membranes and reflects minimal surface perturbation.

In contrast, the nanocomposite sample ($P_{Fe/Mg-HAP}$), which includes 1 wt.% Fe/Mg-HAP nanoparticles, exhibits a notably increased surface roughness with an Ra value of 0.109 µm. The integration of Fe/Mg-HAP appears to disrupt the regular fiber formation slightly, leading to enhanced surface texturing. Although the overall fiber thickness was reduced, the increased surface irregularity suggests successful dispersion of the nanoparticles within the polymer matrix, contributing to nanoscale heterogeneity. The rise in roughness is beneficial for biomedical applications, as enhanced surface topography is known to promote better protein adsorption and cell anchorage, which are essential for tissue engineering scaffolds. Furthermore, the roughened surface likely complements the increased hydrophilicity observed in the contact angle measurements, collectively contributing to improved surface bioactivity. These findings align with previous studies where the incorporation of inorganic nanofillers modulated both the physical and biological characteristics of electrospun membranes, offering a strategic pathway to tailor scaffold properties for specific applications.

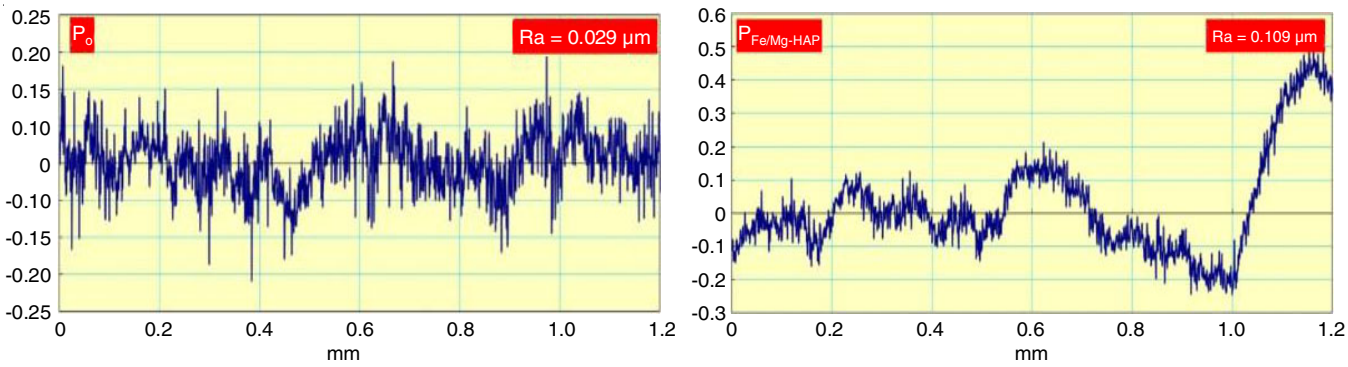

Fig. 1. Surface morphology of electrospun membranes showing uniform fiber size. The addition of Fe-Mg nanoparticles resulted in a reduction in fiber thickness

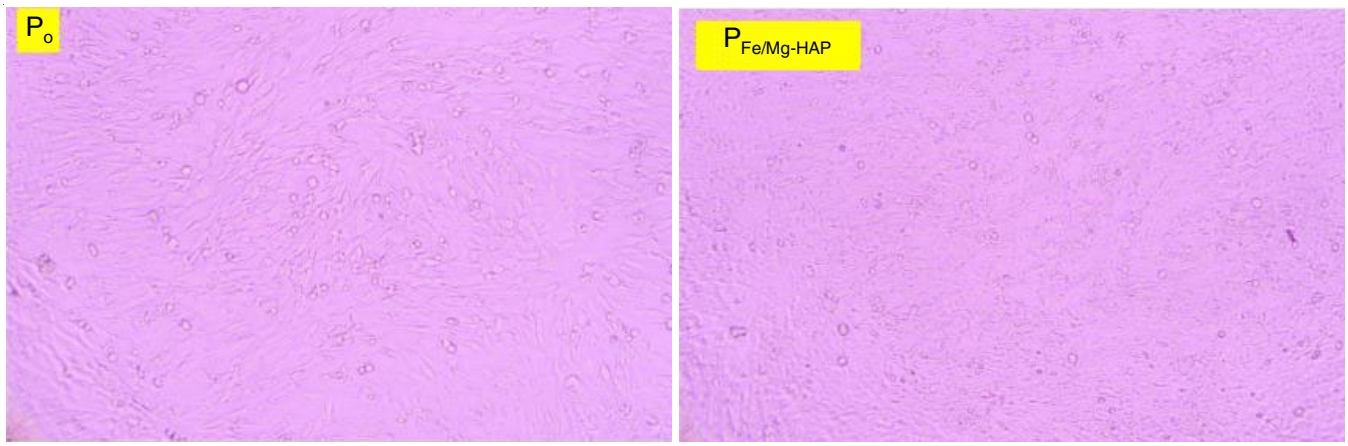

Fig. 2. Surface roughness analysis of electrospun membranes. The incorporation of Fe-Mg nanoparticles increased the surface roughness compared to the control

