

Multifunctional Core-Shell Structured Manganese Nanozyme Incorporated Hydrogel for Spinal Cord Injury Microenvironment Reconstruction

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Abstract. Spinal cord injury (SCI) leads to motor and sensory loss due to damage to the spinal cord's nerve fibers. Secondary inflammation and excessive reactive oxygen species (ROS) create a hostile environment for regeneration. To address oxidative stress and inflammation simultaneously, we developed a composite QUE-Mn₃O₄@PDA-GelMA hydrogel that integrates ROS-scavenging activity of Mn₃O₄ nanozyme with the anti-inflammatory properties of quercetin within a photocrosslinkable GelMA hydrogel matrix. Mn₃O₄ nanozymes were synthesized and encapsulated via dopamine polymerization, then functionalized with quercetin to form Mn₃O₄@PDA-GelMA, which was subsequently embedded in GelMA and crosslinked. Further, structural and physicochemical characterization confirmed successful PDA coating, crystallinity consistent with Mn₃O₄, and uniform nanoparticle distribution. Additionally, the composite hydrogel exhibited suitable mechanical properties and degradation behavior for spinal tissue mimicry. In vitro assays demonstrated that the hydrogel effectively scavenged ROS, reduced pro-inflammatory macrophage polarization, and maintained neural cell viability at the selected concentration. These combined effects suggest that the hydrogel creates a more favorable microenvironment for neural protection and potential regeneration. Therefore, the QUE-Mn₃O₄@PDA-GelMA hydrogel provides a suitable environment for neural regeneration that concurrently attenuates oxidative stress and inflammation, positioning it as a promising candidate for enhancing SCI repair.

Keywords: spinal cord, inflammation, reactive oxygen species, hydrogel, nanozyme

1. Introduction

Spinal cord injury (SCI), resulting from traumatic events or disease, leads to severe neurological deficits. This is primarily due to the breakdown of neural tissue structure and secondary injury processes including inflammation and reactive oxygen species (ROS) accumulation [1]. These factors release pro-apoptotic signals and disrupt the microenvironment required for neuroregeneration. Glial scar formation and diminished neurotrophic support further obstruct axonal regeneration. Recent advancements in SCI repair have focused on regenerative medicine strategies, such as bioactive scaffolds, growth factors, and cell therapies to stimulate neural regeneration [2].

However, despite these innovative strategies, complete functional recovery remains elusive, underscoring the need for treatments that simultaneously address multiple pathological barriers (e.g., oxidative stress, inflammation) to maximize repair potential.

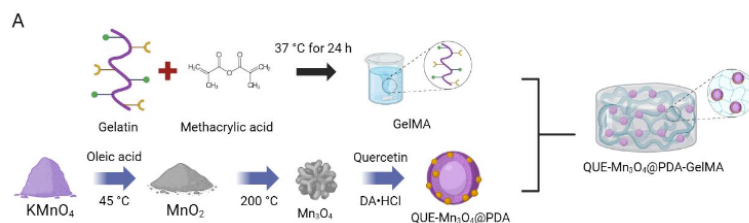
Nanozymes are nanomaterials that mimic natural enzyme catalytic activities while offering better stability, adjustable activity and easy surface modification—advantages critical for SCI applications [3]. Unlike protein enzymes, nanozymes resist denaturation under harsh physiological conditions (e.g., extreme pH, temperature). Moreover, nanozymes can be engineered in size, composition and surface chemistry according to their catalytic performance [4]. Among various nanozyme classes, manganese-based nanozymes have attracted particular interest for biomedical research due to their superoxide dismutase-like and catalase-like activities [5]. The main manganese-based nanozymes include MnO_2 and Mn_3O_4 , and they exploit the $\text{Mn}^{3+}/\text{Mn}^{2+}$ redox couple to convert ROS to less harmful species, effectively diminishing oxidative stress. In SCI, the incorporation of manganese-based nanozymes into biomaterial scaffolds or injectable hydrogels can be a solution to scavenge the excessive ROS and reduce inflammation to make the microenvironment safe enough for regeneration to occur [6]. For example, Zhang et al. demonstrated that an MnO_2 -functionalized hydrogel implanted in a rat model of spinal cord injury achieved over a 60 % reduction in local H_2O_2 levels, attenuated inflammatory cytokine expression, and significantly improved motor function recovery compared to controls [7].

