

Multifunctional core-shell microneedles with sequential release ability for oral ulcer healing

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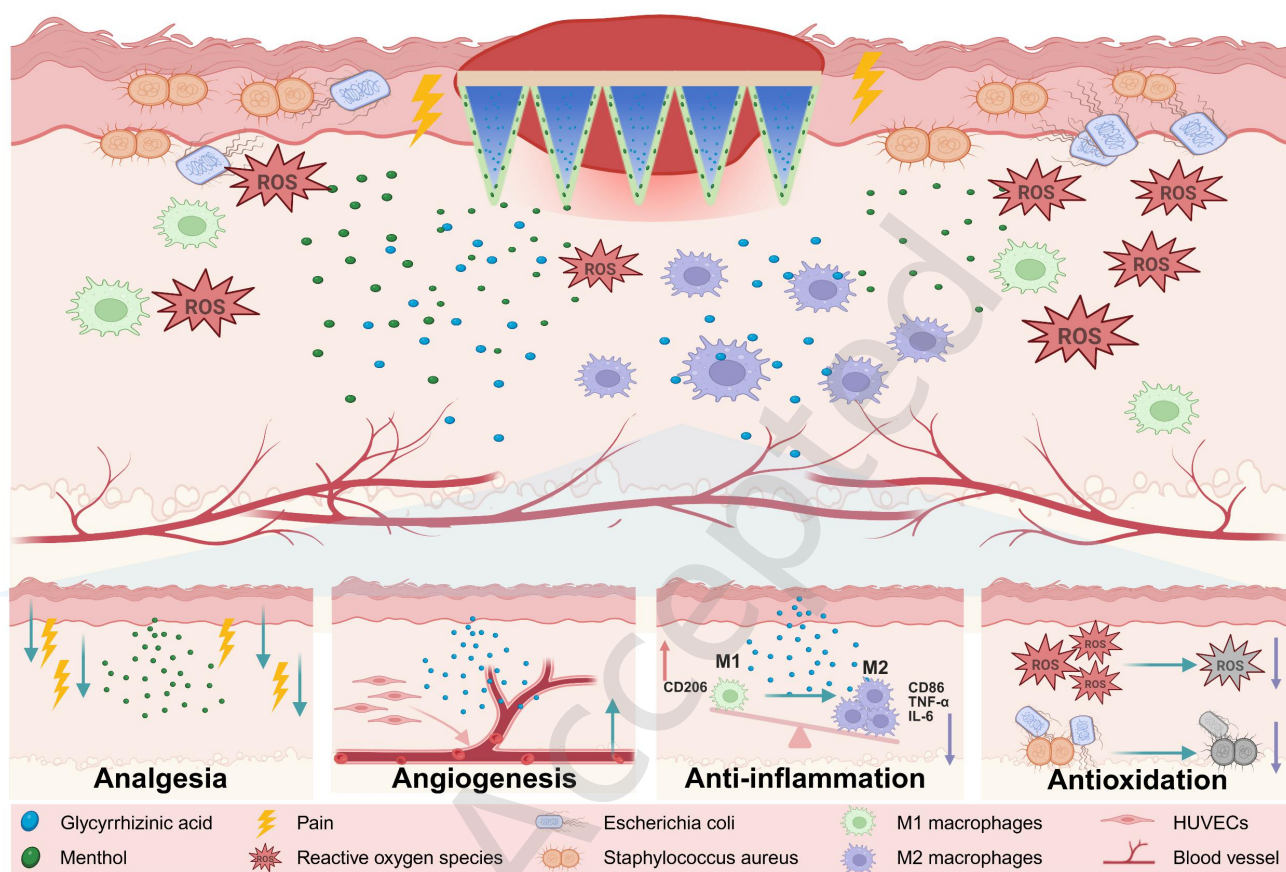
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Multifunctional core-shell microneedles (MG-MNs) accelerate oral ulcer healing through anti-inflammatory, antioxidant, antibacterial, and pro-cell proliferation effects.

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ABSTRACT: Oral ulcers compromise the quality of life of patients due to pain and recurrence. Conventional localized treatments are limited by the dynamic oral environment, resulting in insufficient drug penetration and short retention. Current treatment strategies primarily focus on alleviating clinical symptoms, often overlooking the pathological progression of oral ulcers. The core-shell microneedles (MNs) developed in this study employ a sequential drug release strategy to precisely match the distinct requirements of different ulcer healing stages. The outer hyaluronic acid (HA) shell rapidly dissolves and releases Menthol immediately, producing instant analgesic and cooling effects during the early stage of ulceration. The photocrosslinked gelatin methacryloyl (GelMA) hydrogel core loaded with glycyrrhizic acid (GA) provides a sustained release profile. This release counteracts inflammation, oxidative stress, and bacterial proliferation, while remodeling the pathological microenvironment to promote angiogenesis and cell migration. These combined actions sustain ulcer repair and enable a full-course intervention from acute symptom relief to deep tissue regeneration. In addition, the hydroxypropyl trimethyl ammonium chloride chitosan (HACC) backing layer ensures effective adhesion and enhances antibacterial activity. By integrating rapid symptomatic relief with long-term regenerative therapy, this programmable release platform represents a transformative drug delivery strategy aligned with the pathological stages of oral ulcer healing, serving as a model with strong clinical prospects for next-generation treatments of oral diseases.

KEYWORDS: oral ulcer, core-shell microneedles, drug delivery, glycyrrhizic acid, microneedles

1. Introduction

Oral ulcer is a common type of mucosal disorder characterized by recurrent and periodic round or oval-shaped ulcers[1]. The pathological mechanisms involve multifactorial interactions, including immune dysregulation, local inflammatory responses, oxidative stress, and microbial imbalance. These interactions collectively lead to mucosal epithelial damage, recurrent pain, and impaired quality of life in patients[2-4]. Current clinical management focuses on alleviating symptoms, promoting healing, and prolonging recurrence intervals, including topical patches, gels, mouthwashes, injections of glucocorticoids, lidocaine, etc[5-7]. However, these approaches are commonly limited by inherent drawbacks, such as limited therapeutic scope, short retention time, insufficient drug penetration, poor patient compliance, and potential drug resistance with long-term use[8-10]. Therefore, the development of an advanced drug delivery platform is imperative to address these limitations.

As an emerging drug delivery system, microneedles (MNs) overcome the limitations of traditional administration modes. They can micro-invasively penetrate the mucosal surface layer and directly deliver drugs to the lesion, thereby avoiding dilution and degradation in the oral environment. Simultaneously, through their piercing action, they promote drug penetration into the skin tissue, accelerating wound healing[11-14]. Additionally, MNs can be designed as multifunctional systems to achieve synergistic therapy while avoiding drug entry into the systemic circulation and reducing side effects[15, 16]. However, conventional structured microneedles still face limitations, such as limited drug loading capacity, monotonous release behavior, and difficulty in meeting multi-stage therapeutic requirements. Core-shell structured MNs, through functional zoning and differential material design in the core and shell regions, can effectively address these issues. By loading drugs with different efficacies in separate zones and leveraging the distinct dissolution characteristics of core and shell materials, they enable precise intervention across distinct

healing stages[17-20].

As a hydrophilic natural polymer, hyaluronic acid (HA) can rapidly hydrate and dissolve in the moist oral environment, facilitating the immediate release of drugs. Moreover, it possesses excellent biocompatibility and adhesion, enhancing the fit between microneedles and the ulcerated mucosa[21, 22]. Gelatin methacryloyl (GelMA) hydrogel forms a stable three-dimensional network through photo-crosslinking. Resembling the properties of the natural extracellular matrix (ECM), it provides an ideal scaffold for cell migration and proliferation[23-25]. Additionally, gradual swelling and degradation of the network allow sustained drug release[26]. Hydroxypropyl trimethyl ammonium chloride chitosan (HACC) engages in electrostatic interactions with negatively charged glycoproteins on the mucosal surface, enhancing patch adhesion at the ulcer site[27]. Concurrently, the quaternization modification strengthens the antibacterial activity of chitosan, enabling it to disrupt bacterial cell membrane integrity[28, 29].

The inherent pain of oral ulcers may exacerbate discomfort during microneedle insertion. Menthol can effectively counteract pain signals by producing a cooling sensation, achieving rapid and immediate analgesic effects. This provides patients with an instant sense of comfort during the early stages of treatment[30, 31]. The persistent inflammatory response serves as a critical barrier to oral ulcer healing[32]. Glycyrrhizic acid (GA) is a natural compound with anti-inflammatory, antioxidant, and antibacterial properties. It suppresses signaling pathways such as NLRP3, thereby reducing the release of pro-inflammatory cytokines and creating favorable conditions for tissue repair[33]. Furthermore, the excessive reactive oxygen species (ROS) can lead to cellular dysfunction and delay the wound healing process[34, 35]. GA aids in scavenging free radicals and mitigating oxidative damage[36, 37]. The oral cavity is a moist environment rich in microorganisms, where ulcerated surfaces are susceptible to bacterial colonization, which can interfere with healing[38,

39]. Studies indicates that GA exhibits inhibitory effects against various common pathogenic bacteria, helping to maintain the ecological balance of the local microenvironment[40].

In this study, we developed a multifunctional core-shell MNs system with sequential release capability (designated as MG-MNs, **Figure 1**), aiming to achieve precise stage-specific treatment for oral ulcers. This design overcomes the barriers imposed by the complex oral environment on conventional drug delivery. Meanwhile, it leverages the differential dissolution properties of the core-shell materials to achieve programmed drug release and synergistic effects, thereby precisely matching the therapeutic needs at different stages of oral ulcers. For example, the early stage is characterized by severe pain and inflammation, which require rapid analgesia and anti-inflammatory effects. In the later stage, the emphasis moves to tissue repair and immunomodulation. The MG-MNs in this study use soluble HA as the shell. Upon contact with oral mucosa, the shell rapidly dissolves and releases Menthol, providing immediate cooling and rapid analgesia during the early ulcer stage. The core layer employs GelMA hydrogel loaded with GA, which gradually swells and degrades in the tissue fluid environment, enabling sustained release of GA to exert long-term anti-inflammatory, antioxidant, and antibacterial effects. Simultaneously, the GelMA network mimics the extracellular matrix, supporting cell migration and angiogenesis. The backing layer incorporates HACC, which enhances the adhesion and retention of the patch on the ulcer surface through electrostatic interactions. Furthermore, HACC synergizes with GA to strengthen antibacterial efficacy, collectively maintaining the balance of the local microenvironment. This sequential drug release design based on a core-shell structure ideally matches the pathological progression of oral ulcers. This approach provides a novel drug delivery strategy for oral ulcer treatment that combines an innovative nature with high potential for clinical translation.

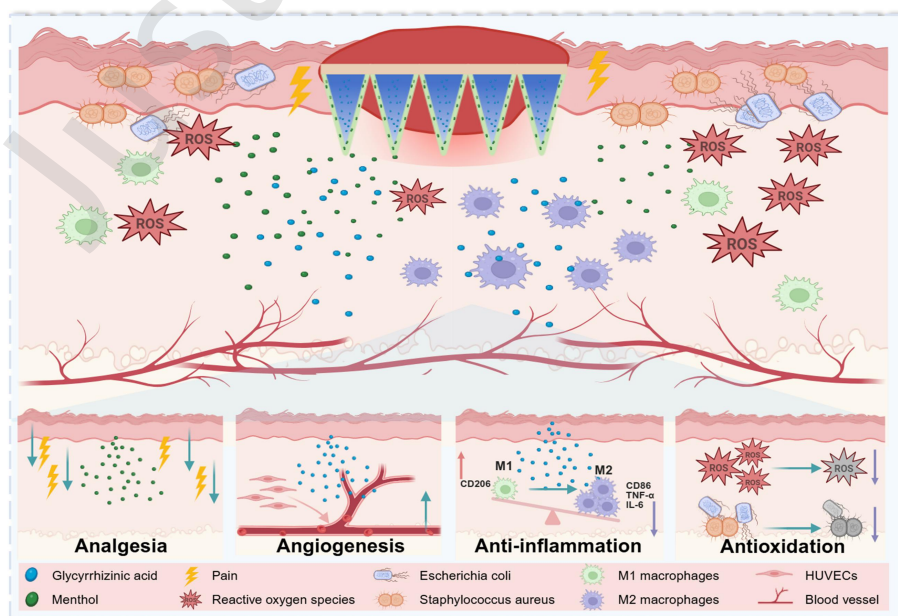


Figure 1 Schematic diagram of the multifunctional core-shell microneedles (MG-MNs) used for oral ulcer therapy. (created with BioRender.com)

2. Results and discussion

2.1 Preparation and characterization of MG-MNs

2.1.1 Morphological characterization of MG-MNs

We utilized GelMA/HA/HACC MNs (MNs) to encapsulate GA and Menthol, thereby fabricating the MG-MNs. The MG-MNs were prepared using a step casting method (**Figure 2a**), and its multifunctional core-shell structure was examined using optical microscopy, stereomicroscopy, and scanning electron microscopy (SEM). **Figure 2b** shows the overall appearance of the prepared MNs array, revealing intact MNs structures and a flat backing layer. The array consists of 15×15 needles (**Figure 2c**), each with a conical shape, a needle length of $700 \mu\text{m}$, a base diameter of $370 \mu\text{m}$, and an inter-needle spacing of $700 \mu\text{m}$ (**Figure S1a and S1b**). SEM images reveal that the MG-MN arrays are well-ordered and the tips are intact (**Figure 2d, 2e and 2f**). Furthermore, to visualize the core-shell structure, different fluorescent dyes were used for labeling: green for the HA shell at the needle tip and the HACC backing layer, and red for the GelMA core. Under a fluorescence microscope, a distinct core-shell architecture was clearly observed, with red fluorescence concentrated in the central region of the MNs representing the core, and green fluorescence distributed around the core representing the shell (**Figure 2g, 2h and 2i**). These intuitive visualization results confirm the successful preparation of the MG-MNs patch with a core-shell structure.