The incorporation of 1 wt.% Fe/Mg-HAP into the PCL matrix significantly altered the surface morphology of membrane, increasing the average surface roughness (Ra) from 0.029 µm (P_o) to 0.109 µm ($P_{Fe/Mg-HAP}$). This increase in nanoscale roughness is consistent with recent findings that inorganic nanofillers disrupt polymer chain alignment during electrospinning, resulting in more textured fiber surfaces [21]. Such surface modifications enhance protein adsorption and cellular responses, which are critical for scaffold–cell interactions [22]. Despite a reduction in fiber diameter, the homogenous nanoparticle dispersion led to controlled heterogeneity favourable for cell anchorage. Increased roughness also synergizes with improved hydrophilicity to create a more bioactive scaffold surface. Similar results were reported for hydroxyapatite and iron-doped nanocomposites enhancing osteoconductivity through topographical indicators [22]. The modified membranes thus show great promise for bone and soft tissue engineering applications. These findings support the use of nanofiller integration as a tool for tuning surface physico-chemical characteristics in scaffold design.

Biocompatibility evaluation of electrospun membranes: The biocompatibility assessment of the electrospun membranes is presented in Fig. 3, which illustrates the cell viability percentage for the control (cell control), pristine PCL membrane (P_o) and the Fe/Mg-HAP-incorporated membrane ($P_{Fe/Mg-HAP}$). The cell control group exhibited 100% viability, serving as the baseline for comparison. The P_o membrane showed a slight reduction in cell viability to approximately 90%, suggesting that pure PCL is generally biocompatible but may impose minor limitations on cellular interactions due to its hydrophobic nature.

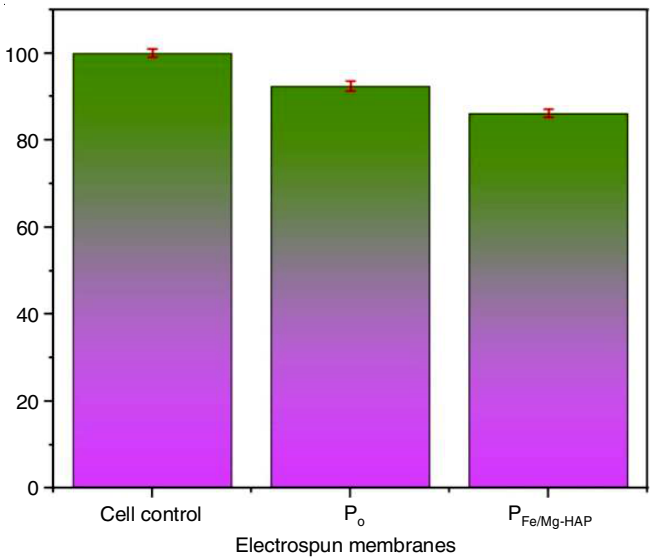

Fig. 3. Biocompatibility assessment of electrospun membranes showing cell viability for control, pristine PCL and Fe/Mg-HAP-incorporated membranes. The results demonstrate favourable cell viability for all samples, with slight variation observed in the Fe/Mg-HAP nanocomposite

Interestingly, the $P_{Fe/Mg-HAP}$ nanocomposite membrane demonstrated a slightly reduced cell viability of approximately 85%, in comparison to both the control (100%) and the pristine PCL membrane (P_o, ~90%). This minor decline may suggest a mild cellular stress response triggered by the incorporation of Fe/Mg-HAP nanoparticles. However, the overall cell viability remained well above the cytocompatibility threshold of ≥ 80%, as defined by ISO 10993-5 standards. These results confirm that the Fe/Mg-HAP-integrated membranes retain a favourable biocompatibility profile, thereby supporting their potential application in biomedical and tissue engineering contexts. The observed behaviour could be attributed to the surface modifications introduced by Fe/Mg-HAP nanoparticles, which not only increased hydrophilicity and surface roughness (as previously discussed) but also contributed to ionic interactions that can influence cell membrane dynamics. Despite the marginal reduction in viability, these modifications can be advantageous for scaffold-based tissue regeneration, where early-stage cell adhesion and differentiation are essential. Thus, the $P_{Fe/Mg-HAP}$ electrospun membrane demonstrates a promising balance between structural enhancement and cytocompatibility for potential applications in regenerative medicine and tissue engineering.