Polydopamine (PDA) is formed when dopamine is exposed to alkaline conditions through oxidation, rearrangement to form indoloquinone, and reacted with catechol. PDA is characterized as a coating layer on almost all material surfaces, including nanoparticles. In fact, it stabilizes nanoparticles against aggregation and harsh physiological conditions while enabling strong interactions to be formed [8]. A study demonstrated that PDA nanoparticles act as a versatile molecular loading platform, exhibiting excellent physiological stability and resistance to aggregation when loaded with multiple therapeutic and imaging agents [9].

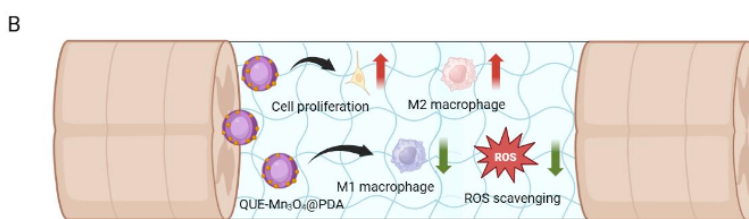
Quercetin (QUE) is a naturally occurring polyphenol that possesses anti-inflammatory and neuroprotective activities as it inhibits pro-inflammatory cytokine signaling and promotes antioxidant pathways [10]. Its effectiveness has been shown in multiple studies, that has shown that quercetin suppresses the production of inflammatory mediators such as $\text{TNF-}\alpha$, $\text{IL-1}\beta$, and IL-6 , modulates key signaling pathways including $\text{NF-}\kappa\text{B}$ and MAPK, and enhances antioxidant defenses through activation of Nrf2, thereby contributing to its protective effects against inflammation and oxidative stress [11].

Hydrogels are biomaterials widely used in biomedical applications, including spinal cord injury treatment methods. Hydrogels are three-dimensional networks of hydrophilic polymers that can absorb and retain large amounts of water, making them soft and elastic for neural cell proliferation [12]. Their structure also contains microscopic pores that enable substances to pass through. Multiple types of hydrogels exist, including natural, synthetic, and hybrid forms. Among them, gelatin, as a natural hydrogel, offers the advantages of biocompatibility and adhesion [13]. For example, Zhang. demonstrated the therapeutic potential of a genipin-crosslinked gelatin hydrogel for SCI repair. They engineered a gelatin scaffold by varying genipin concentrations balancing mechanical strength and degradation rate, then loaded it with basic fibroblast growth factor (bFGF) [14]. In vitro, this hydrogel provided sustained release of bFGF and supported Schwann cell survival and proliferation. In vivo, in a rat spinal cord hemisection model, implantation of the 0.5 % genipin–gelatin hydrogel markedly enhanced axonal regeneration and improved locomotor scores compared to controls. Thus, gelatin hydrogels are the chosen candidates for effectively creating a microenvironment at the site of injury to support the regeneration of damaged tissue.

In this study, we developed the QUE-Mn₃O₄@PDA-GelMA hydrogel, integrating quercetin-modified Mn₃O₄@PDA nanoparticles into GelMA (Scheme 1). This composite hydrogel combines ROS-scavenging and anti-inflammatory properties. It exhibits suitable mechanical properties, degradability, and efficacy in scavenging ROS, reducing inflammation and maintaining neural cell viability. Indeed, its effectiveness at creating a suitable microenvironment for neural cell protection and potential regeneration positions it as a promising candidate for SCI repair.



(A) Schematic representation of integrated QUE-Mn₃O₄@PDA-GelMA hydrogel. PDA was used to modify Mn₃O₄ nanoparticles. The Mn₃O₄ nanozyme was then medicated with quercetin to generate QUE-Mn₃O₄@PDA. The QUE-Mn₃O₄@PDA nanoparticle was then incorporated into the GelMA.



(B) Schematic diagram of the multitarget therapeutic QUE-Mn₃O₄@PDA-GelMA hydrogel with main functions including anti-inflammation and ROS scavenging.

Scheme 1. Illustration of the preparation and application of QUE-Mn₃O₄@PDA-GelMA hydrogel for SCI repair

2. Methods and materials

2.1. Materials

KMnO₄ was obtained from Sinopharm Group Chemical Reagent Co., Ltd. Oleic acid and N, N'-methylenebis-acrylamide were purchased from Aladdin Industrial Co., Ltd (Shanghai, China). Hyaluronic acid (HA, Mw = 40 kDa) was purchased from Bloomage Freda Biopharm Co., Ltd (Shandong, China). Vascular endothelial growth factor was obtained from Novoprotein Co., Ltd (Suzhou, China). Acryl-amide and ammonium persulfate were purchased from Macklin Co., Ltd (Shanghai, China).

