

Two-Dimensional Magnesium Phosphate Nanosheets Promote Antibacterial Effects and Wound Closure

Salma Younes^{1,2}, Salma MS Ahmad¹, Pannawich Thirabowonkitphithan^{3,4}, Shaden H Abunasser¹, Nouran Zein¹, Amir Elhadad⁵, Asada Leelahavanichkul⁶⁻⁸, Wanida Laiwattanapaisal^{9,10}, Awni Al-Otoom¹¹, Khaled A Mahmoud¹², Faleh Tamimi⁵, Gheyath K Nasrallah^{1,2}

¹Department of Biomedical Science, College of Health Sciences, QU Health, Qatar University, Doha, Qatar; ²Biomedical Research Center, Qatar University, Doha, 2713, Qatar; ³Department of Biomedical Science, Faculty of Health and Society, Malmö University, Malmö, 20506, Sweden; ⁴Biofilms - Research Center for Biointerfaces, Malmö University, Malmö, 20506, Sweden; ⁵College of Dental Medicine, Qatar University, Doha, 2713, Qatar; ⁶Translational Research in Inflammation and Immunology Research Unit (TRIRU), Department of Microbiology, Chulalongkorn University, Chulalongkorn, Bangkok, 10330, Thailand; ⁷Department of Microbiology, Faculty of Medicine, Chulalongkorn University, Chulalongkorn, Bangkok, 10330, Thailand; ⁸Immunology Unit, Department of Microbiology, Chulalongkorn University, Chulalongkorn, Bangkok, 10330, Thailand; ⁹Centre of Excellence for Biosensors and Bioengineering (CEBB), Department of Clinical Chemistry, Faculty of Allied Health Sciences, Chulalongkorn University, Chulalongkorn, Bangkok, 10330, Thailand; ¹⁰Department of Clinical Chemistry, Faculty of Allied Health Sciences, Chulalongkorn University, Patumwan, Bangkok, 10330, Thailand; ¹¹Chemical Engineering Department, Jordan University of Science and Technology, Irbid, 22110, Jordan; ¹²Qatar Environment and Energy Research Institute (QEERI), Hamad Bin Khalifa University (HBKU), Qatar Foundation, Doha, 2713, Qatar

Correspondence: Faleh Tamimi, College of Dental Medicine, Qatar University, Doha, 2713, Qatar, Email fmario@qu.edu.qa; Gheyath K Nasrallah, Department of Biomedical Science, College of Health Sciences, Qatar University, Doha, 2713, Qatar, Email gheyath.nasrallah@qu.edu.qa

Background: NeoPhylaxis is a patented two-dimensional (2D) magnesium phosphate (MgP) hydrogel, initially approved in 2023 for dental applications such as implant decontamination, it has demonstrated strong safety and efficacy. This study explores its repurposing for antimicrobial and wound healing applications.

Aim: To synthesize, characterize, and investigate the antibacterial properties, biocompatibility, and wound-healing potential of MgP hydrogel.

Methods: The MgP hydrogel was synthesized via controlled crystallization of a sodium magnesium-phosphate system. Its structural and compositional properties were characterized using Fourier-transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD), and scanning electron microscopy with energy-dispersive X-ray spectroscopy (SEM/EDX). Antibacterial efficacy was evaluated in vitro, while biocompatibility and wound healing efficacy were assessed in vivo using BALB/c mouse model. Mechanistic insights into the hydrogel's antibacterial properties were further investigated via SEM and TEM.

Results: MgP hydrogels exhibited a dose-dependent antibacterial effect, reducing *S. aureus* by at least 10-fold and *E. coli* by over 20-fold compared to controls. SEM and TEM analyses revealed extensive bacterial cell damage, including membrane deformation and compromised cell wall integrity. Treated mice displayed no signs of irritation, erythema, or edema post hydrogel treatment. Wound closure was significantly enhanced in MgP-treated mice, reaching 46% by Day 5 vs 37% in controls ($p=0.008$).

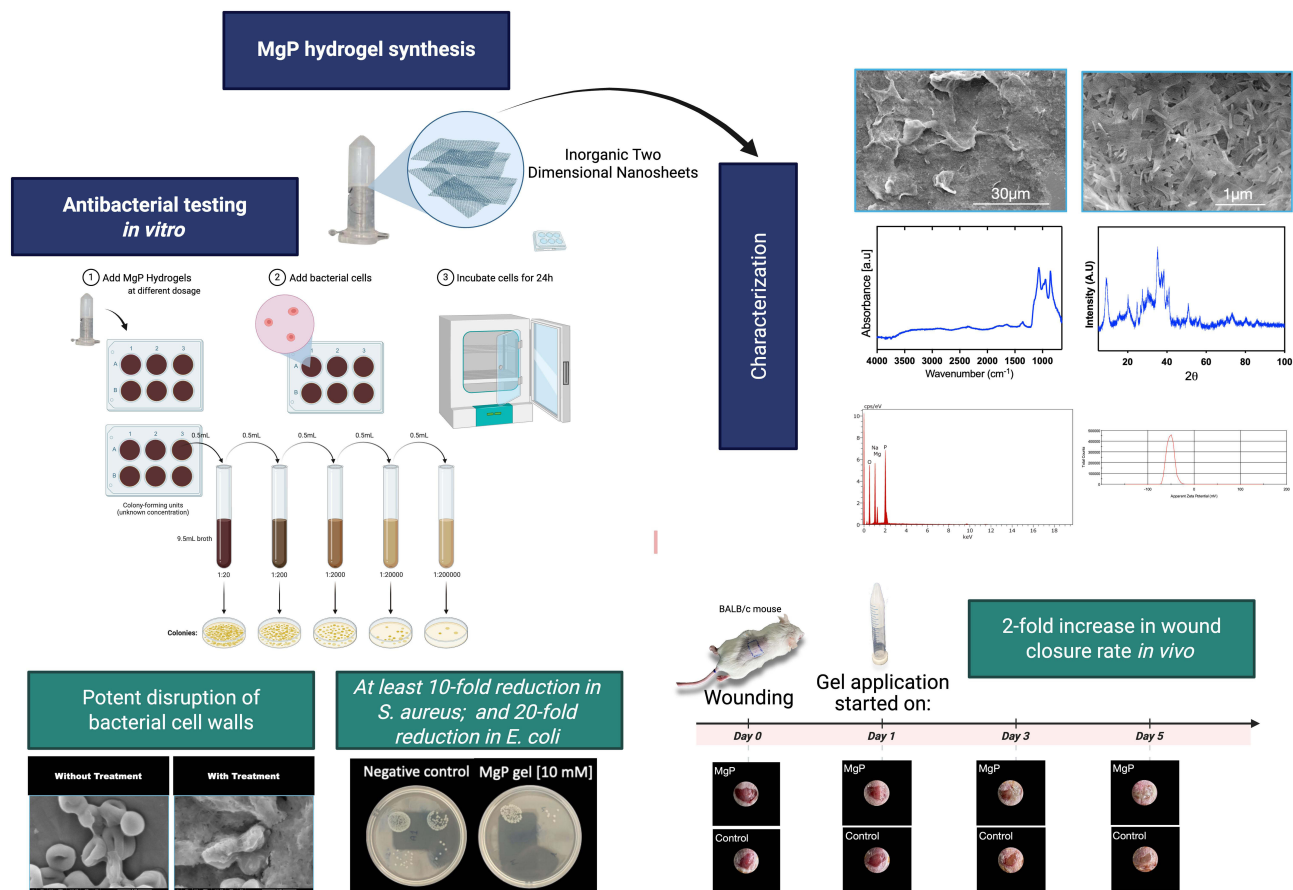
Conclusion: These findings highlight the potential of 2D MgP nanosheets as a multifunctional therapeutic agent for antimicrobial and wound healing applications.