2.1.2 Comprehensive performance evaluation of MG-MNs

Excellent mechanical properties of the MNs are fundamental for penetrating the mucosa and delivering drugs to deep oral tissues. After application to rat oral mucosa and removal, the methylene blue-loaded MNs and MG-MNs left clear blue puncture marks on the mucosa surface (**Figure S2a**), with both types achieving a penetration rate of over 96% (**Figure S2b**). Hematoxylin and Eosin staining (H&E staining) of rat oral mucosa showed that the microneedle tips effectively penetrated the epithelial layer, creating conical microchannels with a penetration depth of $546 \mu\text{m}$ (**Figure S1c**). Due to the elasticity of the oral mucosa, compression deformation occurred during pressing, resulting in an actual penetration depth lower than the microneedle height ($700 \mu\text{m}$). In addition, Parafilm M® is also a commonly used *in vitro* model for verifying insertion depth[41]. **Figure S2c** shows that MG-MNs can penetrate at least about five layers of Parafilm M® ($635 \mu\text{m}$). After pressing MG-MNs into rat tongue mucosa for 5 minutes, the micropores were observed to gradually disappear within 20 minutes, and the mucosa recovered without obvious redness or ulceration (**Figure S3**). We conducted mechanical tests to measure the maximum force supported by the needle tip (N/MNs) (**Figure 2j**). The results showed that the maximum force before crushing of the MG-MNs patch was approximately 0.58 N , which falls within the typical force range ($0.1\text{--}3 \text{ N/needle}$) required for successful penetration

of the human stratum corneum[42].

To determine the drug release rate of MG-MNs, we measured the *in vitro* release behaviors using different methods. GA was detected by ultraviolet-visible spectrophotometer (UV-Vis), and Menthol was detected by gas chromatography (GC) at various time points. **Figure 2k** shows the release profile of GA, which was slowly released and reached about 80% after 7 days. This sustained release behavior is mainly attributed to the dense three-dimensional network structure formed by photo-crosslinked GelMA. **Figure 2l** shows that Menthol release reached 80% at 5 min and was almost complete after 20 min. This biphasic release pattern perfectly matches the design rationale of the core-shell microneedles. The HA shell rapidly releases Menthol for quick symptom relief, while the GelMA core ensures sustained GA release that continuously promotes tissue repair, inflammation resolution, and wound healing during ulcer progression.

The MNs should also possess favorable dissolution properties, which is crucial for drug release and ensuring biocompatibility. **Figure 2m** shows that after application to rat oral mucosa, the MG-MN tips gradually became blunt. After 30 min, they almost completely dissolved into a gel and infiltrated the skin. The remaining tip height was less than $150 \mu\text{m}$, and the residual part was mostly the HACC backing layer, with a final dissolution rate reaching up to 80% (**Figure 2n and 2o**).

The complex oral environment, involving muscle contraction and saliva flushing, poses a key challenge for maintaining effective microneedle fixation on the mucosal surface. We systematically compared the wet adhesion performance of MG-MNs with a commonly used commercial oral ulcer patch (Zizhu film®). In a simulated saliva flushing experiment, MG-MNs gradually detached from porcine oral mucosa when the flow rate reached 250 mL/min . The Zizhu film® detached at 100 mL/min (**Figure S4a**), and both values exceeded the normal adult salivary flow rate of 0.82 mL/min [43]. Compared with Zizhu film®, MG-MNs exhibited longer adhesion time and better resistance to higher flow flushing. Upon wet contact, the backing layer adhered tightly to the mucosa (**Figure S4b**), which is attributed to electrostatic interactions between HACC and mucosal tissues. Adhesion tests also revealed higher adhesion force of MG-MNs than Zizhu film® (**Figure S4c**), supporting effective drug retention at the mucosal site. In addition, rhodamine-loaded microneedles produced red fluorescence immediately upon insertion into porcine oral mucosa. The signal gradually diminished over time, yet the puncture sites were still clearly visible. After 48 h, almost no fluorescence signal was detectable in the insertion area (**Figure S5**). Overall, these results indicate that MG-MNs possess favorable wet adhesion properties, ensuring effective drug retention at the lesion site.

Collectively, the developed core-shell microneedles exhibit suitable mechanical strength, penetration capability, superior wet adhesion, and sequential drug release, representing an ideal platform for transmucosal drug delivery.

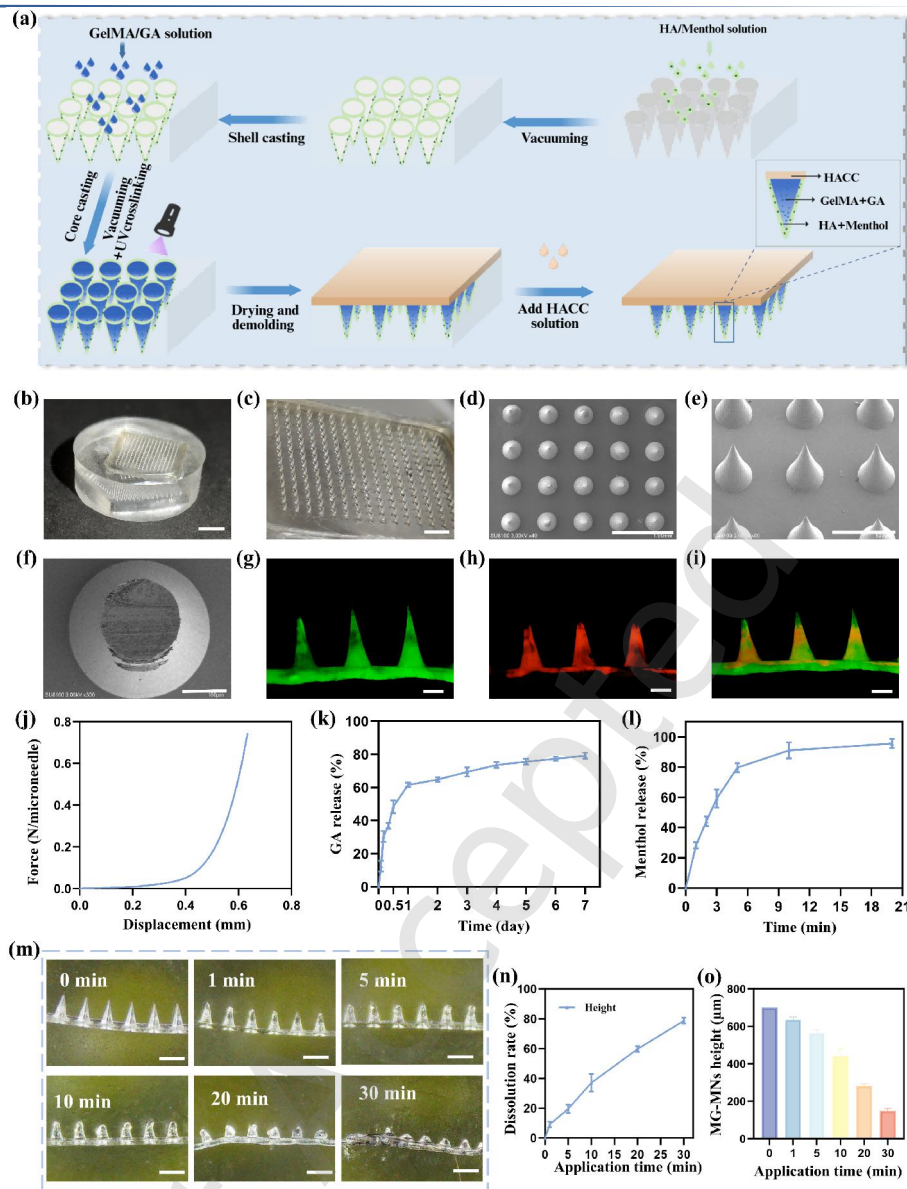


Figure 2 Synthesis and characterization of MG-MNs. (a) Schematic illustration of the preparation of MG-MNs. (b-c) Digital photographs of MG-MNs. (d-f) Scanning Electron Microscope (SEM) images of MG-MNs. (g-i) Fluorescence microscopy image of MG-MNs labeled with fluorescent microspheres (green fluorescence: shell layer and backing layer; red fluorescence: core layer). (j) Mechanical strength of MG-MNs. (k) Release profile of GA from MG-MNs. (l) Release profile of Menthol from MG-MNs. (m) Stereomicroscopic images of MG-MNs applied to rat oral mucosa at designated time points (0, 1, 5, 10, 20, and 30 min). (n) Dissolution profile of MG-MNs. (o) Residual tip height of MG-MNs over time ($n=3$). Scale bars: (b) 5 mm, (c) 2 mm, (d, m) 1 mm, (e) 500 μm , (f) 100 μm (g, h, i) 250 μm

2.2 *In vitro* biosafety assessment of MG-MNs

Biocompatibility is a critical factor for subsequent experiments and for evaluating whether the MNs can be safely applied in practice. We assessed the biosafety of MNs from different groups through *in vitro* studies, employing the CCK-8 assay and Live/Dead staining. Cell viability was examined using fluorescence microscopy after Calcein-AM and PI staining in the following groups. Untreated cells (Control), empty MNs (MNs), MNs loaded only with Menthol (M-MNs), MNs loaded only with GA (G-MNs), and the complete MNs co-loaded with GA and Menthol (MG-MNs). The results showed no alteration in cell morphology in any MNs-treated group, and no significant difference in cell viability was observed compared to the control group (Figure S6a and S6b), indicating that these MNs materials possess good cytocompatibility and low cytotoxicity. In the

CCK-8 assay, HUVEC cells treated with extraction media from the different MNs groups for 24 hours all exhibited cell viability rates above 95% (Figure S6c).

2.3 MG-MNs promote cell migration and vascular formation *in vitro*

During the healing process of oral ulcers, cell migration and angiogenesis play crucial roles. The migration of cells from the wound edge towards the center gradually covers the exposed wound, forming a new epithelial layer, sealing the wound, restoring barrier function, and promoting the healing of oral ulcers. The effect of the MNs on cell migration was assessed via a scratch assay. Compared to the control group, the MG-MNs and G-MNs groups showed a significant enhancement in cell migration ability (Figure 3a and 3c). Additionally, the formation of new blood vessels provides the essential oxygen and nutrients required for the healing of

oral ulcers[44]. The blood flow brought by neovascularization also helps remove metabolic waste and inflammatory mediators, creating favorable conditions for ulcer healing. The tube formation assay revealed a notable increase in intact tubular structures and branching points in

both the G-MNs and MG-MNs groups compared to the control (**Figure 3b and 3d**). This indicates that GA can effectively enhance angiogenesis. In summary, the MG-MNs group promotes both cell migration and angiogenesis, providing strong support for the healing of oral ulcers.