2.2. Synthesis of QUE-Mn₃O₄@PDA-GelMA hydrogel

2.2.1. Synthesis of GelMA

A total of 5.0 g of gelatin and 5.8 mL of MA were dissolved in 100 mL of deionized water. The solution was then maintained at 37–45 °C, with the pH adjusted to 7–8. The mixture was allowed to react for 24 hours. Excess MA was removed through dialysis of 3500 Da for 3 days, 3 times/day.

The GelMA was subsequently freeze-dried to obtain the net weight. For radical polymerization, 0.04 wt% LAP was added, followed by exposure to UV light.

2.2.2. Synthesis of Mn_3O_4 nanoparticles

To prepare Mn_3O_4 nanoparticles, 1.0 g of potassium permanganate was dissolved in 500 mL of ultrapure water and stirred for 30 minutes. Then, 10 mL of oleic acid was added, and the mixture was stirred in a 28 °C water bath for 24 hours. The suspension was centrifuged at 12,000 rpm for 20 minutes to collect the black precipitate, which was washed with ultrapure water and ethanol until the supernatant was clear. The solid was vacuum-dried at 45 °C overnight to obtain MnO_2 nanoparticles. Finally, Mn_3O_4 particles were obtained by calcining MnO_2 at 200 °C for 5 hours.

2.2.3. Preparation of QUE- Mn_3O_4 @PDA

To synthesize Mn_3O_4 @PDA, 0.1 g of Mn_3O_4 nanoparticles was dispersed in 10 mL of deionized water, and 0.3 g of dopamine hydrochloride was added. The pH was adjusted to 8.5 with Tris-HCl buffer, and the mixture was stirred at room temperature for 2 hours for PDA coating. The product was collected by centrifugation, washed with water and ethanol, and freeze-dried.

For quercetin (QUE) functionalization, the dried Mn_3O_4 @PDA was dispersed in 20 mL of ethanol with 0.1 g of QUE and stirred at room temperature for 12 hours. The product was centrifuged, washed with ethanol to remove unbound QUE, and freeze-dried to obtain the final QUE- Mn_3O_4 @PDA nanocomposite.

2.2.4. Preparation of QUE- Mn_3O_4 @PDA-GelMA

The freeze-dried GelMA was dissolved in deionized water at 40°C to prepare a 10% (w/v) solution. A 0.04% (w/v) LAP photoinitiator was added and stirred until fully dissolved. The QUE- Mn_3O_4 @PDA nanocomposite was then dispersed at 10 µg/mL, sonicated for 10 minutes to ensure uniform dispersion and remove air bubbles. The mixture was transferred to a sterile mold, UV-exposed for 3 minutes to induce photocrosslinking, forming the stable QUE- Mn_3O_4 @PDA-GelMA hydrogel. After crosslinking, the hydrogel was rinsed with PBS (pH 7.4) three times to remove unreacted residues and used for characterization and biological assays.

2.3. Characterization of Mn_3O_4 and QUE- Mn_3O_4 @PDA

The Mn_3O_4 and QUE- Mn_3O_4 @PDA were morphologically characterized with transmission electron microscopy (TEM, HITACHI HT7700, Japan) at 100.0 kV and scanning electron microscopy (SEM, HITACHI S4800, Japan). The TEM samples were prepared by adding 10 µL of Mn_3O_4 and QUE- Mn_3O_4 @PDA solutions dropwise onto a copper mesh and allowing them to air-dry. For SEM, the Mn_3O_4 and QUE- Mn_3O_4 @PDA solution was deposited dropwise onto a silicon wafer and sprayed with gold at 10 mV for 2 minutes.

Dynamic light scattering (DLS) was used to measure the zeta potential and hydrated diameter of Mn_3O_4 and QUE- Mn_3O_4 @PDA on a Malvern Zetasizer NanoSeries (Malvern, USA). Prior to measurement, the Mn_3O_4 and QUE- Mn_3O_4 @PDA dispersions were sonicated to ensure even distribution.