Keywords: two-dimensional (2D), nanomaterials, inorganic hydrogel, wound dressings, antibacterial, biocompatibility

Introduction

The skin, as the body's largest organ, serves as a critical barrier against pathogens, physical injuries, and environmental pollutants. Disruption of this barrier exposes underlying tissue to bacterial colonization, significantly increasing the risk of infection. Such infections can complicate wound healing, lead to systemic consequences, and, in severe cases, become life-threatening. The global rise in antibiotic resistance among bacterial pathogens further complicates infection

Graphical Abstract



management in wound care, highlighting an urgent need for innovative therapeutic approaches that circumvent conventional antibiotics.^{1,2}

In recent years, two-dimensional (2D) nanomaterials have emerged as promising candidates in biomedical research due to their ultrathin morphology, large surface area, and tunable surface chemistry. These features enable unique interactions with biological systems, making them particularly attractive for antimicrobial and wound healing applications.^{3,4} Although carbon-based materials like graphene oxide have been widely studied for their antimicrobial efficacy, their clinical translation remains limited due to drawbacks such as non-biodegradability, high cost, and concerns over bioaccumulation and long-term safety.^{5–7} In response, attention has shifted toward **inorganic 2D materials**, which offer superior biocompatibility, chemical stability, and safer degradation profiles.

Metal and metal-phosphate-based 2D materials, in particular, have shown promise as bioactive wound therapeutics due to their ability to disrupt microbial membranes, modulate reactive oxygen species (ROS), and promote tissue regeneration.^{8–15} Despite the increasing interest in inorganic 2D systems, much of the existing research has focused on nanoparticles, which tend to aggregate, exhibit lower surface-to-volume ratios, and may pose toxicity risks in physiological environments.^{16–19} In contrast, **2D nanosheets**, by virtue of their high aspect ratio and layered morphology, can enhance cellular interactions, ion exchange, and controlled bioactivity, positioning them as next-generation candidates for wound care.

Hydrogels have long played a central role in wound care due to their high water content, flexibility, and ability to maintain a moist healing environment, which promotes epithelial migration and reduces infection risk. Traditional hydrogels, however, are often composed of natural or synthetic polymers, such as chitosan, gelatin, or polyethylene

glycol; that require chemical crosslinking agents to maintain structural integrity. However, such additives may introduce concerns regarding biocompatibility, degradation byproducts, and regulatory hurdles. In contrast, Laurenti et al (2016) demonstrated that two-dimensional magnesium phosphate (MgP) nanosheets can spontaneously form physically entangled, thixotropic hydrogels in aqueous media without the need for synthetic gelling agents or crosslinkers.^{20,21} These polymer-free, inorganic hydrogels maintain desirable shear-thinning and injectable properties, while exhibiting notable biological activity in vitro, including upregulation of regenerative genes such as COL1A1, RunX2, ALP, OPN, and OCN. Although direct measurements of ion release were not reported, the authors attributed the regenerative effects in part to the potential local availability of magnesium and phosphate ions, both of which are known to support cellular adhesion, migration, and matrix remodeling.²²

Building on this foundation, we investigate the potential of this polymer-free MgP hydrogel, originally developed for dental implant disinfection under the trade name *NeoPhylaxis* (US Patent No. US10875772B2), as a topical treatment for infected or acute skin wounds.^{20,21,23} The MgP hydrogel consists of 2D nanosheets with a thickness of approximately 4–7 nm, forming a cohesive matrix in water without synthetic gelling agents.²⁰ This structure combines antimicrobial potency, injectability, and biocompatibility, offering potential advantages over both conventional hydrogels and metal nanoparticles. While prior work has focused on its application in dental biofilm control, we hypothesize that this material can be repurposed for skin infection treatment and wound healing.

In this study, we synthesize and characterize the MgP hydrogel and evaluate its safety, antibacterial efficacy, and wound-healing capacity using in vitro cell-based assays and an in vivo murine wound model. By highlighting its dual antimicrobial and regenerative properties, our findings position the 2D MgP nanosheet hydrogel as a multifunctional therapeutic platform for future applications in infection control and skin repair.

Methods

Chemicals and Reagents

The study employed magnesium oxide (Sigma Aldrich, Product No. 1.05862) and phosphoric acid (Sigma Aldrich, Product No. 30417-M) as precursor chemicals. Sodium hydroxide (NaOH) from RICCA Chemical Company (Product No. R7470050) was used as the precipitating reagent. Other reagents included Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), penicillin-streptomycin, Dulbecco's phosphate-buffered saline (DPBS), 0.25% trypsin-EDTA (all from Gibco, CA, USA), Neutral Red solution, Dimethyl sulfoxide (DMSO), and resazurin sodium salt (Sigma Aldrich), and Alamar Blue (Thermo Scientific). All experiments used purified water obtained through a Direct-Q Water Purification System (Merck Millipore).

Synthesis of 2D MgP Hydrogel

Following previously published protocols.^{24,25} A sodium hydroxide solution was gradually added to an aqueous solution of MgP under continuous stirring to initiate gelation and stabilize the hydrogel network to form a standardized MgP hydrogel at a final MgP concentration of 0.29 M. This concentration was predetermined based on prior work by our team and was selected for its optimized physicochemical stability, gelation behavior, and demonstrated biocompatibility, as described in our previous publication published in *Nano Letters*,²⁰ Patent No: US10875772B2²¹ and Provisional Patent No. 63/752,549. The same hydrogel formulation was used consistently across all experiments.