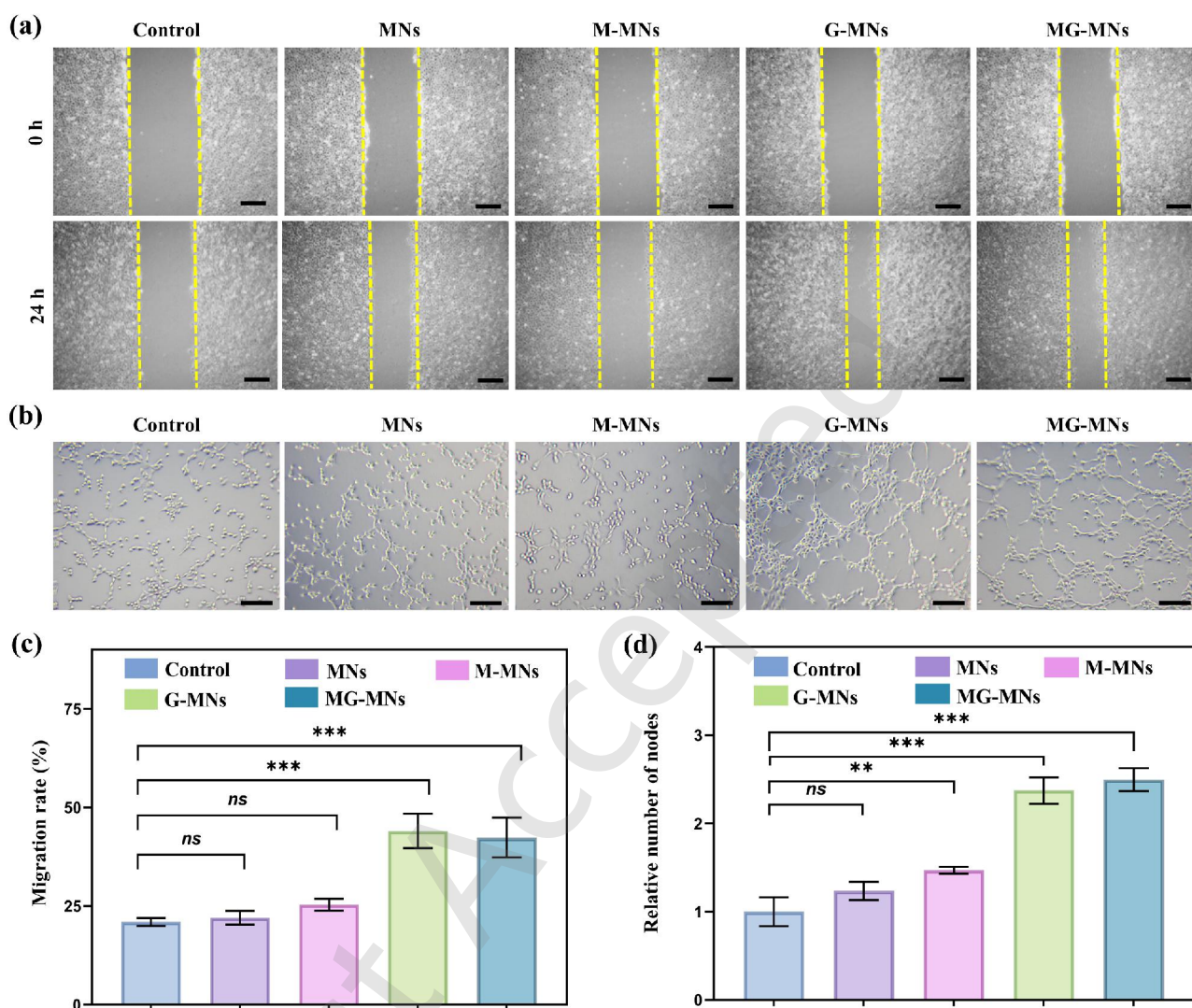


Figure 3 Evaluation of the *in vitro* migration capacity and angiogenesis-promoting capability of MG-MNs. (a) Representative images of the HUVECs migration (scratch) assay under different treatments. (b) Representative images showing the effects of different treatments on HUVECs tube formation. (c) Quantitative analysis of HUVECs migration. (d) Quantitative analysis of the tube formation assay. Each experiment was performed three times in repetition. Scale bar: (a) 100 μ m, (b) 300 μ m. ** P <0.01, *** P <0.001. ns: not significant.

2.4 Synergistic antibacterial effect of HACC and GA

It is widely recognized that bacterial infection is closely associated with the progression of oral ulcers[45]. To investigate the effectiveness of the MN patches against bacterial infection, this study conducted four main experiments focusing on *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*), and *Candida albicans* (*C. albicans*). Our research group had previously conducted antibacterial tests on HACC, confirming its broad-spectrum antimicrobial activity[46]. Results from the plate coating method showed a significant reduction in bacterial counts in the HACC-containing MNs and M-MNs compared to the control group. The G-MNs containing GA exhibited even fewer colonies. Notably, results for all three types of bacteria indicated that the MG-MNs group, containing both HACC and GA, possessed superior antibacterial capability compared to groups

containing HACC or GA alone (**Figure 4a and 4c**), suggesting a synergistic relationship in inhibiting bacterial growth and proliferation. SEM observations revealed pronounced abnormal morphology in the microscopic morphology of the bacteria in the experimental groups (**Figure 4b**). Subsequently, the zone of inhibition tests demonstrated that the diameters of the inhibition zones for the G-MNs and MG-MNs were larger than those of the control group or the group containing only HACC (**Figure S7a and S7b**). These experiments collectively confirmed that both HACC and GA exhibit significant inhibitory effects against the three common oral pathogens mentioned above. The MG-MNs, leveraging the dual action of GA and HACC, demonstrated a more pronounced antibacterial effect.

HACC, as a cationic quaternized chitosan derivative, exerts its antibacterial activity primarily through electrostatic interactions with negatively charged bacterial

membranes[47]. This interaction disrupts membrane integrity and leads to leakage of intracellular contents. GA has been reported to interfere with bacterial cell wall synthesis and to induce oxidative stress[48]. To explore their synergistic mechanism, intracellular reactive oxygen species (ROS) levels were measured using DCFH-DA in bacteria treated with different microneedle groups. Fluorescence imaging revealed no green signal in controls, but all treated

groups showed positive fluorescence, with MG- MNs exhibiting significantly higher ROS levels than others (**Figure S7c and S7d**). This suggests that HACC and GA can penetrate bacterial membranes and trigger oxidative stress. The resulting excessive ROS accumulation damages membrane structure and interferes with metabolism, ultimately leading to bacterial inactivation.

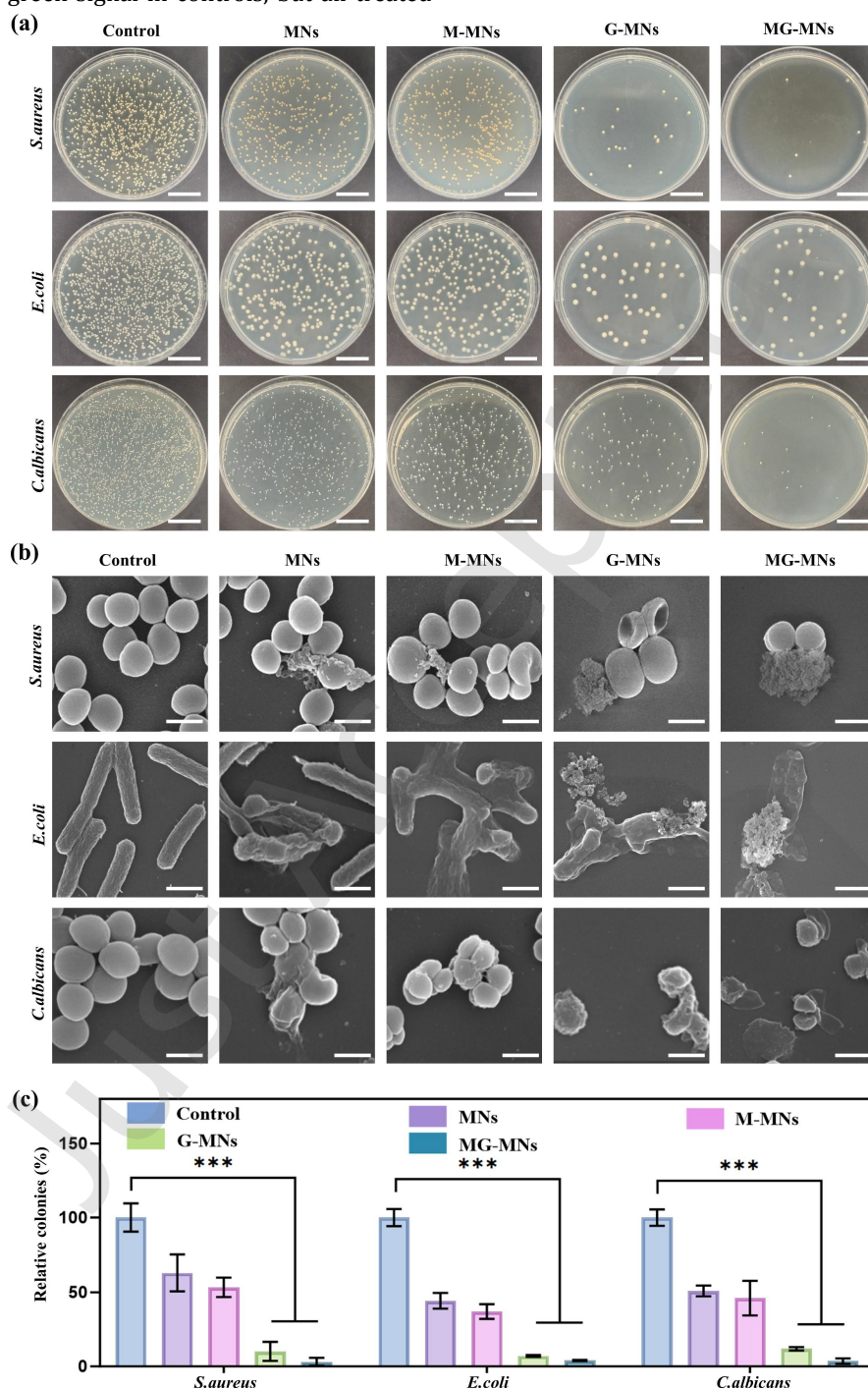


Figure 4 Evaluation of the antibacterial properties of MG-MNs. (a) Digital photographs of colony formation for *S. aureus*, *E. coli*, and *C. albicans* after different treatments on agar plates. (b) SEM images of *S. aureus*, *E. coli*, and *C. albicans* after different treatments. (c) Quantitative analysis of colony formation for *S. aureus*, *E. coli*, and *C. albicans* after different treatments. Each experiment was performed three times in repetition. Scale bars: (a) 2 cm, (b) 1 μ m, *** P <0.001.

2.5 Evaluation of the *in vivo* antioxidant and anti-inflammatory efficacy of MG-MNs

The pathological process of oral ulcers is often

accompanied by increased generation of ROS and an imbalance in oxidative stress, which can negatively impact cellular repair processes and consequently delay wound healing[49-51]. GA has been demonstrated to possess

antioxidative stress effects and has been used in the treatment of diseases such as hepatitis and diabetes[37, 52]. This study evaluated the intracellular ROS levels using the fluorescent probe DCFH-DA. We exposed RAW264.7 cells to 2 mM H_2O_2 to induce an oxidative stress state. The results showed that the H_2O_2 group exhibited intense green fluorescence, successfully modeling the oxidative stress condition (**Figure 5a**). Notably, in the groups treated with GA (G-MNs and MG-MNs), a distinct reduction in green fluorescence was observed, and the ROS levels were significantly decreased (**Figure 5b**). This precisely indicates that the MG-MNs help ameliorate the oxidative stress status at the oral ulcer site and promotes ulcer healing.

Elevated inflammation is a key contributing factor in oral ulcers. Therefore, demonstrating the ability of the MNs to inhibit pro-inflammatory factors is crucial for treating oral ulcers. This study investigated the effects of different treatments on the polarization of RAW 264.7 cells through immunofluorescence staining and quantitative analysis. Results from the CD86 assessment showed that following LPS stimulation, both the fluorescence intensity of CD86 and the proportion of CD86-positive cells increased significantly, indicating that LPS successfully induced macrophage polarization towards the M1 phenotype. Compared to the Control, MG-MNs exhibited markedly weakened CD86 fluorescence signals and a significant reduction in the proportion of CD86-positive cells (**Figure 5c and 5e**). Furthermore, MG-MNs showed significantly enhanced

CD206 fluorescence intensity and a substantially increased proportion of CD206-positive cells (**Figure 5d and 5f**). These results indicate that MG-MNs can effectively suppress macrophage polarization towards the pro-inflammatory M1 phenotype and promote their shift towards the anti-inflammatory M2 phenotype, thereby exerting an anti-inflammatory regulatory role.

NLRP3 serves as the core component of the most critical inflammasome in the pyroptosis pathway. Damage to the oral mucosal epithelial barrier leads to the release of danger signals such as ATP and ROS, which are recognized by macrophages, triggering the assembly of the NLRP3 inflammasome. This subsequently activates Caspase-1, a key executioner protease in pyroptosis. Activated Caspase-1 not only cleaves the pro-forms of IL-1 β and IL-18 but also cleaves GSDMD, inducing pyroptosis. This process results in the massive release of inflammatory factors, exacerbating tissue edema and pain at the ulcer site[53, 54]. The effect of different MNs treatment groups on the protein expression levels of NLRP3, Caspase-1, and GSDMD-N in LPS-induced macrophages was assessed by Western Blot. The results demonstrated that LPS stimulation significantly upregulated the protein expression levels of NLRP3, Caspase-1, and GSDMD-N compared to the control group (**Figure 5g**). In contrast, treatment with G-MNs and MG-MNs markedly reduced the protein expression levels of NLRP3, Caspase-1, and GSDMD-N (**Figure S8**).