The crystal structure of Mn_3O_4 was analyzed by X-ray diffraction (XRD, D8 Advance, Bruker AXS, USA) using Cu K α radiation over $2\theta = 10\text{--}85^\circ$ at a scan rate of $8^\circ/\text{min}$. The diffraction

patterns were compared with standard reference cards, and the inter-crystalline spacing was calculated according to Bragg's equation:

$$n\lambda = 2d\sin\theta \quad (1)$$

2.4. Characterization of QUE-Mn₃O₄@PDA-GelMA hydrogel

The QUE-Mn₃O₄@PDA-GelMA hydrogel was freeze-dried and sputter-coated with a conductive gold layer at 20 mV for 3 minutes to enable morphological observation by SEM (HITACHI S 4800, Japan).

The mechanical properties of the QUE-Mn₃O₄@PDA-GelMA hydrogel were measured using a rotational rheometer (Kinexus Pro, Malvern, USA) with an 80 mm parallel plate configuration. The elastic modulus and viscous modulus of the hydrogels were measured over a frequency range of 0.1–100 Hz to evaluate whether their mechanical properties were compatible with spinal cord tissue.

2.5. Cell viability assay

HT22 cell line was digested by adding 1 mL Trypsin-EDTA to obtain single cells. 2000 cells were planted into the 96 well plate. QUE-Mn₃O₄@PDA-GelMA hydrogel was added into the cells (concentration from 0 to 200 µg/mL) to co-culture. After 24 hours, the culture media was removed and 10 % cell counting kit-8 (CKK-8) solution was added to the 96-well plate and incubated for 1 hour. The absorbance was measured using the multimode reader (Cytation 3, BioTek, USA) to calculate the cell viability.

$$\text{Cell Viability} = \frac{A - A_b}{A_c - A_b} \times 100\% \quad (2)$$

where A = absorbance of sample, A_b = absorbance of blank and A_c = absorbance of control.

2.6. Regulation of ROS in cells under oxidative stress

8×10⁵ HUVEC were planted in 3.5 cm culture dishes and supplemented with biomaterials, then cultured at 37 °C in a 5 % CO₂ environment. After 12 hours, 0.4 mM H₂O₂ was added to each dish and the cultures were incubated for 24 hours. Following this, cells were stained with a concentration of 5 µM DCFH-DA probe (Sigma, D6883, USA) to assess the antioxidative capacity of the biomaterials, and imaged using a laser confocal microscope (Olympus, FV3000, Japan).

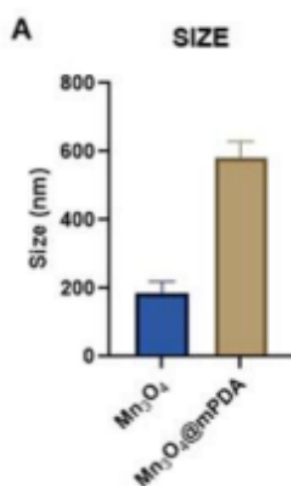
2.7. Regulation of inflammation in THP-1

THP-1 cells were seeded in a 10 cm culture dish and treated with 0.1 µg/mL 12-O-tetradecanoylphorbol-13-acetate (PMA) to induce differentiation into mature macrophages, followed by incubation for 24 h at 37 °C in a 5% CO₂ atmosphere. The macrophages were subsequently stimulated with 20 ng/mL interferon-γ (Novoprotein, C74650, USA) and 250 ng/mL lipopolysaccharide (Invitrogen, 00497693, USA) for an additional 24 h to polarize them into the M1 phenotype. The biomaterials were then applied to the M1 macrophages for 24 h, and the regulation of inflammation was assessed by flow cytometry.