Characterization of the 2D Diphosphate Materials

The freeze-dried hydrogel powders were analyzed using Fourier Transform Infrared Spectroscopy (FT-IR) on a Nexus 470 instrument (Thermo Fisher Scientific) to identify functional groups, with spectra recorded over the range of 500–4000 cm^{-1} .²⁵ The powders were also washed with deionized water and alcohol before assessment by X-ray Diffraction (XRD) using a D8 Advance diffractometer (Bruker) with a Copper K-alpha source, scanning a 2θ range of 10° to 80° . Surface morphology was examined using Scanning Electron Microscopy (SEM) on a Nova Nano 450 (FEI), while elemental composition of the freeze-dried gels was analyzed through Energy-Dispersive X-ray Spectroscopy (EDX). The synthesized hydrogel nanomaterials were characterized to determine their zeta potential using a Zetasizer

Nano Series (Malvern Instruments, Malvern, United Kingdom). Prior to analysis, hydrogel samples were diluted at a ratio of 1:40 (v/v) using deionized water. The diluted samples were transferred to clean Eppendorf tubes and gently vortexed to achieve homogeneity. Approximately 1 mL of each sample was then loaded into a disposable zeta cell for analysis. Measurements were conducted at room temperature (25°C) with three readings per sample to ensure reliability. The zeta potential values were calculated based on the electrophoretic mobility of the particles using Smoluchowski's equation. The results were recorded as the mean $\pm$ standard deviation of the triplicate measurements.

In vitro Assessment of Bacterial Growth Inhibition

Colony-Forming Unit (CFU) Assay

The study utilized *Staphylococcus aureus* (ATCC BAA-976) and *Escherichia coli* (ATCC 8739) obtained from the American Type Culture Collection. Nutrient agar was used as a solid growth medium. The microorganisms were grown in LB broth and subjected to hydrogel treatments at different concentrations (10–160 mM). After 24-hour incubation with the hydrogel, colony-forming units per milliliter (CFU/mL) were quantified using the plate serial dilution methods.

Bacterial Morphological Analysis Using SEM, TEM and EDS Analyses

The impact of MgP hydrogel on the surface structures of *S. aureus* was examined using scanning electron microscopy (SEM), transmission electron microscopy (TEM), and energy-dispersive X-ray spectroscopy (EDS) as describe in our previous protocol.²⁶ MgP hydrogel samples at concentrations of 40 mM and 60 mM were prepared in 6-well plates. *S. aureus* cultures were grown in LB broth at 37°C with shaking at 200 rpm for 24 hours. The following day, bacterial cultures were standardized to an optical density (OD) of 0.05 at 600 nm. Standardized bacterial suspensions were added to the MgP hydrogel samples in the 6-well plates and incubated at 37°C with shaking at 600 rpm for 24 hours to facilitate interaction between the hydrogel and bacterial cells.

After incubation, untreated and treated bacterial samples (40 mM and 60 mM) were transferred to 2 mL Eppendorf tubes, washed three times with phosphate-buffered saline (PBS) to remove residual medium, and then fixed in a 2.5% glutaraldehyde solution at 4°C for 24 hours. The samples were subsequently washed three times with distilled water to remove residual glutaraldehyde and dehydrated through a graded ethanol series (25%, 50%, 80%, and 100%), with each concentration applied for 10 minutes. Following dehydration, 1 μ L of each sample was placed onto SEM stages, sputter-coated with platinum, and imaged using an FEI Quanta 650 FEG scanning electron microscope (Hillsboro, OR, USA) to assess surface morphology changes in the treated bacterial cells. EDS analysis was performed concurrently to determine elemental composition changes on the bacterial surfaces, providing insights into the interaction between the hydrogel and bacterial cells.

For TEM analysis, fixed and dehydrated bacterial samples were embedded in resin, sectioned into ultrathin slices, and mounted on copper grids. TEM imaging was conducted on a JEM-1400 transmission electron microscope (JEOL Ltd., Tokyo, Japan) at 120 kV to observe internal cellular alterations in *S. aureus* after treatment. This combination of SEM, TEM, and EDS analyses allowed for a comprehensive assessment of the morphological, structural, and elemental effects of MgP hydrogel on bacterial cell integrity.

In vivo Safety and Wound Healing Assessments in Animal Model

Safety Assessment in BALB/c Mouse Model

The in vivo safety assessment of the MgP hydrogel was conducted in collaboration with Chulalongkorn University and the Centre of Excellence for Biosensors and Bioengineering (CEBB). 6 ~ 8-week-old female BALB/c mice were purchased from Nomura Siam International (Pathumwan, Bangkok, Thailand). The experiments were conducted in accordance with protocols approved by the Institutional Animal Care and Use Committee (IACUC) of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand (protocol number 011/266), and complied with the guidelines established by the National Institutes of Health (NIH), USA.

Mice were randomly divided into groups (3 mice/group). Following this, the dorsal hair of each mouse was carefully removed using an electric shaver. . To evaluate biocompatibility and potential skin irritation, volume of ~100 μ L of the standardized MgP hydrogel was applied topically to each animal. Signs of erythema, edema, or adverse reactions were documented photographically over 7 days.

Wound Healing Assessment in BALB/c Mouse Model

For the wound healing assessment, tramadol was prepared at a final concentration of 1 mg/kg in normal saline (NSS) and administered orally once daily for up to three days prior to the wounding procedure. Mice were randomly divided into groups (3 mice/group). Following this, the dorsal skin of a mouse was depilated and disinfected using Povidone-iodine, and a 10 mm diameter excisional wound was created at the center of the back using a sterile skin biopsy punch under isoflurane anesthesia on a sterile sheet. A volume of ~100 μ L of the standardized MgP hydrogel was applied daily to the wound area. Mice were monitored over five days, and wound closure was evaluated and documented through daily photographs.

Statistical Analysis

Statistical analyses were performed using GraphPad Prism software (version 9.3.1, GraphPad Software, San Diego, CA, USA). Results are presented as mean $\pm$ standard deviation (SD), with statistical significance defined as $p < 0.05$. A one-way ANOVA was used to assess CFU/mL bacterial data, evaluating differences across various concentrations. For in vivo safety assessments, the percentage of wound closure was calculated using the following formula: Wound closure (%) = $((A_0 - A_t) / A_0) \times 100$ where A_0 represents the initial wound area on day 0 and A_t represents the wound area at the corresponding time point. A two-way ANOVA was applied to analyze grouped data, followed by Dunnett's post hoc test for multiple comparisons.

Results

Comprehensive Characterization of MgP Hydrogel: Morphology, Composition, and Structural Validation

The MgP hydrogel was characterized using various analytical techniques, with results presented in Figure 1. Panel A displays SEM images of the MgP hydrogel at multiple magnifications, revealing its distinct layered and platelet-like structure. This morphology, characteristic of 2D materials, shows the porous and textured surface that could facilitate interactions with biological tissues or pathogens (Figure 1A). Panel B illustrates the FTIR spectrum of the MgP hydrogel,