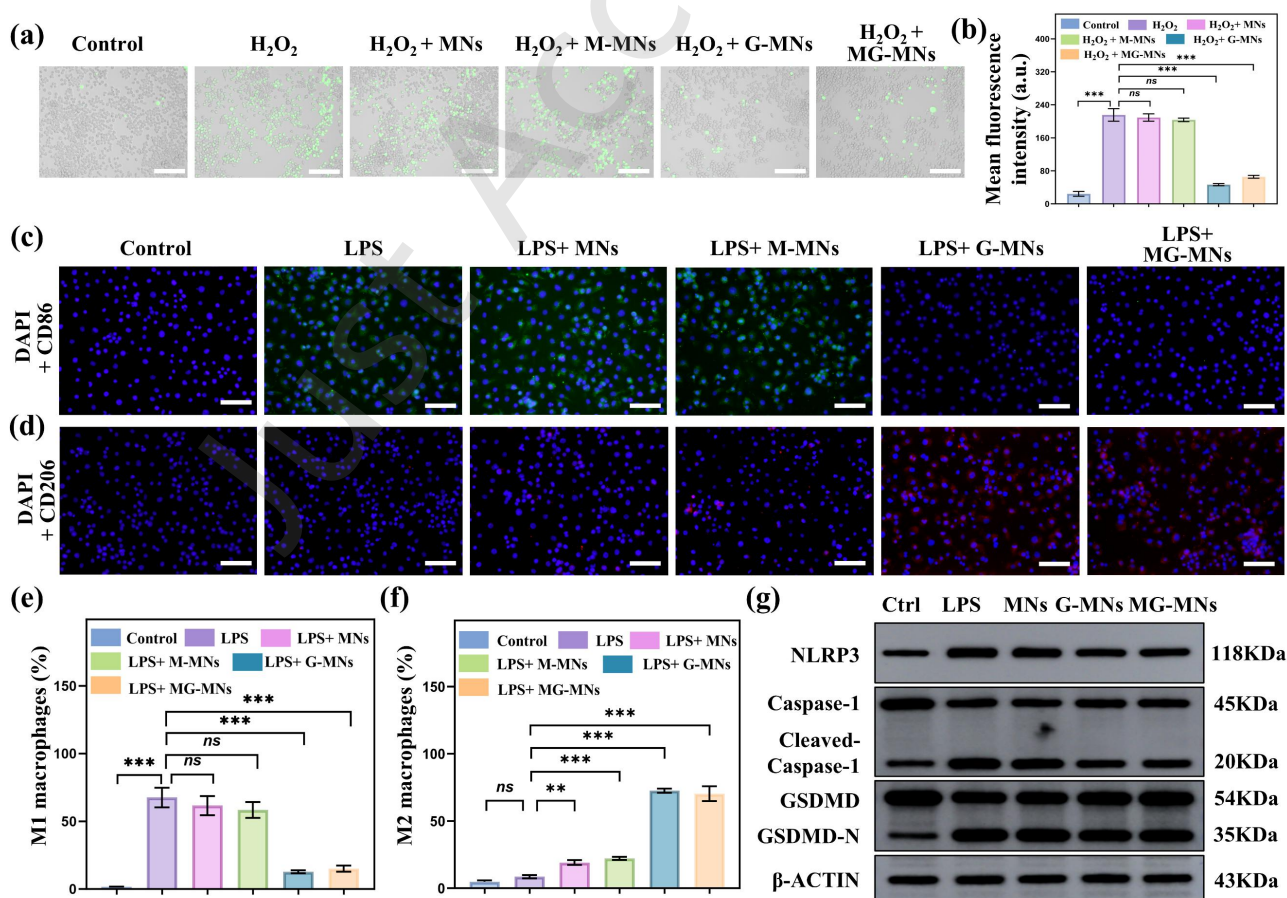


Figure 5 Evaluation of *in vitro* antioxidant and anti-inflammatory capacity of MG-MNs. (a) Fluorescence images indicating intracellular Reactive Oxygen Species (ROS) levels in RAW264.7 cells. (b) Quantitative analysis of intracellular ROS levels in RAW264.7 cells. (c) Immunofluorescence staining images showing CD86 (green) and DAPI (blue) in RAW264.7 cells after different treatments. (d) Immunofluorescence staining images showing

DAPI (blue) and CD206 (red) in RAW264.7 cells after different treatments. (e) Quantitative analysis of M1 macrophage polarization in RAW264.7 cells (f) Quantitative analysis of M2 macrophage polarization in RAW264.7 cells. (g) Western blot analysis of NLRP3, Caspase-1, and GSDMD protein expression in different treatment groups. Each experiment was performed three times in repetition. Scale bar: 100 μm . ** $P < 0.01$, *** $P < 0.001$, ns: not significant.

These findings indicate that MG-MNs can effectively suppress the expression of pyroptosis-related proteins in LPS-induced macrophages, thereby alleviating the inflammatory response in oral ulcers.

2.6 Animal experiments

2.6.1 Validation of the analgesic effect of Menthol

This study employed the water bath method to simulate the burning pain associated with oral ulcer episodes, aiming to validate the analgesic effect of Menthol[55]. To avoid false-positive pain responses caused by male rodent testes contacting the heat source, female mice were selected for the experiment (**Figure S9a**). The tail-flick latency in the control group and experimental groups without Menthol was approximately 3-5 seconds. In contrast, groups treated with Menthol or lidocaine cream exhibited prolonged tail-flick latencies, extending to around 20 seconds (**Figure S9c**). Three minutes after administration, the percentage of maximum possible effect (%MPE) in the Menthol-containing MNs groups exceeded 60%, demonstrating an immediate analgesic effect (**Figure S9d**). This result aligns with our initial design principle for the core-shell structure, wherein

the rapid dissolution of the shell enables prioritized release of Menthol to achieve immediate pain relief.

2.6.2 MG-MNs effectively promotes oral ulcer healing

We validated the ability of MG-MNs to promote oral ulcer healing by establishing a rat oral ulcer model. Rats were first randomly divided into six groups: Control, MNs, M-MNs, G-MNs, MG-MNs, and Dexamethasone ointment (DXMS). After model establishment, the animals were observed for five consecutive days, with regular photographs taken following daily MNs treatments (**Figure 6a**). Results showed that 24 hours post-modeling, distinct ulcers accompanied by yellow-white exudate appeared in the experimental areas, confirming successful model establishment (**Figure S9b**). After five days, compared to the untreated Control group, both the MNs and M-MNs groups showed significant improvement in ulcer conditions. However, rats treated with G-MNs and MG-MNs exhibited faster ulcer healing rates, second only to the group treated with the commercially available dexamethasone ointment (**Figure 6b and S10**). The MG-MNs group achieved an 80% healing rate, reducing the ulcer area to 20%, demonstrating excellent healing-promoting efficacy (**Figure 6c and 6d**).

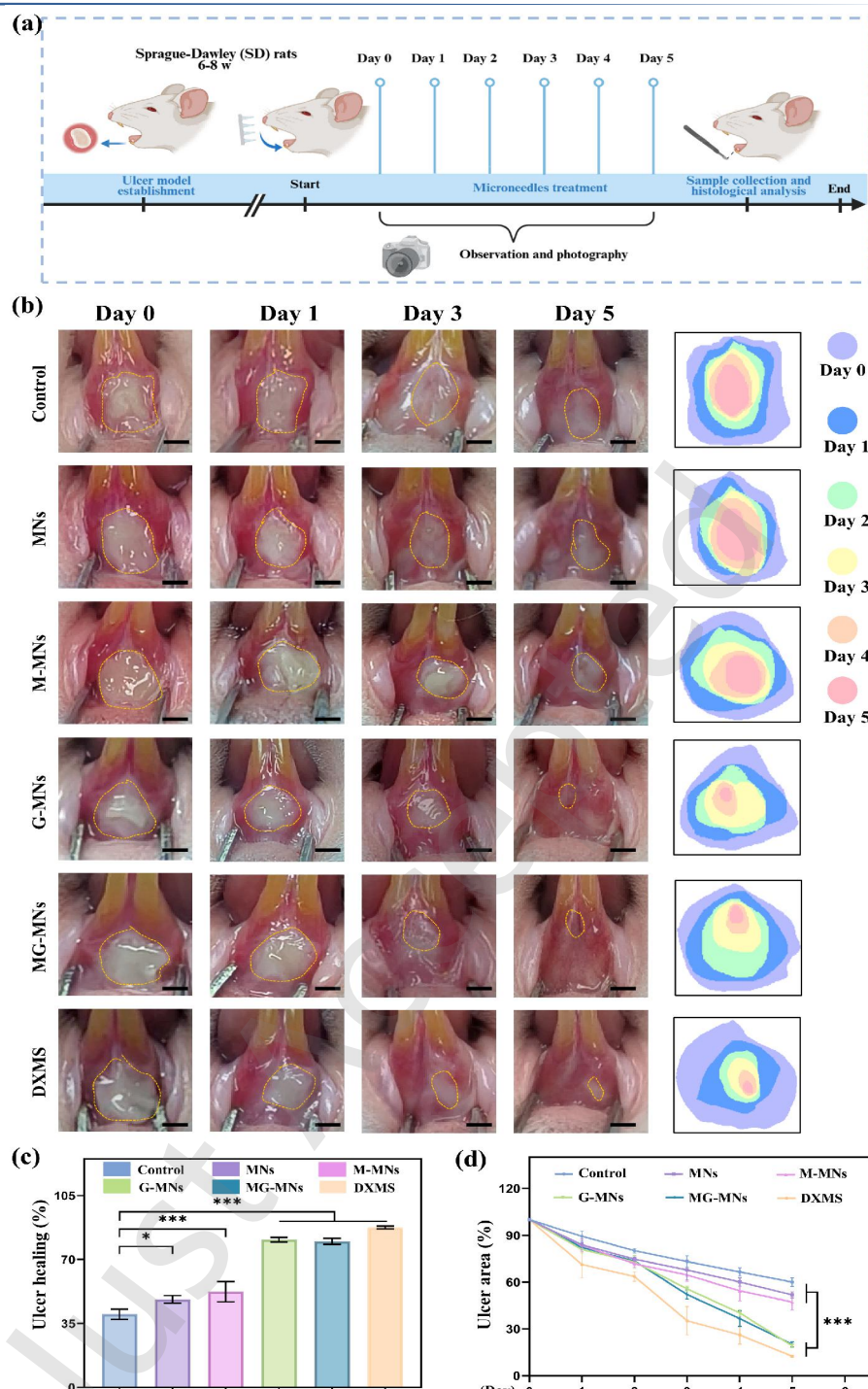


Figure 6 Assessment of oral ulcer healing in rats following treatment. (a) Schematic diagram of oral ulcer model establishment and microneedle drug administration. (b) Representative images of rat oral ulcers at day 5 post-treatment. (c) Ulcer healing rate in rats at day 5 post-treatment. (d) Percentage of ulcer area remaining in rats at day 5 post-treatment (n=6). Scale bar: 2 mm. * $P < 0.05$, *** $P < 0.001$, ns: not significant.

Histological H&E staining revealed marked differences. The ulcer areas in the G-MNs and MG-MNs groups, loaded with GA, maintained integrity across all tissue layers, exhibited significantly thickened epithelium, and displayed reduced inflammatory response, indicating superior performance in promoting epithelial regeneration (**Figure 7a**). Masson's trichrome staining results showed that the ulcer tissues treated with G-MNs and MG-MNs exhibited significantly higher levels of collagen deposition-related indicators compared to the control group (**Figure 7b and**

7d). This indicates that the finished MNs groups can effectively promote collagen deposition in the ulcer area, providing a solid foundation for tissue repair.

Reduced inflammation is a key indicator of healing progression. To evaluate healing progression, the inflammatory response was analyzed by immunohistochemical staining. We observed that the expression levels of TNF- α and IL-6 were significantly lower in all treatment groups relative to the control, with the groups containing GA achieving effects comparable to the DXMS group (**Figure 7c and 7e**).

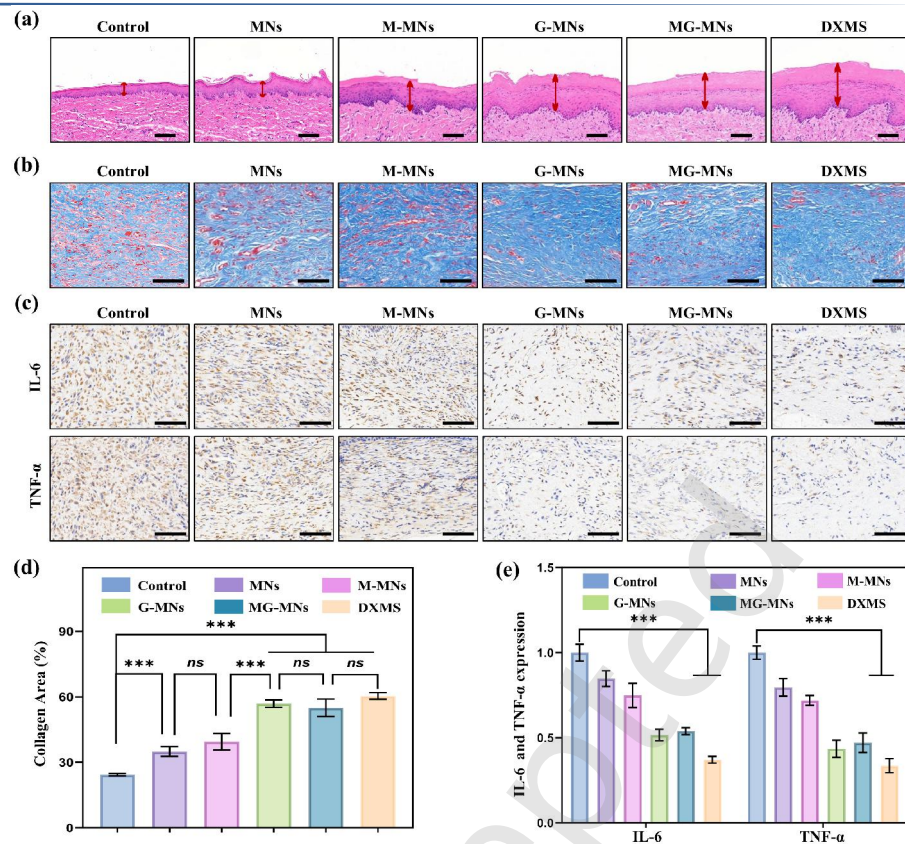


Figure 7 Characterization of collagen deposition and inflammatory factors. (a) Representative H&E-stained images of oral ulcer tissues from different treatment groups at day 5 post-treatment. (b) Masson's trichrome staining showing collagen deposition. (c) Representative immunohistochemical (IHC) staining images of interleukin-6 (IL-6) and tumor necrosis factor α (TNF- α) expression. (d) Quantitative analysis of the collagen area fraction. (e) Quantitative analysis of IL-6 and TNF- α expression levels (n=6). Scale bar: 100 μ m, *** P <0.001, ns: not significant.