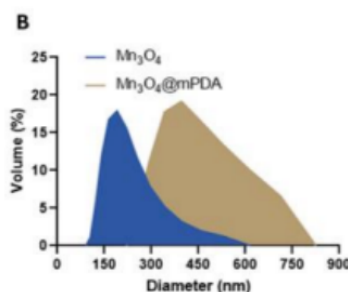
3. Results and discussion

3.1. Characterization of Mn_3O_4 and $\text{Mn}_3\text{O}_4@\text{PDA}$

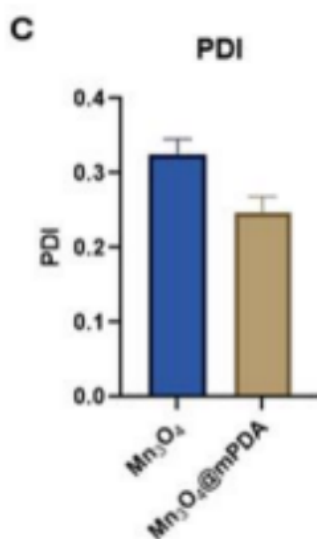
Dynamic light scattering (DLS) was used to characterize the physicochemical properties of Mn_3O_4 nanoparticles before and after polydopamine (PDA) modification. The average hydrated size of pristine Mn_3O_4 was 187.17 ± 32.3 nm, while $\text{Mn}_3\text{O}_4@\text{PDA}$ nanoparticles increased to 580.33 ± 48.34 nm, confirming PDA coating deposition (Figure 1A). Size distribution profiles (Figure 1B) further showed a shift from a narrow distribution for Mn_3O_4 (centered around 150 nm) to a broader one for $\text{Mn}_3\text{O}_4@\text{PDA}$ (300–400 nm), indicating successful PDA polymerization. The polydispersity index (PDI) of $\text{Mn}_3\text{O}_4@\text{PDA}$ (0.248 ± 0.02) was lower than that of Mn_3O_4 (0.326 ± 0.02), reflecting improved size uniformity after PDA modification (Figure 1C). Zeta potential measurements (Figure 1D) revealed a shift from -33.47 ± 0.71 mV (Mn_3O_4) to -21.17 ± 1.14 mV ($\text{Mn}_3\text{O}_4@\text{PDA}$), attributed to the neutralization of surface charges by the PDA layer, which reduces electrostatic repulsion but maintains colloidal stability via steric hindrance. These DLS results confirm the successful formation of $\text{Mn}_3\text{O}_4@\text{PDA}$ nanoparticles with enhanced stability, providing a platform for further quercetin loading and biological application.



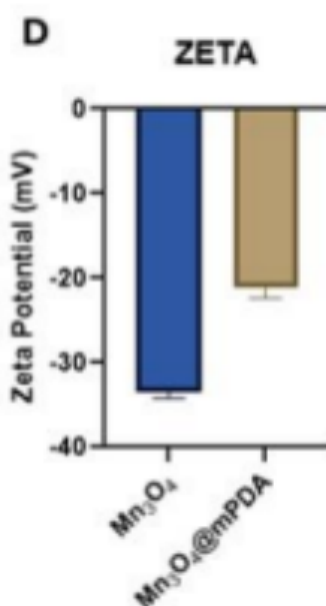
(A) Size of Mn_3O_4 and $\text{Mn}_3\text{O}_4@\text{PDA}$ nanoparticles.



(B) Percent volume according to the diameter of Mn_3O_4 and $\text{Mn}_3\text{O}_4@\text{PDA}$ nanoparticles.



(C) Polydispersity index of Mn₃O₄ and Mn₃O₄@PDA nanoparticles.



(D) Zeta potential of Mn₃O₄ and Mn₃O₄@PDA nanoparticles.

Figure 1. Characterization of Mn₃O₄ and Mn₃O₄@PDA

Six broad peaks in the X-ray diffraction (XRD, Figure 2) pattern were observed at 2θ angles of 21.5° , 29.15° , 32.42° , 36.45° , 59.89° , and 66.35° , respectively corresponding to the (101), (112), (103), (211), (224), and (314) planes. And the result is in line with Mn₃O₄, JCPDS 80-0382, confirming the XRD results. The presence of these characteristic diffraction peaks, with no additional impurity peaks, confirms the successful synthesis of pure-phase Mn₃O₄ nanoparticles. The broad nature of the peaks suggests the nanocrystalline structure of the synthesized Mn₃O₄, which is typical for nanoparticles due to the small crystallite size and increased surface to volume ratio. This

crystalline phase confirmation is crucial as it ensures the Mn_3O_4 nanoparticles possess the desired redox properties for subsequent ROS scavenging applications in the context of spinal cord injury treatment.