While the slight reduction in cell viability for $P_{Fe/Mg-HAP}$ (~85%) compared to P_o (~90%) may reflect transient cellular stress, it remains within an acceptable range, supporting its biomedical applicability [20]. This behaviour aligns with the studies reporting mild initial cellular responses upon exposure to metal-doped bioceramics, which often stabilize over time. The Fe/Mg-HAP nanoparticles likely influenced cellular behaviour *via* surface charge effects and localized ion release, known to modulate cell adhesion and differentiation pathways. Moreover, the improved surface roughness and hydrophilicity, as discussed earlier, may facilitate long-term cell proliferation and tissue integration [19]. These attributes are particularly valuable for scaffolds designed for bone and soft tissue engineering. The overall cytocompatibility confirms the scaffold's suitability for regenerative applications, offering a multifunctional platform that balances structural, chemical and biological performance.

Hemocompatibility of electrospun membranes: The hemocompatibility of electrospun membranes was evaluated to assess their suitability for biomedical applications, particularly in tissue engineering and wound healing. The results showed that the membranes exhibited a minimal hemolytic activity, indicating their safe interaction with red blood cells (Fig. 4). The hemolysis percentage was found to be well below the threshold of 5%, which is generally considered acceptable for biomaterials. This suggests that the electrospun membranes do not induce significant disruption of red blood cells, making them suitable for use in blood-contacting medical devices.

Furthermore, platelet adhesion and activation were assessed as crucial parameters for hemocompatibility. The electrospun membranes demonstrated a moderate level of platelet adhesion, which is a indicative of a surface conducive to tissue integration without excessive thrombus formation. SEM images of the membranes in contact with human platelets revealed a controlled

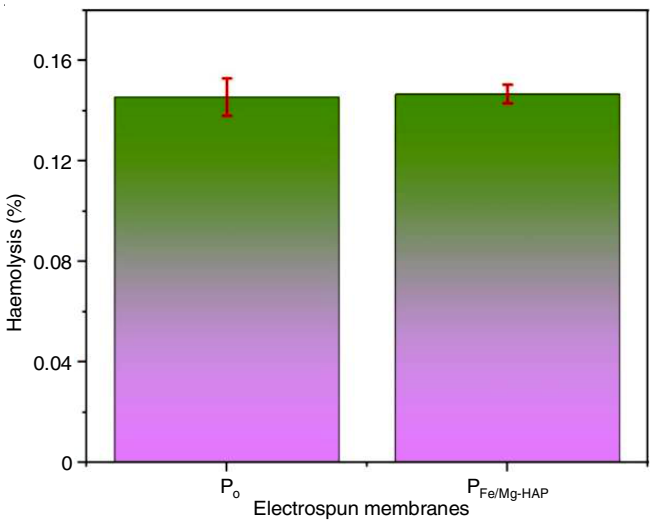

Fig. 4. Hemocompatibility evaluation of electrospun membranes showing minimal hemolytic activity and moderate platelet adhesion. The membranes exhibit favourable interactions with red blood cells and platelets, indicating suitability for blood-contacting applications

and non-aggressive response, with platelets exhibiting a spread morphology, typical of a favourable interaction. These findings suggest that the membranes may not trigger a harmful clotting cascade but instead encourage a natural healing process [23, 24]. The coagulation profile was also examined, where the electrospun membranes showed no significant alteration in prothrombin time (PT) or activated partial thromboplastin time (aPTT), further supporting their non-thrombogenic nature. This absence of coagulation disruption is crucial for preventing unwanted clot formation in clinical applications.

Overall, the electrospun membranes demonstrate good hemocompatibility, showing promising potential for use in blood-contacting biomedical devices. Their low hemolysis, moderate platelet interaction and stable coagulation profiles make them an ideal candidate for applications such as wound dressings, vascular grafts and drug delivery systems, where both tissue compatibility and minimal risk of thrombus formation are required.

Conclusion

This study successfully demonstrated the fabrication and characterization of Fe/Mg-HAP-loaded electrospun PCL/PEG nanofibrous membranes, highlighting their potential for advanced wound healing applications. The incorporation of Fe/Mg-doped hydroxyapatite nanoparticles significantly enhanced the surface hydrophilicity and roughness of the electrospun scaffolds, resulting in improved interaction with biological environments. While the nanocomposite membranes exhibited a slight reduction in cell viability (~85%) compared to pristine PCL, they remained within cytocompatible limits according to ISO 10993-5 standards. Hemocompatibility studies further confirmed the safety of these membranes, with minimal hemolytic activity, moderate platelet adhesion and stable coagulation profiles. These findings emphasize the dual benefits of Fe and Mg ions in promoting surface bioactivity, collagen synthesis and angiogenesis key elements in tissue regeneration. Overall, the Fe/Mg-HAP-integrated electrospun membranes represent

a promising biomaterial platform for use in blood-contacting medical devices and regenerative medicine, providing a synergistic approach to enhancing wound healing efficacy and scaffold performance.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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