Macrophages play a pivotal role in the inflammatory mechanism by secreting a series of inflammatory factors to regulate the immune response. Their polarization state determines the tissue fate. M2 macrophages highly express CD206 and produce IL-10 and TGF- β , promoting angiogenesis and collagen deposition, thereby accelerating mucosal repair. We utilized F4/80 (green) and CD206 (red) immunofluorescence staining to analyze macrophage phenotype switching following different groups treatment. The results showed that compared to the control group, MNs and M-MNs groups had a marked increase in CD206-positive cells (**Figure 8a**). The groups containing GA showed a higher

M2 polarization rate (**Figure 8c**), indicating that GA can effectively modulate the polarization direction of macrophages, thereby further reducing the inflammatory response in oral ulcers and promoting ulcer healing.

The formation of new blood vessels supplies adequate oxygen and nutrients to the ulcer tissue, promotes cell proliferation and migration, and accelerates the healing process. Here, we used fluorescence staining to observe the expression of vascular markers (CD31 and α -SMA). Fluorescence staining results demonstrated that the MG-MNs treatment group exhibited superior angiogenesis effects compared to the control group (**Figure 8b and 8d**).

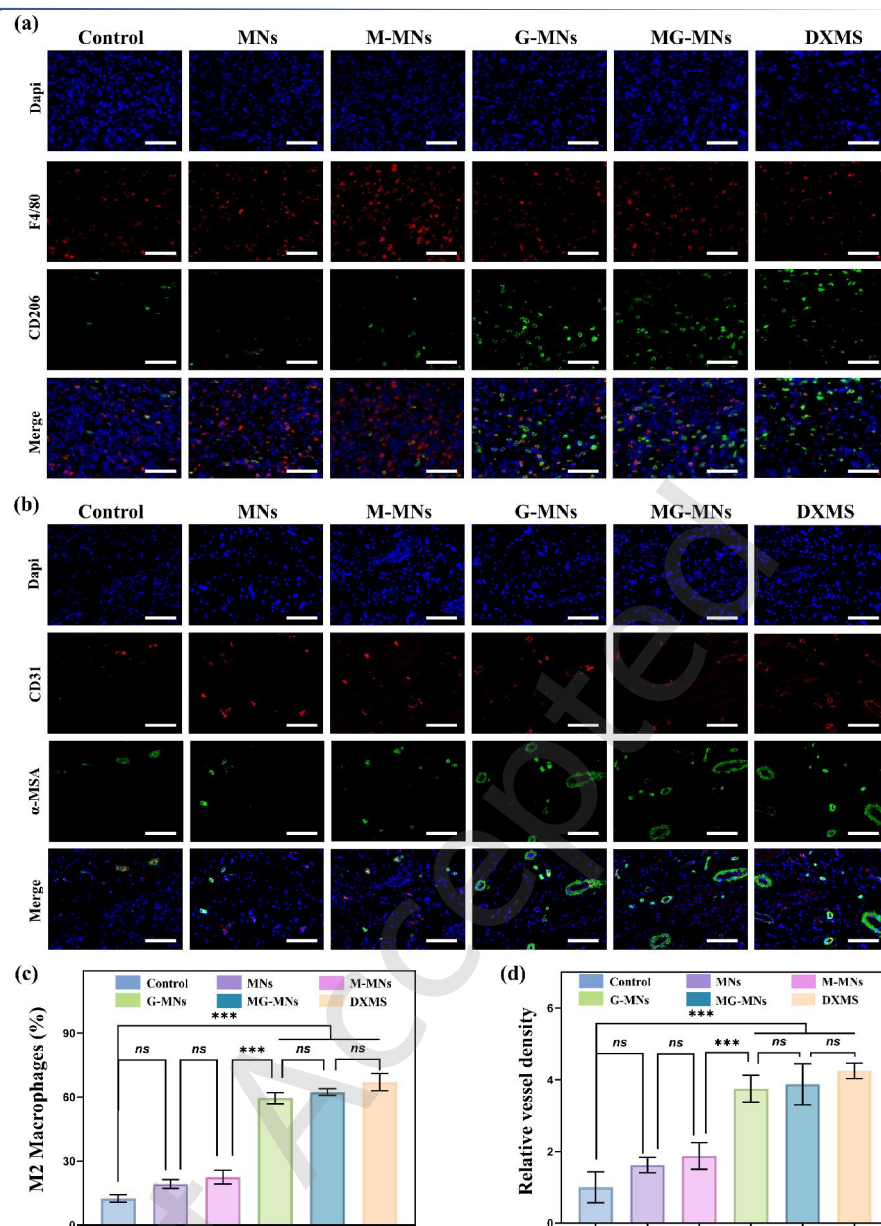


Figure 8 Characterization of angiogenesis and macrophage polarization in oral ulcer tissues. (a) Immunofluorescence staining images showing F4/80 (red), CD206 (green), and DAPI (blue). (b) Immunofluorescence staining images showing CD31 (red), α-SMA (green), and DAPI (blue). (c) Quantitative analysis of M2 macrophages. (d) Quantitative analysis of neovessel formation (n=6). Scale bar: 100 μm. *** $P < 0.001$, ns: not significant.

We also investigated the *in vivo* biotoxicity of the different groups. H&E staining was performed on five representative organs (heart, liver, spleen, lung, and kidney) on day 5 after ulcer treatment in rats to investigate potential organ abnormalities. The results revealed no significant organ damage in any treatment group (**Figure S11a**). Furthermore, results from the hemolysis test demonstrated that none of the MNs groups induced hemolysis, indicating favorable hemocompatibility of the materials (**Figure S11b**).

In conclusion, MG-MNs not only promotes collagen deposition and angiogenesis but also modulates the immune response and reduces inflammation levels, providing a comprehensive and effective solution for oral ulcer therapy.

3. Materials and methods

3.1 Materials

The reagents and consumables used in this study were procured as follows: Hyaluronic Acid (HA) was obtained from Bloomage Biotech; Gelatin Methacryloyl (GelMA) was sourced from EFL; Hydroxypropyltrimonium Ammonium Chitosan (HACC) was purchased from Aladdin; Polydimethylsiloxane (PDMS) was acquired from Shiling Laker Mold Merchant; Menthol and Glycyrrhizic Acid (GA) were procured from Shanghai Yuanye Bio-Technology; Calcein-AM/PI Cell Viability/Cytotoxicity Assay Kit, Reactive Oxygen Species Assay Kit, and Cell Counting Kit-8 (CCK-8) were purchased from Beyotime Biotechnology; Fetal Bovine Serum (FBS) was obtained from Nanjing Keygen Biotech; F-12/DMEM and DMEM/High Glucose media were sourced from HyClone; Phosphate Buffered Saline (PBS) and Lipopolysaccharide (LPS) were purchased from Biosharp; Matrigel was purchased from CORNING; The oral simulant fluid (pH 7) was purchased from Feijing Biotechnology Co., Ltd (composed of magnesium chloride hexahydrate, sodium bicarbonate, calcium chloride, and other substances).

3.2 Cell culture and animals

HUVEC and RAW264.7 cells were purchased from the Xiangya Central Laboratory (Changsha, China). HUVECs were cultured in F-12/DMEM medium supplemented with 10% fetal bovine serum, while murine RAW264.7 cells were cultured in high-glucose DMEM supplemented with 10% fetal bovine serum (FBS) and maintained at 37°C under a humidified 5% CO₂ atmosphere. Six- to eight-week-old Sprague-Dawley (SD) rats and eight-week-old C57 mice (weighing 17-19 g) were supplied by Hunan Silaike Jingda Experimental Animal Co., Ltd. (Changsha, China) and housed under specific pathogen-free conditions. All animal experiments were approved by the Animal Ethics Committee of the Second Xiangya Hospital (approval No: 20260047).

3.3 Fabrication of MG-MNs

Core-shell polydimethylsiloxane (PDMS) molds were purchased from Shiling Laker Mold Merchant (Guangzhou, China). These molds consist of a PDMS negative mold and a corresponding PDMS positive mold. The MG-MNs were fabricated using a step casting method. First, HA powder was dissolved in PBS to prepare a 10% (w/v) solution. Menthol was added to this solution, and the mixture was centrifuged to obtain a homogeneous 10% (w/v) HA/Menthol mixture. This mixture was then injected into the cavities of the PDMS negative mold. The PDMS mold was placed in a vacuum desiccator and subjected to a negative pressure of -0.08 MPa for 10 min to ensure complete filling of the precursor solution into the microcavities. Subsequently, the matching PDMS positive mold was pressed into the negative mold, ensuring proper alignment of the cavities, and excess material was removed. The assembly was placed in a drying oven at 37°C for 2-3 hours. The shell structure was obtained after demolding.

Next, solid GelMA powder was dissolved in PBS to prepare a 15% (w/v) solution. The photoinitiator LAP (0.25% w/v) and GA were added to this solution. After centrifugation, the mixture was injected into the remaining voids of the PDMS mold. Bubbles were removed using a vacuum pump under negative pressure (-0.08 Mpa) for 2-3 minutes, and excess liquid on the back of the MNs array was scraped off. The construct was then exposed to UV light for 60 seconds for cross-linking. Finally, a pre-prepared 8% (w/v) HACC gel was applied to cover the base, ensuring that the amount injected reached the highest level of the mold each time. After air-drying the entire construct, it was stored overnight at room temperature. The drug-loaded GelMA/HA/HACC core-shell MNs (MG-MNs) were carefully demolded for subsequent analysis.

3.4 Performance testing of MG-MNs

Morphological characterization: the morphological characteristics of the MG-MNs were observed using optical microscopy, stereomicroscopy, and SEM. To visualize the core-shell structure, fluorescent MG-MNs were prepared by incorporating green fluorescent microspheres into the HA shell layer and the HACC backing layer, and red fluorescent microspheres into the GelMA core layer. The samples were then imaged using fluorescence microscopy.

Penetration test: for the rat oral mucosa penetration experiment, methylene blue-loaded microneedles were

applied to rat oral mucosa and pressed for 5 min, then removed. After removal, puncture sites were counted. For the *in vitro* Parafilm M® penetration depth test, Parafilm M® was folded into 6 layers (each layer 127 μm). After microneedle insertion, the Parafilm M® was unfolded and the number of puncture holes on each layer was counted. Both tests were calculated using the following formula.

$$\text{Penetration efficiency (\%)} = N_1 \div N_0 \times 100\% \quad (1)$$

In this content, N1 represents the number of puncture sites, and N0 represents the original number of microneedle tips.

Mechanical properties test: the mechanical properties of the MNs patch were tested using a force gauge (CMT6103, MTS Industrial Systems, China). The MNs patch was fixed on a horizontal platform with the needle tips facing upwards. A probe moved vertically downward at a constant speed of 0.1 mm/minute. The maximum supporting force per needle tip (N/microneedle) was calculated by dividing the measured force value by the number of needle tips.

Dissolution characteristics test: MG-MNs were applied to rat oral mucosa. At specific time points (0 s, 1, 5, 10, 20, 30 min), the MNs were removed, observed, and photographed. Residual tip height was measured using ImageJ software.

$$\text{Dissolution rate (\%)} = (H_0 - H) / H_0 \times 100\% \quad (2)$$

where H₀ is the initial tip height and H is the residual height at time. The kinetic curve was then plotted.

Dynamic adhesion stability test: porcine oral mucosa was wetted with oral simulant fluid to simulate the moist oral environment[56]. MG-MNs and Zizhu film® were then applied onto the flat mucosal surface and pressed for 3 min. Continuous flushing with flowing water was initiated at 50 mL/min, with the flow rate increased by 50 mL/min every 30 s. The failure criteria were defined as edge curling, breakage, or detachment. The flow rate and status at failure were recorded.

Adhesion strength measurement: two pieces of porcine buccal mucosa (20 mm × 80 mm) were prepared. The mucosal surfaces were pre-wetted with oral simulant fluid. Each sample (MG-MNs or Zizhu film®) was placed between the two mucosa pieces, forming a 5 mm × 5 mm adhesion area (A). The maximum failure load (F_{max}) was measured using a lap shear test. Adhesion strength (kPa) was calculated as:

$$\text{Adhesion strength (kPa)} = F_{\text{max}} \div A \quad (3)$$

where F_{max} = maximum failure load (N), and A = adhesion area (mm²).

Drug retention test: rhodamine was used as a model drug to evaluate the retention time of MG-MN-loaded drugs in porcine oral mucosa[57]. MG-MNs were inserted into the mucosa and removed after 5 min. Fluorescence retention at the insertion sites was continuously observed under a fluorescence microscope.

3.5 In vitro drug release test

The microneedles were immersed in oral simulant fluid and shaken at 200 rpm and 37°C. At predetermined time intervals, 100 μL aliquots were withdrawn and replenished with equal pre-warmed fluid. After filtration and centrifugation, supernatants were assayed: Glycyrrhizic acid release was measured by ultraviolet-visible

spectrophotometry (Thermo Fisher Scientific) at 254 nm. Menthol release was determined by gas chromatography (GC). Release curves were constructed. All release experiments were performed in triplicate.