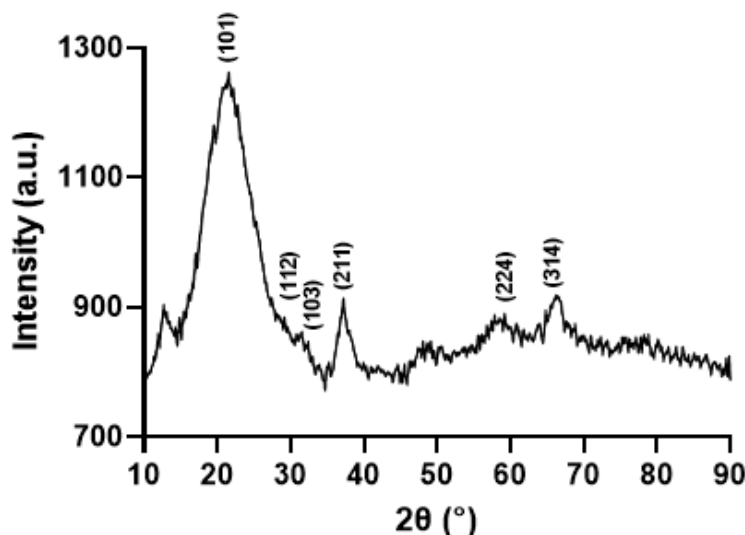
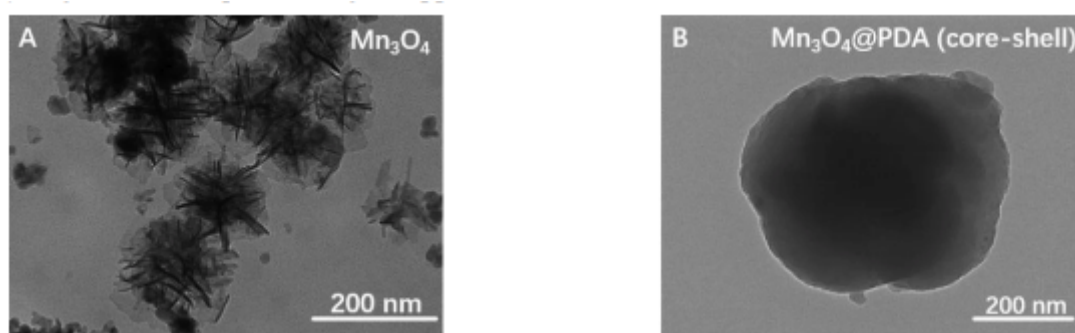


Figure 2. X-ray diffraction of Mn_3O_4

TEM of Mn_3O_4 and $\text{Mn}_3\text{O}_4@\text{PDA}$ nanoparticles were detected by TEM (Figure 3A and B). The sizes of the Mn_3O_4 nanoparticles had diameters between 150 and 200 nm and presented a flower-like shape, exposing more surface area and thus ROS scavenging sites. The $\text{Mn}_3\text{O}_4@\text{PDA}$ nanoparticles had diameters around 450 nm. They represented a core-shell structure significantly larger than the Mn_3O_4 nanoparticles, thus showing successful encapsulation. The formation of the core-shell structure not only provides a protective layer for Mn_3O_4 but also can potentially improve the biocompatibility and stability of the nanoparticles, which is beneficial for their integration into the hydrogel and subsequent biological applications.



(A) Mn_3O_4 nanoparticle shown at a voltage of 100 kV by TEM.

(B) $\text{Mn}_3\text{O}_4@\text{PDA}$ nanoparticle shown at a voltage of 100 kV by TEM.

Figure 3. TEM of Mn_3O_4 and $\text{Mn}_3\text{O}_4@\text{PDA}$ nanoparticle

3.2. Characterization of QUE-Mn₃O₄@PDA-GelMA hydrogel

Furthermore, we mixed GelMA and QUE-Mn₃O₄@PDA nanoparticles to prepare QUE-Mn₃O₄@PDA-GelMA hydrogel. Characterized via SEM (300× magnification), the QUE-Mn₃O₄@PDA-GelMA hydrogel shows a porous structure (Figure 4). These pores, about 100 nm in diameter, are regularly shaped and interconnected. This porous network offers space for cell adhesion, proliferation, and migration. It also facilitates transport of nutrients, oxygen, and waste, mimicking the extracellular matrix to support cell function. The hydrogel's moisture-retaining ability maintains a stable, moist microenvironment, reducing cell apoptosis. Thus, it holds promise as a cell scaffold for tissue engineering, like spinal cord injury repair.