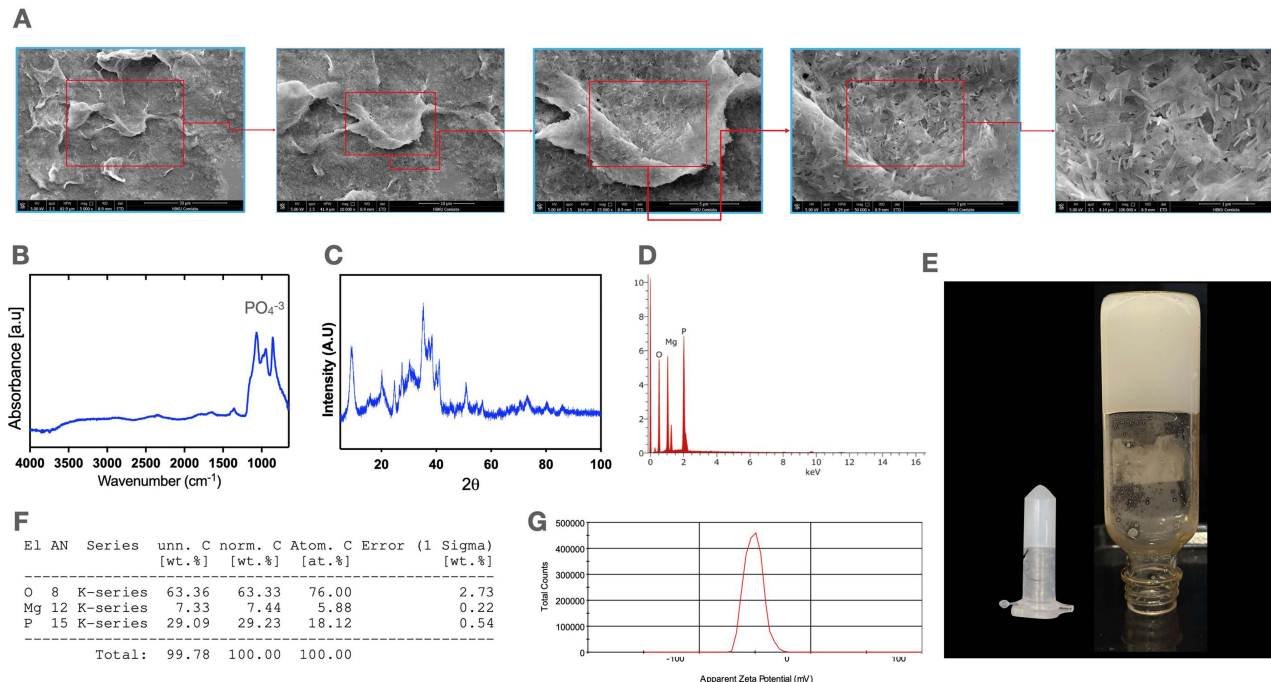


Figure 1 Characterization of the Synthesized MgP Hydrogel. Panel (A) SEM images of the MgP hydrogel at multiple magnifications, revealing its layered, platelet-like morphology and porous structure. Panel (B) FTIR spectrum of the MgP hydrogel, showing characteristic peaks associated with phosphate groups (PO_4^{3-}) within the hydrogel matrix. Panel (C) XRD pattern of the MgP hydrogel, indicating its crystalline structure consistent with magnesium phosphate. Panel (D) EDX spectrum of the MgP hydrogel, confirming the presence of magnesium (Mg), phosphorus (P), and oxygen (O) as the primary elements. Panel (E) Photograph of the MgP hydrogel, demonstrating its transparency and homogeneous appearance. Panel (F) Detailed elemental composition of the MgP hydrogel, providing quantitative analysis of magnesium, phosphorus, and oxygen content, verifying the intended formulation. Panel (G) Zeta-potential analysis.

where absorption peaks characteristic of phosphate groups (PO_4^{3-}) is visible around 1000 cm^{-1} . These peaks confirm the presence of phosphate groups in the hydrogel, while additional absorption bands further validate the structural composition, specifically indicating P=O stretching vibrations associated with phosphate-based compounds within the matrix (Panel 1B). Panel C shows the XRD pattern of the MgP hydrogel. The diffraction peaks align with expected crystalline phases of magnesium phosphate (Panel 1C). Panel D presents the EDX spectrum, which confirms the elemental composition of the MgP hydrogel. The spectrum shows prominent peaks for magnesium (Mg), phosphorus (P), and oxygen (O) (Panel 1D). Panel E is a photograph of the MgP hydrogel, showcasing its physical appearance, highlighting the hydrogel's transparency and homogeneity, demonstrating the uniformity and clarity achieved in the synthesis process (Panel 1E). Panel F provides a detailed elemental composition of the MgP hydrogel, quantitatively analyzing the constituent elements (Panel 1F). The zeta potential of the NMP nanocrystals showed an overall negative charge comprised between $-45.3 \pm 7.1\text{ mV}$ (Panel 1G).

Dose-Dependent Bacterial Inhibition by MgP Hydrogels

The antibacterial effectiveness of MgP hydrogel against *S. aureus* and *E. coli* was assessed using CFU assays, as shown in Figure 2. The CFU assay demonstrated a substantial reduction in the number of viable *S. aureus* colonies with MgP hydrogel treatment compared to the control (Figure 2A and B). Specifically, *S. aureus* CFU counts decreased by approximately 10-fold at 40 mM and up to 10,000-fold at 160 mM relative to the untreated control after 24 hours, indicating a potent inhibitory effect of the MgP hydrogel. Similarly, the CFU assay revealed significant antibacterial activity