3.6 Cytotoxicity assay

First, MNs, M-MNs, G-MNs, and MG-MNs were dissolved in DMEM medium containing 10% fetal bovine serum (FBS) (1 ml) and filtered for subsequent use. Unless otherwise specified, all cell culture experiments in this study utilized extraction media (or PBS solutions) obtained using this method with the corresponding base medium. HUVECs were seeded in 96-well plates (3000 cells/well) and exposed to the different extraction media for 24 hours. Cell viability was detected using the CCK-8 reagent. For Live/Dead staining, cells were fluorescently labeled with Calcein-AM and propidium iodide (PI) mixed at a 1:1000 ratio in buffer, and fluorescence images were acquired using an inverted fluorescence microscope.

3.7 Hemolysis assay

Blood samples were collected from the periorbital venous plexus of Sprague-Dawley (SD) rats. Fresh blood (1 mL) was gently defibrinated by stirring. It was then centrifuged at 2000 rpm for 5 minutes, and the supernatant was discarded. The resulting red blood cells (RBCs) were washed three times with PBS to obtain the packed RBCs. A 100 μ L aliquot of the packed RBCs was mixed with 5 mL of PBS. Subsequently, ultrapure water, PBS, and different MNs extraction media were added to separate aliquots of this RBC suspension. After incubation at 37°C for 3 hours, the samples were examined and photographed.

3.8 Scratch assay

HUVECs were seeded into 6-well plates. When the cell density reached approximately 90%, a linear scratch wound was created in the center of each well. The cells were then washed three times with PBS. Subsequently, the cells were incubated for 24 hours in either normal serum-free medium (Control group) or in the respective extraction media (prepared in serum-free medium).

3.9 Tube formation assay

The procedure was as follows: Extracellular matrix gel was diluted using serum-free DMEM on ice. Then, 50 μ L of the mixture was added to a 96-well plate and incubated at 37°C with 5% CO₂ for 1 hour to allow the gel to solidify. Subsequently, 100 μ L of HUVEC cell suspension (3 $\times$ 10⁵ cells/mL) from the control or each MNs extraction media groups was added onto the prepared gel. After incubation for 4-6 hours, the formation of tubular structures was observed under a microscope.

3.10 In vitro antibacterial testing

The antibacterial activity of the MNs were evaluated using various methods against *E. coli* (ATCC 25922), *S. aureus* (ATCC 25923), and *C. albicans* (ATCC 90028). In the agar plate method, a specified quantity of *E. coli*, *S. aureus*, and *C. albicans* was first inoculated into LB broth and cultured for 6 hours. The bacterial suspensions were then co-cultured

in different microneedle extracts for 24 hours. The mixtures were evenly spread on culture plates, incubated statically for 12 hours, and then photographed and determined the bacterial inhibition rate. In the inhibition zone assay, a specified volume of bacterial suspension was first evenly spread over the surface of a solid agar plate using a sterile spreader. Wells were then punched into the agar surface. Subsequently, 200 μ L of microneedle extract solution, along with PBS as the control solvent, was added into each well. After 24 h of incubation, images were taken and the diameters of the inhibition zones were measured; Bacterial suspensions were co-incubated with the various extraction media for 12 hours. The mixtures were then added to 6-well plates containing sterile coverslips, and incubation continued for over 12 hours. Subsequently, the coverslips with attached bacteria were fixed with 2.5% glutaraldehyde solution for 8 hours, washed with PBS, dehydrated through an ethanol gradient, immersed in tert-butanol, and finally mounted. The bacterial samples were imaged using a SEM.

The procedure for measuring intracellular Reactive oxygen species (ROS) levels in bacteria was as follows. Bacterial suspensions were diluted to 1 $\times$ 10⁶ CFU/mL. The diluted bacterial solutions were then co-incubated with extracts of different microneedle groups at 37°C for 6 h with shaking. After incubation, the bacteria were harvested by centrifugation, and the supernatant was discarded. The bacterial pellets were washed twice with sterile PBS, resuspended in PBS containing 10 μ M DCFH-DA, and incubated in the dark at 37°C for 30 min. The bacteria were then washed three times with PBS. ROS levels were observed under a fluorescence microscope.

3.11 Reactive oxygen species (ROS) scavenging assay

The ROS-sensitive fluorescent probe DCFH-DA was used to quantify intracellular ROS levels. RAW 264.7 cells were seeded in a 24-well plate. After 24 hours, the cells were treated with 2 mM hydrogen peroxide (H₂O₂) in the dark environment for 30 minutes, with untreated cells serving as the control group. Subsequently, the supernatant was removed, and the cells were washed with PBS. The cells were then further treated with different MNs extraction media for 24 hours. After discarding the supernatant, serum-free high-glucose DMEM medium containing DCFH-DA was added, followed by incubation in the dark for 30 minutes. The cells were then washed three times with serum-free DMEM. Finally, the fluorescent signals of the cells were observed using a fluorescence microscope, and the mean fluorescence intensity was quantitatively analyzed using ImageJ software.

3.12 Immunofluorescence analysis

For the immunofluorescence analysis, RAW 264.7 cells were seeded in 24-well plates. The cells were first stimulated with 1 μ g/mL LPS to induce polarization towards the M1 phenotype, followed by treatment with MNs extraction media for 24 hours. Subsequently, the cells were fixed with 4% paraformaldehyde solution for 30 minutes, washed three times with PBS buffer, and blocked with 4% bovine serum albumin (BSA) for 1 hour. The cells were then incubated overnight at 4°C with anti-CD86 antibody and anti-CD206 antibody. After incubation, the cells were

washed three times with PBST buffer, followed by incubation with fluorescent secondary antibodies at room temperature in the dark. Finally, the nuclei were stained with DAPI, and the cells were washed three times with PBST. The stained cells were observed and analyzed using a fluorescence microscope.

3.13 Western blotting analysis

RAW 264.7 cells were seeded in 6-well plates and cocultured with MNs extraction media and LPS (1 µg/mL) for 24 hours. Cells from the 6-well plates were then harvested for Western blotting analysis to detect the expression of inflammation-related signaling proteins. After protein quantification, the samples were loaded onto a 10% sodium dodecyl sulfate-polyacrylamide gel (SDS-PAGE) (Servicebio, China) and subsequently transferred onto PVDF membranes. The membranes were blocked with 5% skim milk and then incubated sequentially with primary and secondary antibodies[58]. The primary antibodies used included those against: NLRP3, GSDMD full length, GSDMD-N terminal, Caspase-1, and Cleaved Caspase-1. After incubation, the blots were washed with TBST and visualized using an enhanced chemiluminescence detection system.

3.14 Establishment of the *in vivo* ulcer oral model

Thirty-six male rats were anesthetized using isoflurane. An oral ulcer model was established on the lower lip mucosa by applying a 50% glacial acetic acid chemical burn. The rats were then randomly divided into six groups (n=6 per group): Control (receiving no treatment), MNs, M-MNs, G-MNs, MG-MNs (all receiving respective MNs treatments), and a DXMS group (treated with dexamethasone ointment). Treatment began on the first day after ulcer induction. For the MNs groups, the patches were cut to an appropriate size, adhered to the ulcer surface, and gently pressed for 3 minutes to achieve tight attachment. In the positive control DXMS group, dexamethasone ointment was applied evenly to the ulcer surface. Treatments were administered consecutively for 6 days. Ulcer changes were recorded daily, and wound area and oral ulcer healing rate were measured from day 1 to day 6 using ImageJ software. The ulcer healing rate and relative ulcer area were calculated.

$$\text{Ulcer Healing Rate (\%)} = (A_0 - A_t) \div A_0 \times 100\% \quad (4)$$

where A_0 = initial ulcer area at Day 0, and A_t = ulcer area at the indicated time point.

$$\text{Ulcer Area (\%)} = A_t \div A_0 \times 100\% \quad (5)$$

where A_0 = initial ulcer area at Day 0, and A_t = ulcer area at the indicated time point.

3.15 Analgesia experiment

The analgesic effect was evaluated using the mouse tail-flick test, conducted via a water bath method as described in the literature[55]. C57 mice were restrained on a surgical platform with their tails exposed. The MNs patches from different groups were applied and pressed onto the mouse tails to ensure firm adhesion. The positive control group received lidocaine cream applied topically to the tail. After an adaptation period, the tail was immersed in a 45 °C water bath, and the latency time was recorded. The response was measured at specific time points (0, 2, 5, 6, and 7 minutes; n = 6) after 3 minutes of MNs or lidocaine cream application.

The timing was stopped immediately when the mouse exhibited a vigorous tail-flick or withdrew its tail from the water. Data are expressed as the percentage of maximum possible effect (%MPE).

$$\%MPE = [(T_1 - T_0)] \div [(T_c - T_0)] \times 100\% \quad (6)$$

where T_1 is the latency after drug administration, T_0 is the baseline latency before treatment, and T_c is the cutoff time set to prevent tissue damage.

3.16 Histological analysis

On day 5, the experimental rats were anesthetized and subsequently euthanized. Oral ulcer tissue samples were collected and fixed in paraformaldehyde. The samples were then dehydrated through a graded ethanol series, embedded in paraffin, and sectioned. After deparaffinization in xylene and rehydration through a descending ethanol series, the sections were subjected to H&E staining and Masson's trichrome staining to evaluate the healing status of the lesion area and collagen deposition. Three staining protocols were employed: immunohistochemistry to detect TNF-α and IL-6 expression; immunofluorescence to evaluate angiogenesis (via CD31 and α-SMA) and macrophage polarization (via F4/80 and CD206); and H&E staining of major organs (heart, liver, spleen, lungs, kidneys) to assess potential *in vivo* toxicity.

3.17 Statistical analysis

Data were obtained from at least three independent experiments and are presented as the mean ± standard deviation (SD). Statistical analysis in this study was performed using GraphPad Prism (GraphPad Prism version 10.1.2) and ImageJ software with unpaired two-tailed Student's t-tests for pairwise comparisons and one-way ANOVA for significance testing. Statistical significance was defined as nonsignificant (*ns*) for $P > 0.05$, $*P < 0.05$, $**P < 0.01$, and $***P < 0.001$.

4. Conclusion

In summary, this study has developed a multifunctional core-shell microneedles (MG-MNs) that significantly promotes oral ulcer healing. MNs can effectively penetrate the mucosal barrier to directly deliver drugs into deep lesion sites, thus enhancing drug bioavailability while reducing discomfort during treatment. The designed MG-MNs leverages the differential dissolution properties of the core-shell structure to achieve sequential drug release, thereby providing dual efficacy of rapid pain relief and long-term tissue repair. This release pattern better matches the pathological progression of oral ulcers. The HA-based shell serves to rapidly release Menthol upon dissolution, alleviating acute pain. The GelMA core encapsulates GA and supports ulcer tissue repair and regeneration through multiple pathways. These include facilitating the polarization of macrophages toward a pro-repair phenotype, accelerating cell migration and angiogenesis, and exerting antioxidant activity. The HACC backing layer adheres firmly to the ulcer surface and exerts synergistic antibacterial effects with GA, thereby creating a favorable microenvironment for ulcer healing. Furthermore, MG-MNs demonstrates excellent biosafety and mechanical properties,

indicating significant potential for clinical application in oral ulcer treatment. Both the design concept and experimental findings of this study can provide novel insights and experimental evidence for future research on the treatment of oral ulcers. However, the oral microenvironment is complex and influenced by numerous factors, making it difficult to precisely predict its actual efficacy in clinical practice. Future studies should involve long-term clinical trials to validate its safety and efficacy in broader populations. Additionally, ongoing optimization of diverse core-shell material combinations and the design of responsive release profiles is necessary to achieve more precise targeted release control. This will ultimately provide a more promising therapeutic strategy for oral mucosal diseases.

Electronic Supplementary Material: Supplementary material (The results of microneedles adhesion testing, *in vivo* and *in vitro* biosafety evaluation, and analgesic effect assessment, etc.) is available in the online version of this article at <https://doi.org/10.26599/NR.2026.94909131>.

Data availability

All data needed to support the conclusions in the paper are presented in the manuscript and the Electronic Supplementary Material. Additional data related to this paper may be requested from the corresponding author upon request.

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Declaration of competing interest

All the contributing authors report no conflict of interests in this work.

Author contribution statement

XX, SX and LL designed the study. YY performed most of the experiments and data analysis. JL, TJ and YP assisted in sample collection and data analysis. YY and XX wrote the manuscript. JL revised the manuscript. YY prepared figures. SX and LL supervised the research. All authors have read and approved the article.

Informed consent

Not applicable.

Ethics statement

Ethical approval for all animal experimentation procedures was granted by the Animal Investigation Ethics Committee of Second Xiangya Hospital (approval No: 20260047) and followed institutional ethical guidelines.

Use of AI statement

None.