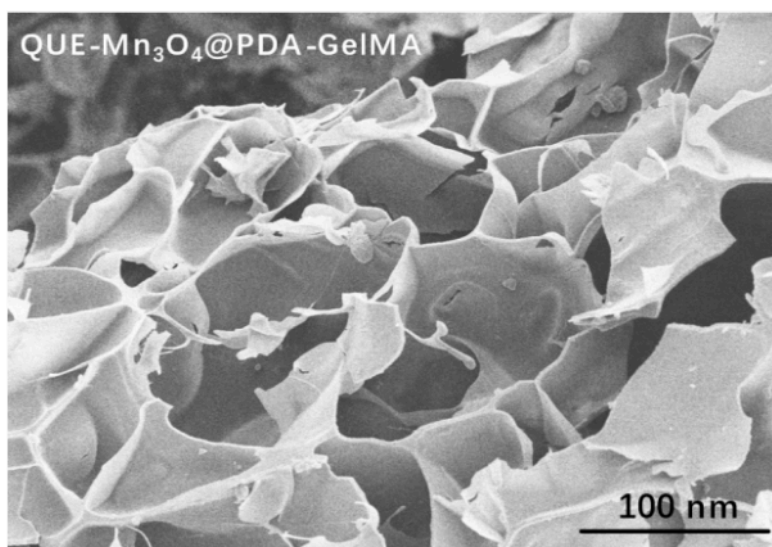


Figure 4. SEM of QUE-Mn₃O₄@PDA-GELMA hydrogel shown at 10 kV by SEM

The rheological properties of GelMA and QUE-Mn₃O₄@PDA-GelMA hydrogels were evaluated by assessing elastic modulus and viscous modulus across 0.1–100 Hz (Figure 5). For pure GelMA, both elastic modulus and viscous modulus are relatively low and fluctuate with frequency, indicating a softer, more deformable network with prominent viscous characteristics. In contrast, QUE-Mn₃O₄@PDA-GelMA exhibits much higher and more stable G' and G'' across the frequency range. The stable, high G' reflects a rigid, elastic network resistant to deformation, while the consistent G'' implies controlled energy dissipation. The enhanced properties stem from interactions (electrostatic, hydrogen bonding, or chemical bonding) between QUE-Mn₃O₄@PDA nanoparticles and GelMA chains, forming additional cross-links to increase network density. These superior mechanical properties make QUE-Mn₃O₄@PDA-GelMA a promising candidate for tissue engineering and regenerative medicine. Its stable, tissue-mimicking rheology can support cell processes involved in wound healing and tissue repair, outperforming pure GelMA.

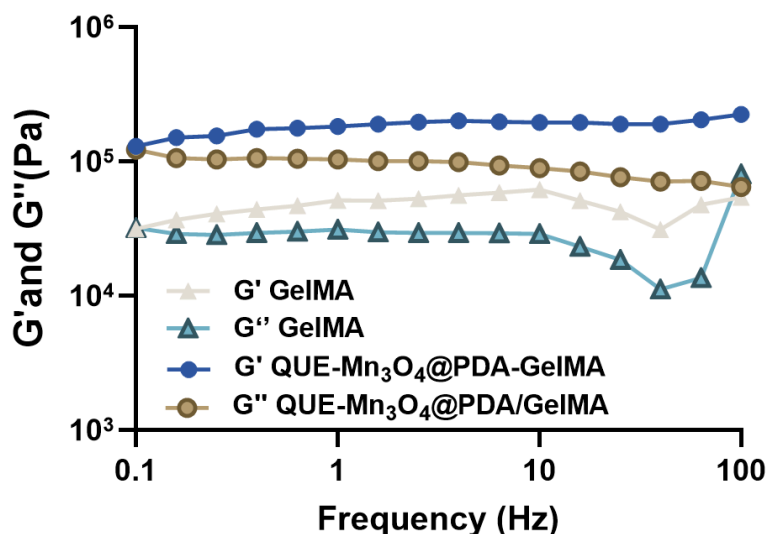
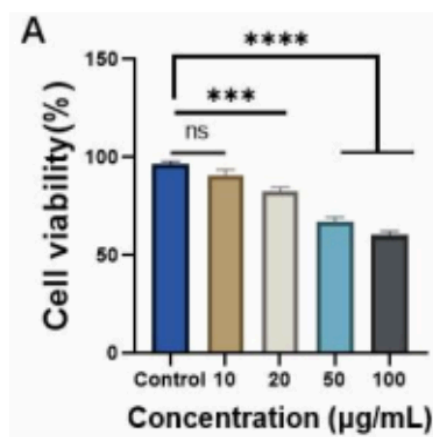


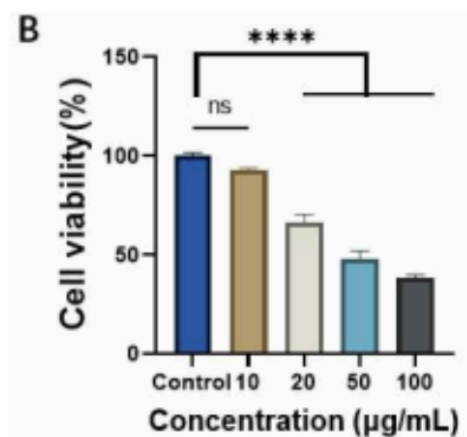
Figure 5. Frequency sweep rheology of GelMA and QUE-Mn₃O₄@PDA-GelMA hydrogels. Storage modulus (G') and loss modulus (G'') were measured from 0.1–100 Hz