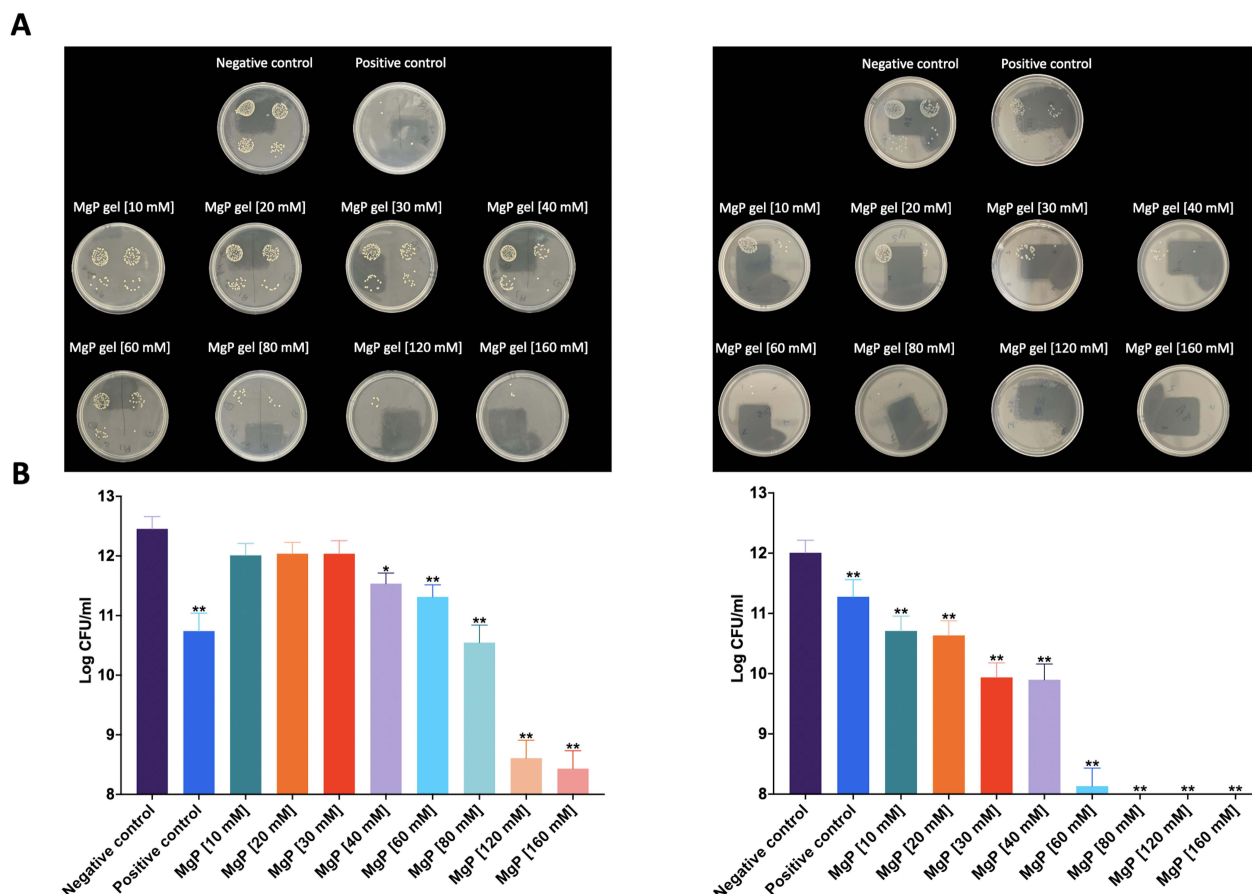


Figure 2 Colony Forming Unit (CFU) Assay of *S. aureus* and *E. coli* treated with various concentrations of MgP hydrogels. Panel (A) Representative images of *S. aureus* CFU assay plates after 24 hours of treatment with increasing MgP hydrogel concentrations (10 mM to 160 mM), along with negative and positive controls. Panel (B) Bar chart depicting the CFU/mL of *S. aureus* after 24-hour treatment, with data presented as mean $\pm$ standard deviation from triplicate experiments. Significant reduction in colony count is observed with increasing hydrogel concentration. Panel (C) Representative images of *E. coli* CFU assay plates under similar treatment conditions. Panel (D) Bar chart illustrating the CFU/mL of *E. coli* after 24-hour treatment, demonstrating a dose-dependent antibacterial effect of MgP hydrogels. Data are presented as mean $\pm$ standard deviation from triplicate experiments.

against *E. coli*, with at least a 20-fold reduction in CFU counts at 10 mM MgP hydrogel concentration compared to the untreated control (Figure 2C and D), further underscoring the hydrogel's effectiveness against both bacterial strains.

MgP Hydrogel Induces Bacterial Cell Wall Damage

The SEM and TEM images in Figure 3 demonstrate the morphological and structural changes in *S. aureus* cells following treatment with MgP at concentrations of 0 mM, 40 mM, and 60 mM. In Panel A, SEM images illustrate that untreated cells (0 mM MgP) display smooth, intact surfaces, characteristic of healthy bacterial morphology. In contrast, cells treated with 40 mM MgP exhibit roughened surfaces and begin to aggregate, suggesting initial bacterial stress or early membrane disruption due to MgP exposure. At the higher concentration of 60 mM MgP, the SEM images show pronounced morphological changes, including significant membrane deformation, irregular shapes, and dense aggregation, indicative of extensive cell damage. Such structural disruptions suggest that MgP may exert antimicrobial effects by compromising the bacterial cell wall, aligning with mechanisms reported in literature where bacterial cell wall integrity is compromised under antimicrobial stress.

TEM images in Panel B complement the aforementioned SEM findings by providing insights into the internal structural changes of *S. aureus* at each MgP concentration. Untreated cells (0 mM) maintain well-defined internal structures, consistent with healthy morphology. At 40 mM, signs of intracellular aggregation and slight density changes are observed, potentially indicating initial MgP interaction within the cell and an onset of cellular stress. With 60 mM MgP treatment, TEM images reveal substantial internal damage, including electron-dense regions, and less-defined cellular structure, which suggests severe intracellular interaction with MgP.

EDS analysis (Figure S1) further corroborates these observations by demonstrating changes in elemental composition, reflective of bacterial disruption. Untreated cells (0 mM) show typical cellular elements, such as carbon, nitrogen, and oxygen. In contrast, cells treated with 40 mM and 60 mM MgP exhibit increased levels of magnesium and oxygen, coupled with a decrease in nitrogen.