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Just Accepted

Multifunctional core-shell microneedles with sequential release ability for oral ulcer healing

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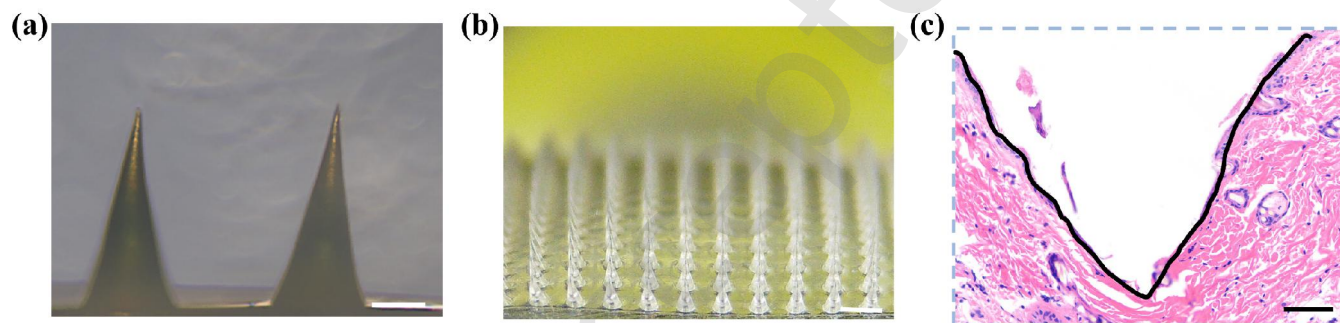
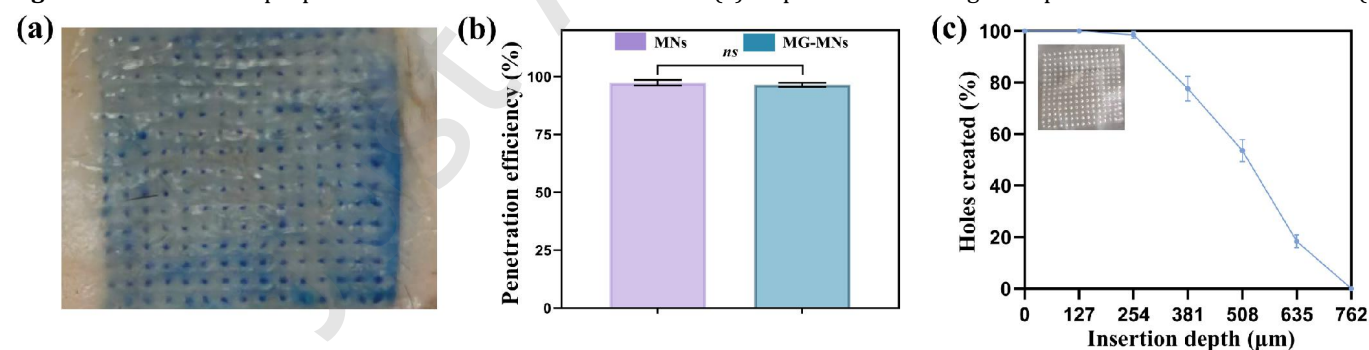


Figure S1 Morphological characterization of MG-MNs. (a) Optical microscopy image of MG-MNs. (b) Stereomicroscope image of MG-MNs. (c) H&E-stained image of rat oral mucosa after MG-MNs insertion. Scale bars: (a) 200 μm , (b) 1mm, (c) 100 μm .

Figure S2 Mechanical properties characterization of MG-MNs. (a) Representative images of punctured rat oral mucosa. (b)



Statistical analysis of the penetration efficiency of MNs and MG-MNs. (c) Insertion of MG-MNs into Parafilm M® at different depths. Inset: holes created by the microneedle array on Parafilm M® after insertion. Each experiment was performed three times in repetition. Scale bar: 100 μm . ns: not significant.

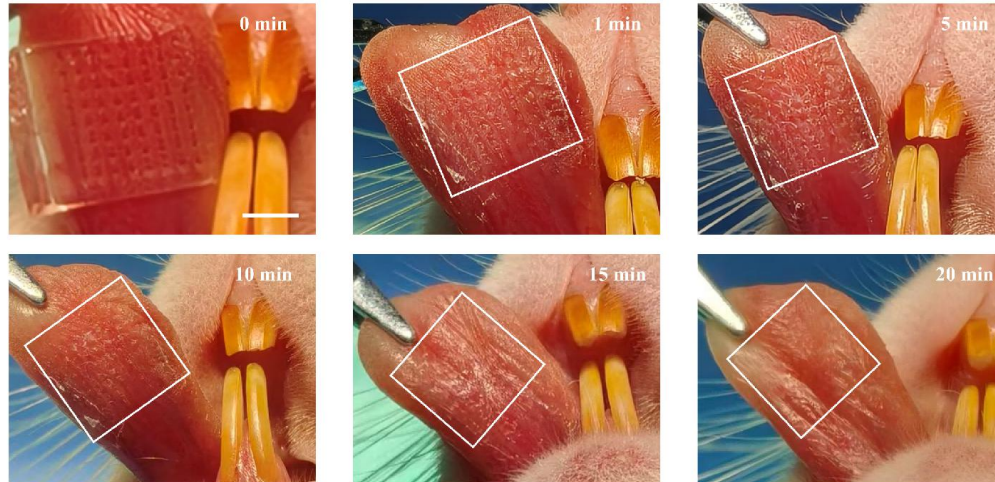


Figure S3 *In vitro* irritancy test on rat tongue mucosa. Mucosal images obtained at different time points (0, 1, 5, 10, 15 and 20 minutes) after microneedles was applied to the tongue of rats. Scale bar: 5mm.

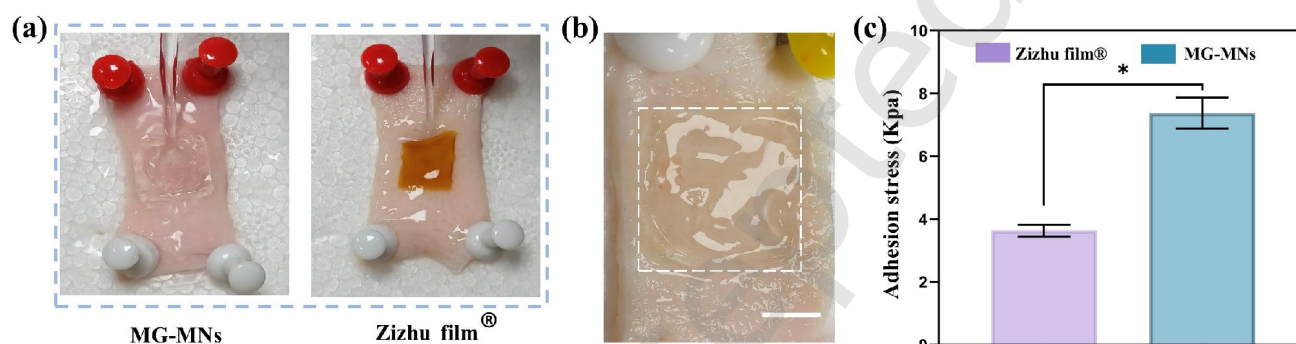


Figure S4 Dynamic adhesion stability and shear strength tests of MG-MNs and the commercial oral ulcer patch (Zizhu film®). (a) Adhesion behavior of MG-MNs and Zizhu film® under flowing water conditions. (b) Morphological changes of MG-MNs under oral simulated liquid wetting conditions. (c) Comparison of adhesion strength between MG-MNs and Zizhu film®. Each experiment was performed three times in repetition. Scale bar: 5 mm. *P<0.05.

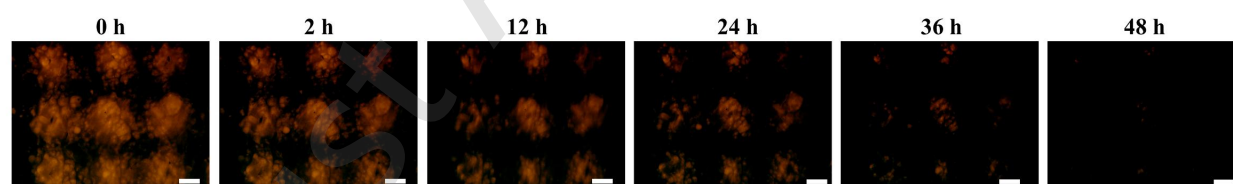
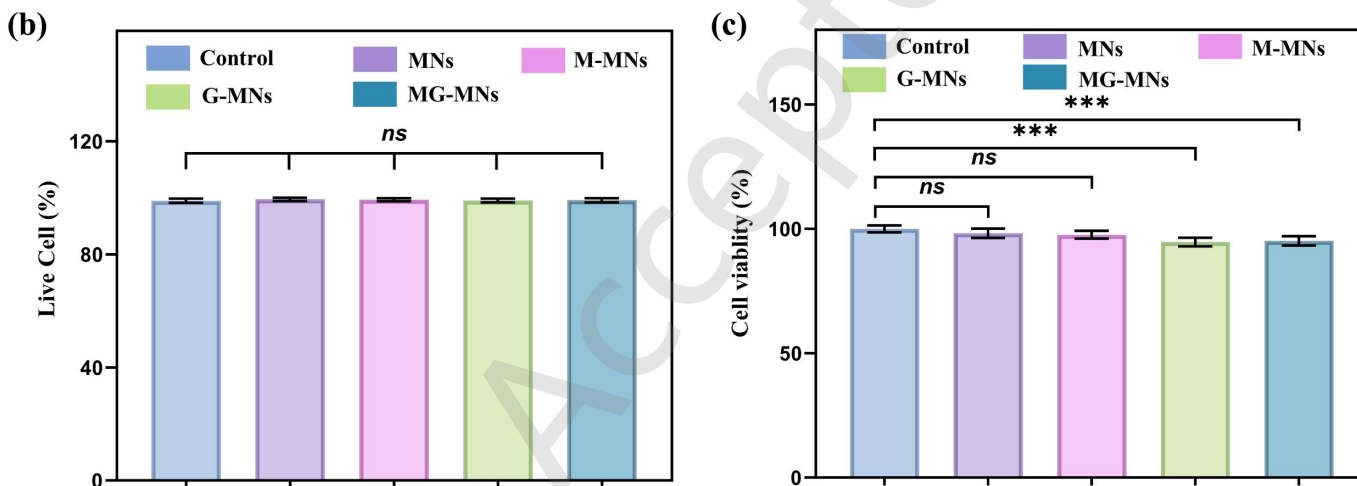
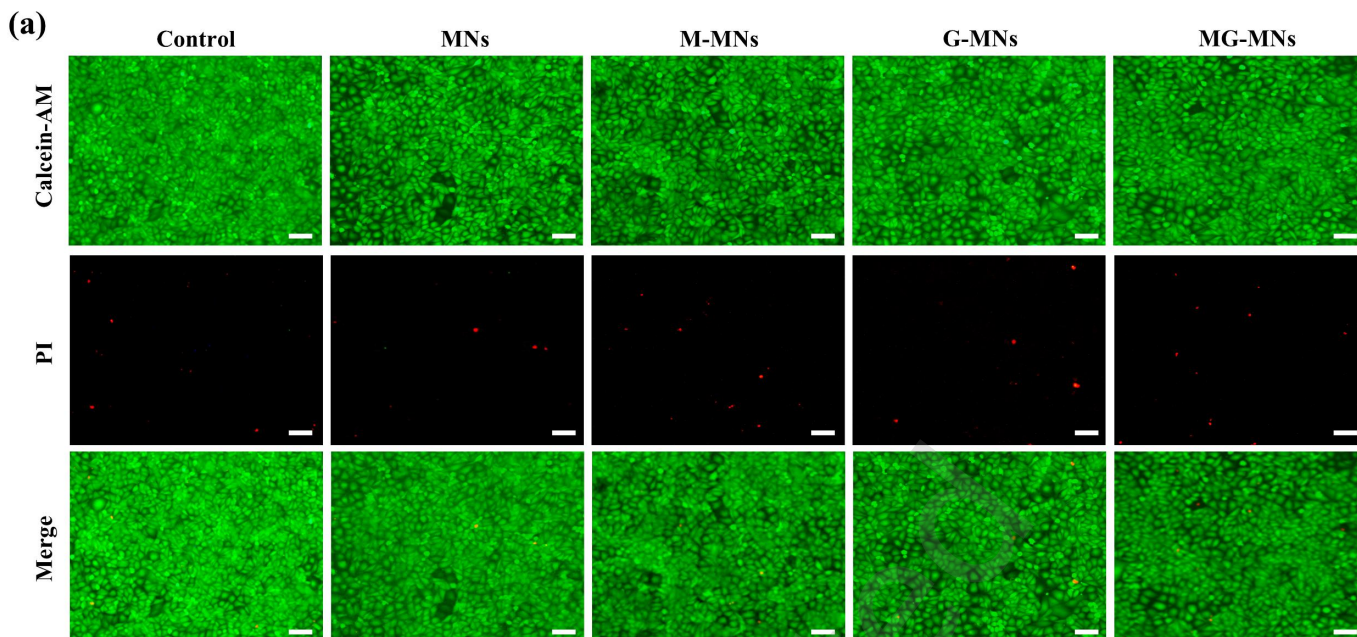


Figure S5 Retention images of loaded rhodamine microneedles captured at different time points (0, 2, 12, 24, 36, and 48 h). Scale bar: 200 μ m.

Figure S6 Evaluation of biocompatibility of various microneedles patch groups. (a) Live/Dead fluorescence images after



different treatments, showing Calcein-AM (green) and PI (red) staining. (b) Quantitative analysis of Live/Dead staining. (c) Cell viability of HUVECs measured by the CCK-8 assay. Each experiment was performed three times in repetition. Scale bar: 100 μ m. * P <0.05, *** P <0.001, *ns*: not significant.