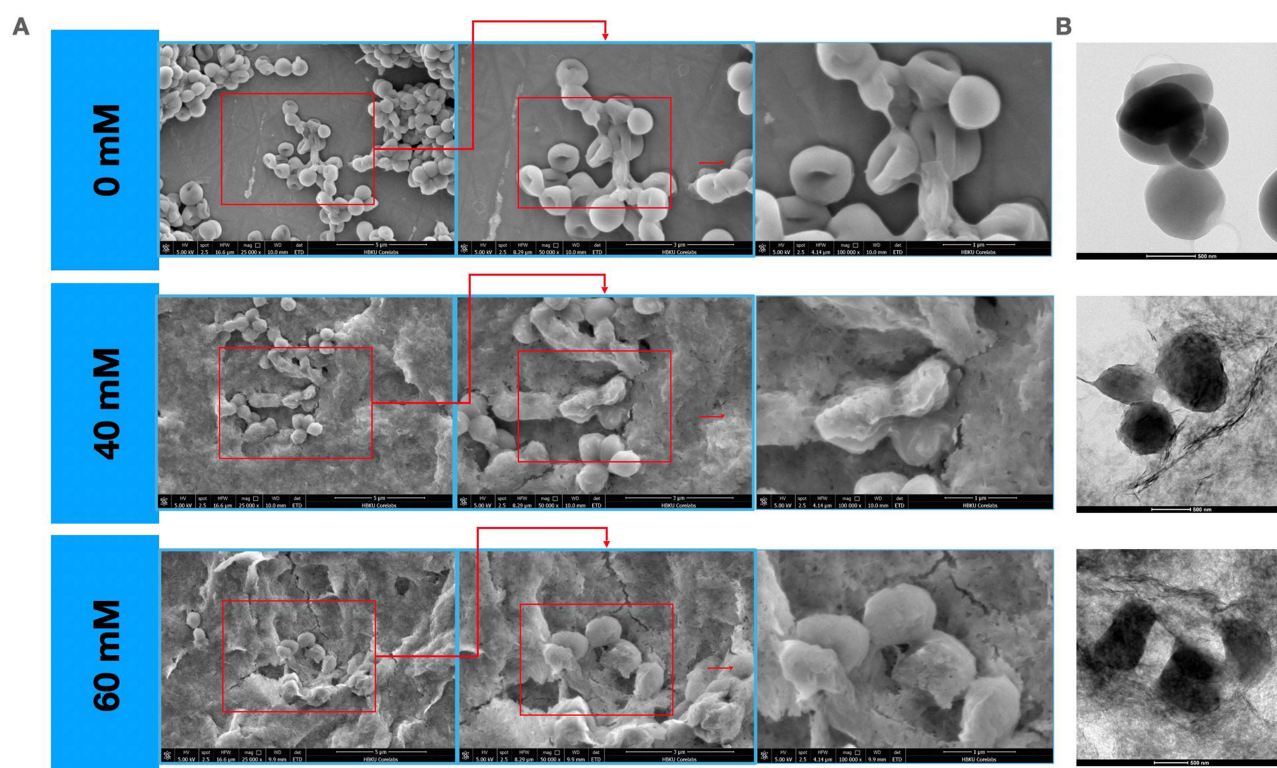


Figure 3 SEM and TEM Analysis of *Staphylococcus aureus* Post-Treatment with MgP at Varying Concentrations. (A) SEM images of *S. aureus* treated with MgP at 0 mM, 40 mM, and 60 mM, illustrating the progressive changes in surface morphology with increasing MgP concentration. (B) TEM images of *S. aureus* corresponding to each MgP concentration, providing an internal view of cellular structures.

MgP Exhibited No Significant Cytotoxicity in vivo

In the in vivo evaluation using a BALB/c mouse model, the MgP hydrogel was applied topically, and the treated skin was observed daily for seven days for any signs of irritation, erythema, or edema (Figure 4). Photographic documentation at various time points (Days 1, 3, 5, and 7) revealed no visible indications of adverse skin reactions, suggesting that the MgP hydrogel is well-tolerated and does not induce irritation or other negative dermal effects over the observed period.

In vivo Wound Healing Assessment Shows Rapid Wound Closure Rate in MgP-Treated Mice

The in vivo wound healing efficacy of the MgP hydrogel was assessed using a BALB/c mouse model with full-thickness excisional wounds treated with MgP hydrogel (Figure 5). Wound healing progression was documented daily through photographs for up to five days, allowing for visual and quantitative assessment of the wound closure rate in both the MgP-treated and control groups.

Photographic documentation (left panel of Figure 5) illustrates the wound healing trajectory over time, revealing noticeably accelerated wound closure in the MgP-treated group compared to the control. Quantitative analysis of wound closure rate (right panel of Figure 5) confirmed these findings. On Day 1, the MgP group showed a wound closure rate of 29% compared to 11% in the control group. On Day 2, closure reached 39% in the MgP group versus 15% in the control. This significant difference persisted through Day 3 (31% vs 14%), Day 4 (45% vs 22%), and Day 5 (46% vs 37%). At all-time points, the MgP-treated wounds exhibited statistically significant improvements in closure compared to control ($p=0.008$ across all days). These results demonstrate the enhanced wound healing potential of the MgP hydrogel and support its utility as an effective topical therapeutic for skin repair.

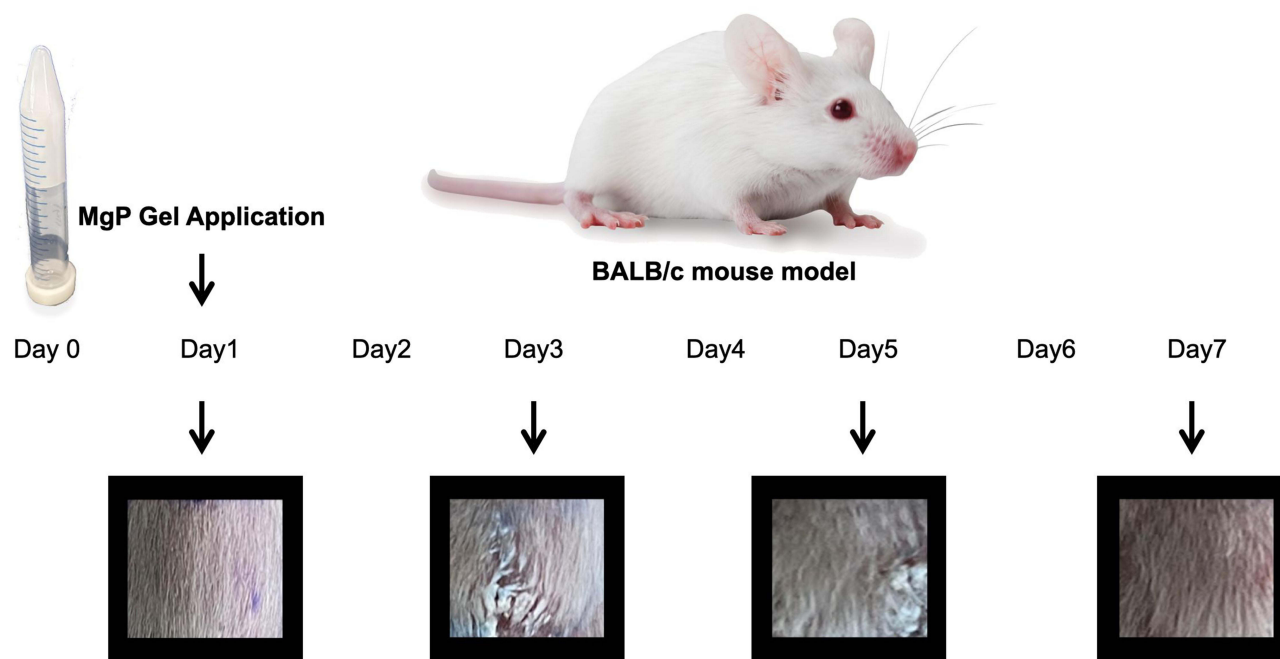


Figure 4 In Vivo Safety Evaluation of MgP Hydrogel Application in a BALB/c Mouse Model. The MgP hydrogel was applied to the wounded area on Day 1, and the site was monitored over a seven-day period. Photographs of the treated skin on Days 1, 3, 5, and 7 display the progressive healing process with no visible signs of irritation, erythema, or edema, indicating that the MgP hydrogel is well-tolerated and safe for dermal application in this model.

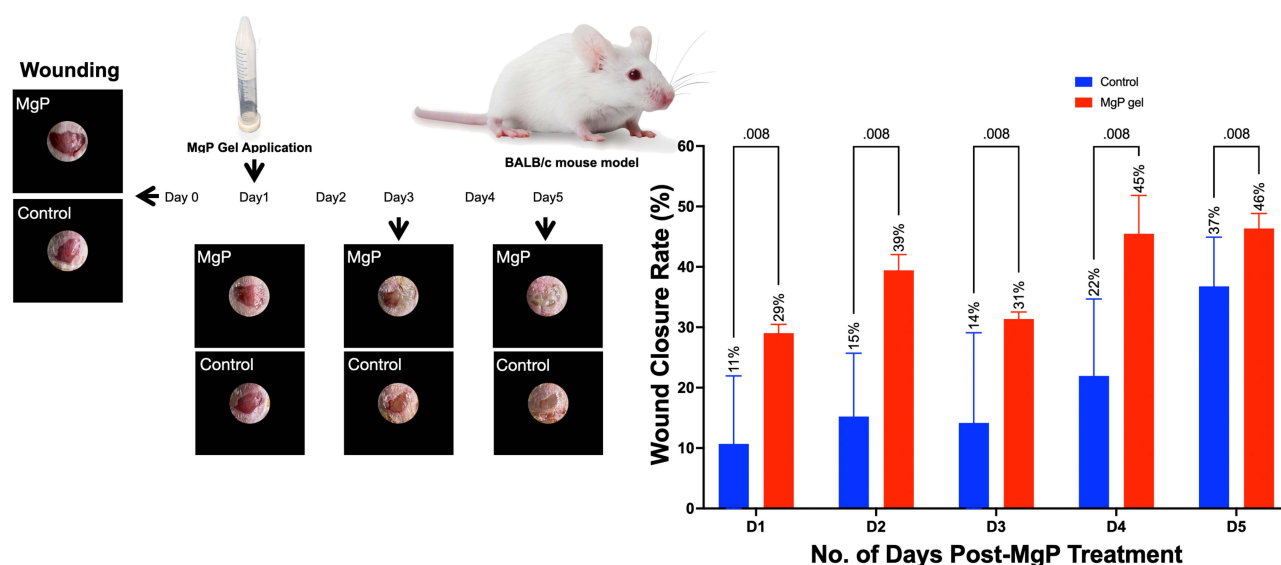


Figure 5 The left panel displays photographic evidence of wound healing progression from Day 0 to Day 5 in both MgP-treated and control groups. The right panel shows the wound closure rate (%) over time post-treatment. The MgP-treated group exhibited significantly enhanced wound closure compared to the control group at all time points, with statistical significance noted at $p < 0.001$. These findings support the potential of MgP hydrogel to accelerate wound healing in vivo.

Discussion

The escalating global challenge of antibiotic resistance has intensified the demand for innovative therapeutic solutions, particularly in wound care. Our study presents a novel application of magnesium phosphate (MgP) nanosheets, highlighting their dual role as both antibacterial agents and wound-healing enhancers. These findings build upon prior work, notably the *Nano Letters* 2016 publication by Laurenti et al,²¹ which introduced a thixotropic hydrogel composed of 2D MgP nanosheets that demonstrated regenerative potential in bone models.

The structural characterization of the MgP hydrogel in this study, supported by FTIR and XRD analyses, confirms the formation of a stable magnesium phosphate phase with strong phosphate-related bonds and crystalline integrity. FTIR spectra (Figure 1B) revealed distinct peaks corresponding to phosphate groups, while the XRD pattern (Figure 1C) confirmed the presence of crystalline MgP, consistent with prior findings by Laurenti et al (*Nano Letters*, 2016).²⁰ SEM and EDX analyses (Figures 1A and D) further demonstrated a characteristic platelet-like, layered morphology, validating the successful formation of two-dimensional nanosheet structures.

These MgP-based 2D nanosheets represent a substantial advancement over conventional metallic nanoparticle systems, which, although widely explored in nanomedicine, are often hindered by key limitations such as aggregation, low surface area, uncontrolled ion release, and cytotoxicity arising from undesired cellular uptake and long-term tissue retention.^{16–19,24,25} In contrast, the 2D MgP nanosheets developed in this study, and previously described by our group in *Nano Letters*, 2016,²⁰ exhibit a high surface-to-volume ratio, superior colloidal stability, and enhanced dispersibility, reducing the risk of systemic toxicity and improving biological performance.

One of the defining advantages of this system lies in its phosphate-based composition. Phosphate-containing materials are generally recognized as more biocompatible than oxide-based nanomaterials,^{27,28} making MgP nanosheets inherently more suitable for biomedical applications. Furthermore, the 2D morphology promotes stronger bacterial surface interaction, improved access to active sites, and enhanced antimicrobial efficacy even at lower concentrations.^{29–31} Importantly, unlike conventional polymeric hydrogels that often require synthetic crosslinkers and organic additives to maintain structure, the MgP nanosheets spontaneously form a physically entangled, thixotropic hydrogel in aqueous media, achieving gelation without chemical modification.²⁰ Together, these features position MgP nanosheet hydrogels as a multifunctional, low-toxicity platform well-suited for next-generation wound care applications.

Recent literature highlights that 2D nanomaterials exhibit intrinsic antimicrobial properties, largely due to their distinct physicochemical features—such as atomic-scale thickness, tunable band gaps, and localized surface charge

density.³² These properties enable multiple bactericidal mechanisms, including photocatalysis, electrostatic interactions, and direct physical disruption of microbial membranes. Sharp-edged nanosheet morphologies can penetrate and compromise bacterial cell walls, while negatively charged surfaces enhance electrostatic repulsion and destabilize membrane integrity.⁵ Additionally, the high surface area of 2D materials facilitates close contact with microbial cells, enabling effective wrapping, adsorption, and hydrogen bonding, which can further disrupt cellular processes. These synergistic antimicrobial mechanisms have been widely documented across various 2D systems, including graphene oxide, graphitic carbon nitride, transition metal dichalcogenides, and MXenes.⁵

In the current study, the MgP hydrogel demonstrated potent antimicrobial effects against two clinically relevant pathogens, *Staphylococcus aureus* and *Escherichia coli* (Figure 2). SEM and TEM analyses (Figure 3) revealed significant bacterial membrane deformation and cell aggregation following treatment with MgP, particularly at 40- and 60-mM concentrations. These alterations are indicative of direct membrane compromise, likely driven by the high surface reactivity, sharp edges, and localized surface charge of the 2D nanosheet architecture. Complementing these findings, EDS analysis (Figure S1) revealed a shift in elemental composition following treatment. In untreated cells, carbon and nitrogen were predominant, consistent with intact organic cell structures. However, treated cells showed increased levels of magnesium, phosphate, and oxygen, with a concurrent reduction in carbon and nitrogen content. This compositional shift suggests that the inorganic MgP nanosheets either masked or displaced the native organic matrix and potentially degraded nitrogen-rich biomolecules such as proteins and nucleic acids. Collectively, these data support a multi-pronged antibacterial mechanism involving both membrane disruption and intracellular interference. Similar mechanisms have been reported for other 2D materials, such as MXenes and graphene-based systems, which exert antimicrobial effects through a combination of physical slicing, oxidative stress induction, and charge-transfer interactions that disrupt membrane integrity and intracellular homeostasis.^{33–36} For instance, MXenes have been shown to wrap around bacterial cells, producing nanoblade-like effects, while simultaneously generating reactive oxygen species (ROS) and adsorbing vital surface biomolecules, actions that collectively lead to bacterial lysis and death.^{33–36} By analogy, the MgP nanosheets likely act via multivalent mechanisms, leveraging their inorganic composition and 2D morphology to disrupt membrane structures, interfere with nutrient uptake, and impair bacterial bioenergetics. These mechanistic insights, supported by the significant reductions in colony-forming units (Figures 2B and 2D), underscore the efficacy of the MgP hydrogel as a multifunctional, polymer-free wound dressing capable of mitigating infection in the early stages of healing.