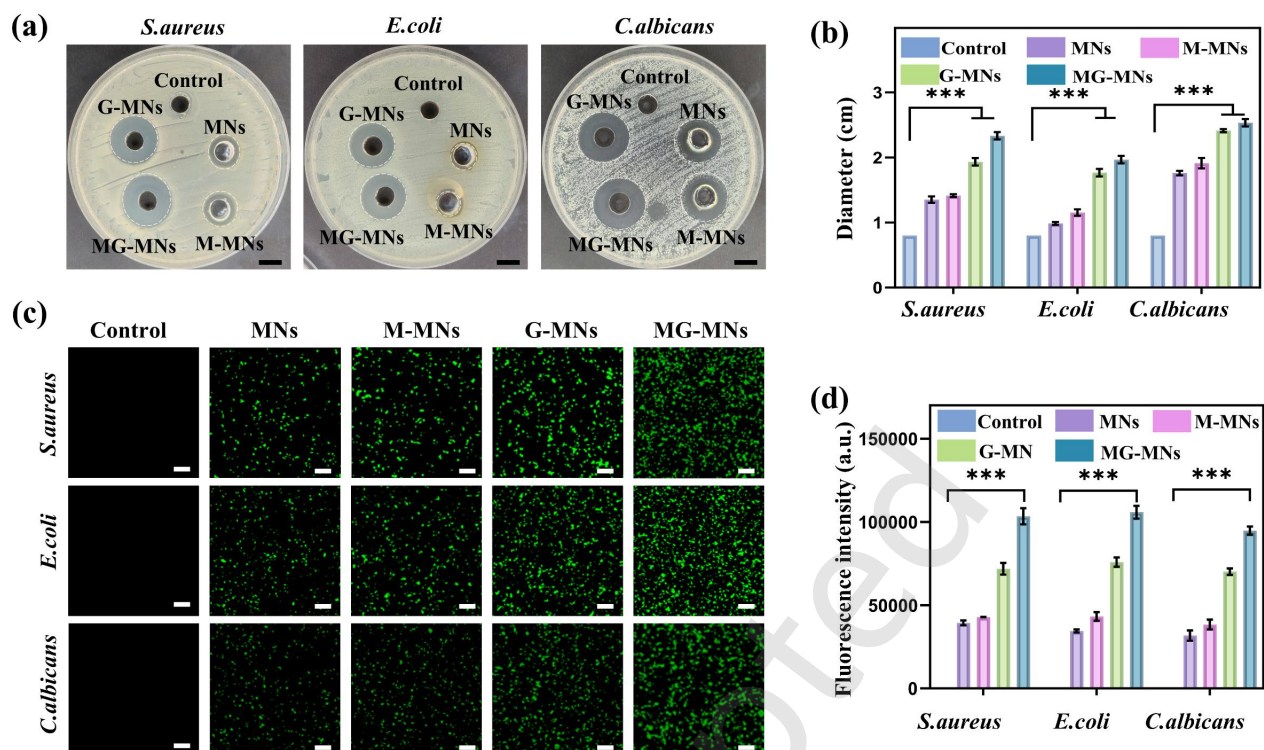


Figure S7 *In vitro* antibacterial performance. (a) Images of inhibition zones. (b) Quantitative results of inhibition zone diameters. (c) Fluorescence images of ROS in *S. aureus*, *E. coli*, and *C. albicans* subjected to different treatments via DCFH-DA. (d) Quantitative analysis of ROS in *S. aureus*, *E. coli*, and *C. albicans* subjected to different treatments via DCFH-DA. Each experiment was performed three times in repetition. Scale bar: (a) 1 cm, (c) 50 μ m *** P <0.001.

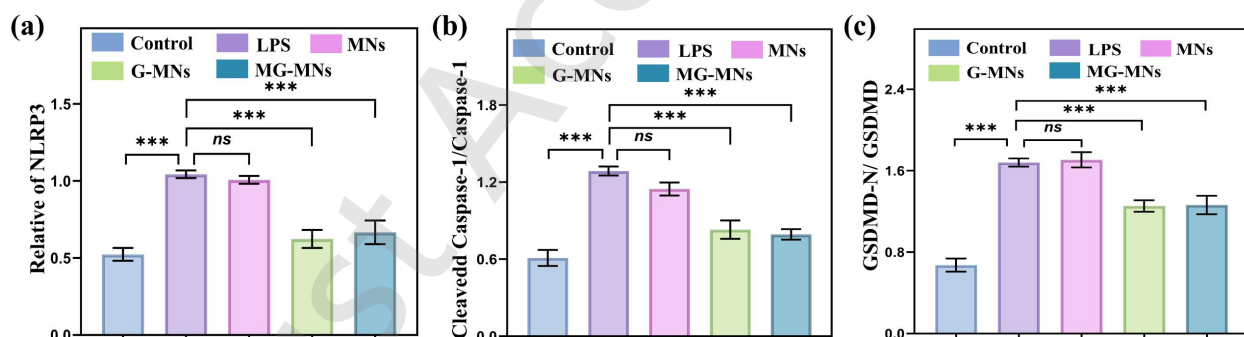


Figure S8 Analysis of the regulatory effect of MG-MNs on the expression of NLRP3 inflammasome-related proteins. (a) Quantitative analysis of the relative expression level of NLRP3 protein. (b) Quantitative analysis of the relative expression level of Caspase-1. (c) Quantitative analysis of the relative expression level of GSDMD. *** P <0.001, ns: not significant.

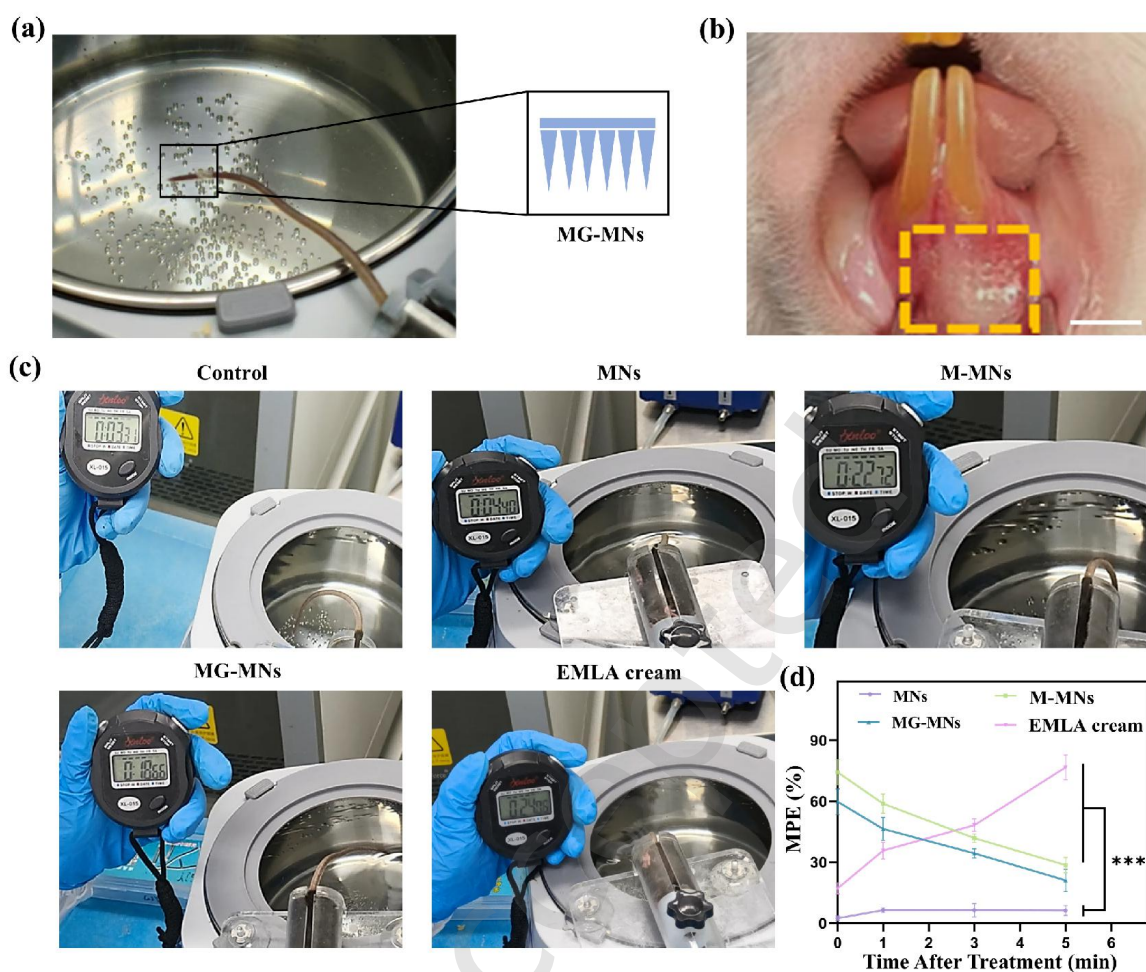


Figure S9 Schematic diagram of *in vivo* experiments and evaluation of the *in vivo* analgesic efficacy of MG-MNs. (a) Schematic illustration of the analgesia experiment. (b) Demonstration of microneedles application to the oral ulcer area in rats. (c) Schematic representation of tail-flick latency in mice following different treatments. (d) Quantitative analysis of the percentage of maximum possible effect (%MPE) of analgesia. Each experiment was performed three times in repetition (n=6). Scale bar: 5mm. *** $P < 0.001$.



Figure S10 Oral ulcer healing progression in rats across different treatment groups from day 0 to day 5 (n=6). Scale bar: 1 cm.

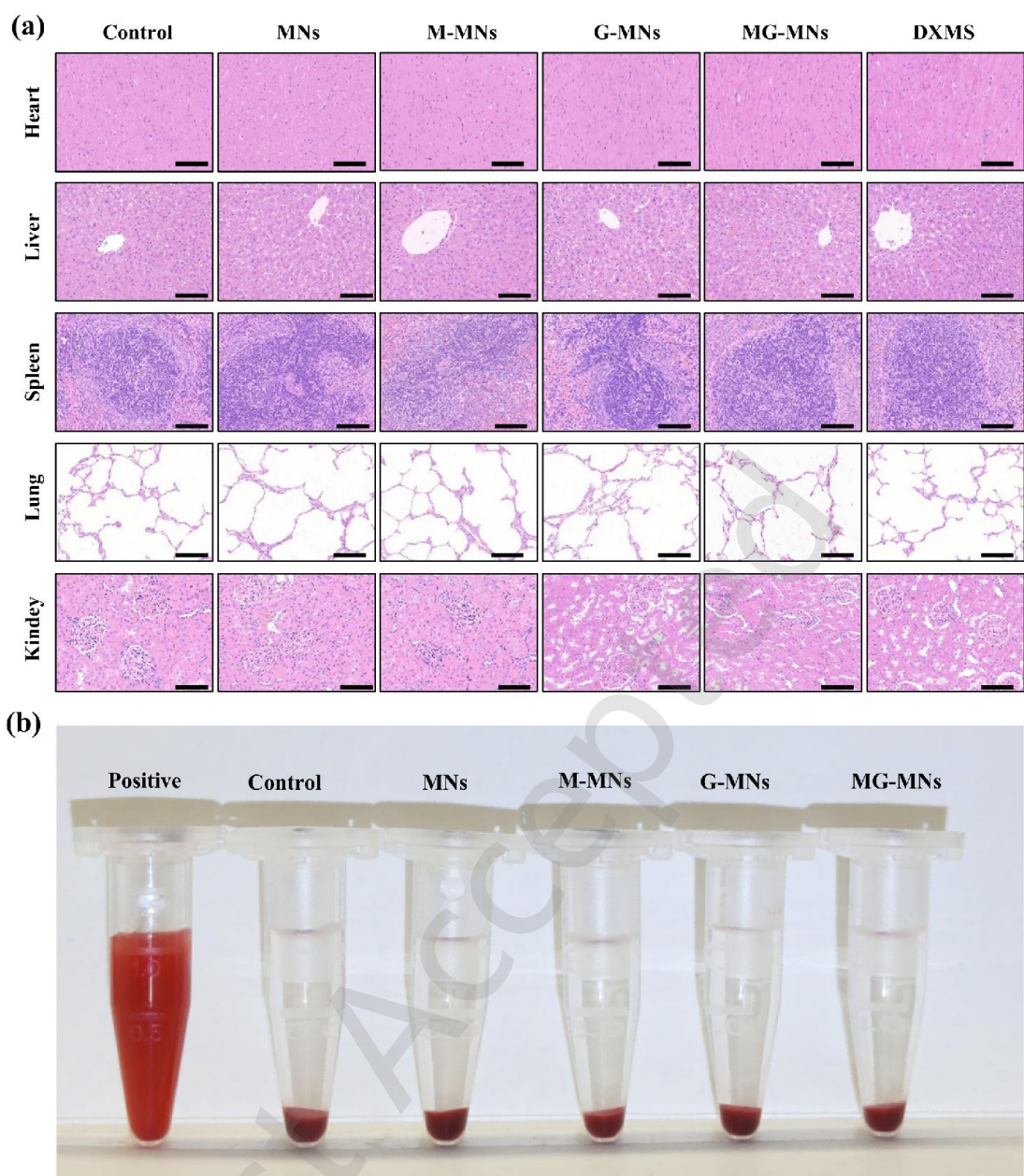


Figure S11 *In vivo* Biocompatibility and Safety Evaluation. (a) H&E-stained images of major organs (heart, liver, spleen, lung, kidney) from rats. (b) Results of hemolysis assay following treatment with different groups (n=6). Scale bar: 100 μ m.