The in vivo evaluation of the MgP hydrogel demonstrated excellent biocompatibility, with no signs of erythema, edema, or other adverse dermal reactions in BALB/c mice following topical application (Figure 4). These findings reinforce the hydrogel's safety profile and support its suitability for clinical translation, addressing a critical challenge in wound care, where many antimicrobial agents pose risks of cytotoxicity or delayed healing.¹⁹ In support of these findings, our prior study published in *Nano Letters* 2016,²⁰ investigated the same MgP nanosheet formulation in vitro and demonstrated its long-term cytocompatibility with human fibroblasts. That study employed Alamar Blue metabolic assays, live/dead viability staining, scanning electron microscopy, and gene expression profiling of osteogenic markers, collectively confirming excellent cell viability, adhesion, and functional activity. Together, these in vitro and in vivo results underscore the safety and bioactivity of the MgP nanosheet hydrogel.

Additionally, the MgP hydrogel significantly accelerated wound closure in the treated group compared to untreated controls (Figure 5), suggesting that the material not only controls infection but also enhances early tissue repair. This observation is consistent with our previous findings,²⁰ where MgP nanosheets upregulated pro-regenerative genes such as COL1A1, RunX2, and ALP and promoted fibroblast proliferation and differentiation. These regenerative effects are likely enhanced by the nanosheets' high surface area, ionic composition, and unique 2D morphology, which together create a favorable microenvironment for wound healing. Compared to conventional nanoparticles, these nanosheets offer improved dispersion and reduced risk of aggregation or long-term accumulation, key factors that support safe and effective tissue integration.

This study has several strengths, including the comprehensive characterization of the MgP hydrogel through FTIR, SEM, and EDX, which provides a detailed understanding of its structural and compositional properties. The antimicrobial efficacy against both *S. aureus* and *E. coli* underscores its potential in treating skin infections. The biocompatibility

assessments in both in vitro and in vivo settings further confirm its safety for therapeutic use, positioning it as a viable candidate for clinical applications.

Nevertheless, several limitations should be acknowledged. First, the antibacterial evaluations were restricted to two bacterial species as representative Gram-positive and Gram-negative pathogens. Broader testing, including multidrug-resistant strains like *Pseudomonas aeruginosa* and *Enterococcus faecalis*, is warranted to fully evaluate the hydrogel's antimicrobial spectrum. Second, while the 5-day in vivo wound healing model aligns with rapid murine re-epithelialization kinetics, it does not fully capture chronic or infected wound scenarios. Extended studies incorporating histological analyses and longer-term endpoints are needed to elucidate the material's regenerative capacity. Third, the volume of hydrogel applied in the in vivo model (approximately 100 μ L per wound) was selected empirically to ensure full coverage, based on gel consistency and wound dimensions. However, this was not established through a formal dose-response optimization. Further investigations are warranted to identify the minimum effective dose, assess concentration-dependent effects, and support clinical translation. Fourth, minor variability in wound area measurements may have occurred due to hydrogel thickness variability, natural skin contraction, animal movement, or manual imaging inconsistencies, factors commonly encountered in in vivo wound models. Fifth, although porosity was not directly quantified in the present work, the platelet-like 2D architecture, previously characterized via electron microscopy and detailed in our US patent (US10875772B2), suggests an inherently porous, biointeractive matrix. Future studies should include surface area and pore distribution analyses (eg, BET or mercury intrusion porosimetry) to better understand its fluid handling and cellular interface characteristics. Lastly, while prior cytocompatibility data supports safety,²⁰ validation in skin-specific models and 3D tissue systems would further substantiate its long-term bioperformance. Despite these limitations, this work provides a strong foundation for advancing MgP nanosheet hydrogels as multifunctional wound care platforms with antimicrobial and regenerative potential.

Conclusion

This study highlights the potential of MgP nanosheet hydrogels as an effective antimicrobial and wound-healing agent. MgP hydrogels demonstrated significant antibacterial activity against *S. aureus* and *E. coli*, while exhibiting biocompatibility in both in vitro and in vivo models. The accelerated wound closure observed in MgP-treated mice suggests these hydrogels support rapid tissue regeneration, making them promising candidates for clinical wound care. Future studies should aim to elucidate the precise mechanisms by which MgP nanosheets disrupt bacterial membranes and evaluate the long-term stability and potential for resistance development associated with repeated use.

Ethical Approval

All characterization and in vitro experiments presented in this paper were conducted under the Qatar University's Institutional Biohazard Committee (QU-IBC) approval. The in vivo experiments were conducted in accordance with protocols approved by the Institutional Animal Care and Use Committee (IACUC) of the Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand (protocol number 011/266), and complied with the guidelines established by the National Institutes of Health (NIH), USA.

Acknowledgments

We sincerely thank the Environmental Science Center at Qatar University for their assistance with the freeze-drying of samples. We also extend our gratitude to the Central Laboratories Unit (CLU) at Qatar University for their invaluable support in the material characterization, which greatly contributed to the depth and accuracy of our study. Additionally, we appreciate the Center for Advanced Materials (CAM) at Qatar University for their generous support, particularly in conducting the Fourier Transform Infrared (FTIR) analysis. The authors would like to thank the Qatar National Library for supporting the publication fees.

Author Contributions

G.K. and F.T. conceptualized and designed the study. S.Y. wrote the initial draft. S.Y., conducted: synthesis, characterization, and antimicrobial testing of the MgP nanosheets. S.A., and P.T. conducted the *invitro* cytotoxicity and in vivo work, respectively. A.E. provided support and contributed to the characterization work. W.L. supervised the in vivo studies and oversaw the work conducted in Thailand. G.K.N. and F.T. provided overall supervision and contributed to the study design, data analysis, and manuscript preparation. All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Funding

This research was funded by the Qatar National Research Fund (QNRF) under grant ARG01-0525-230341 (a member of Qatar Foundation) and the Qatar University Collaborative Grant 2021 (ID: 2307). Additionally, the first author, Salma Younes, is a PhD candidate supported by a Graduate Assistantship Grant from Qatar University. The funding agencies had no role in the study design, data collection, analysis, interpretation, or manuscript writing. The findings presented are solely the responsibility of the authors.

Disclosure

Ms Salma Younes, Professor Faleh Tamini and Dr Gheyath Nasrallah report a pending patent “Two-dimensional nanomaterials, magnesium, silver, copper, zinc, antimicrobial, wound healing” to Qatar University. Professor Faleh Tamini is a share holder in the Company Invicare Inc. that produces and commercializes 2D magnesium phosphate. The authors declare no other conflicts of interest in this work.

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