3.3. The Biocompatibility of QUE-Mn₃O₄@PDA nanoparticle

The toxicity of Mn₃O₄ and QUE-Mn₃O₄@PDA was evaluated using CCK-8 (Figure 6A & B). Different concentrations of Mn₃O₄ nanoparticles were used to treat HT22 cells to assess cell viability. The results showed that NSCs remained viable at concentrations up to 10 µg/mL, indicating biosafety at this level. Similarly, different concentrations of QUE-Mn₃O₄@PDA were used to treat HT22 cells to assess cell viability. The results showed that NSCs remained viable at concentrations up to 10 µg/mL, confirming biosafety at this level. Therefore, 10 µg/mL was selected for all subsequent cell experiments.



(A) CCK-8 results for NSCs treated with different concentrations of Mn₃O₄ nanoparticles.

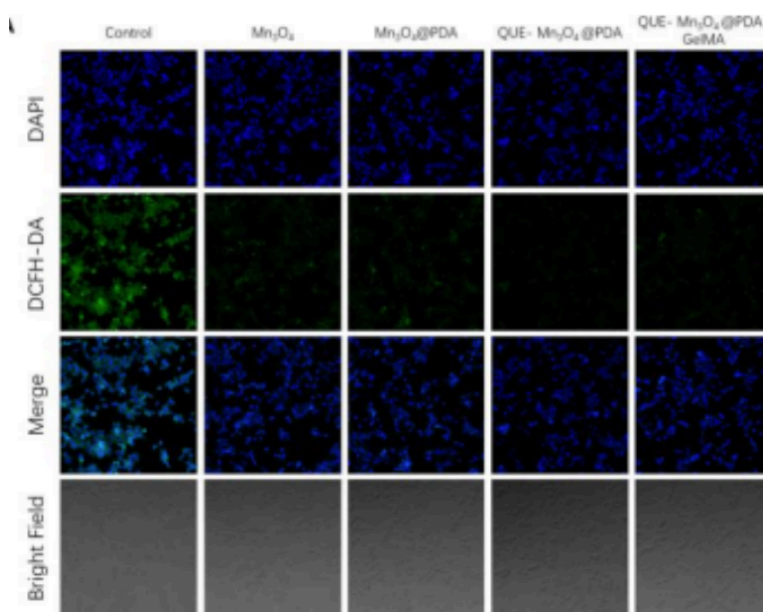


(B) CCK-8 results for NSCs treated with different concentrations of QUE-Mn₃O₄@PDA.

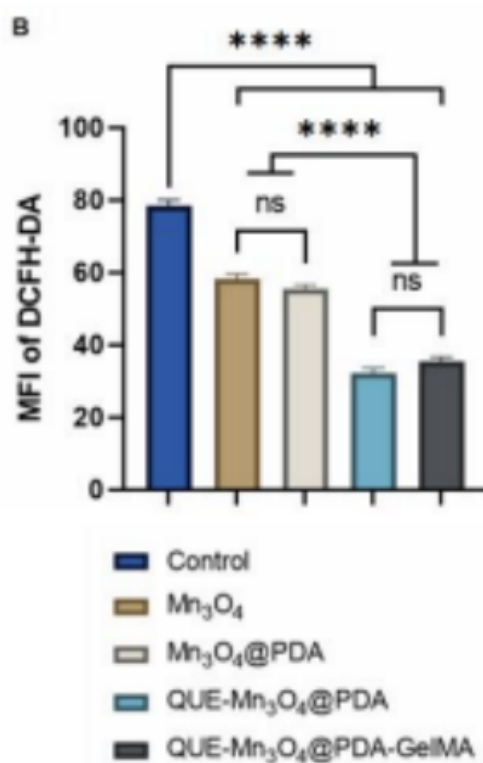
Figure 6. NSCs viability treated by Mn₃O₄ and QUE-Mn₃O₄@PDA nanoparticles. Data are presented as mean ± SD. Statistical analysis was performed using a one-way ANOVA/T-Test followed by Dunnett's post hoc test, *p < 0.05; **p < 0.01; ***p < 0.001; ns, no statistical significance

3.4. QUE-Mn₃O₄@PDA-GelMA's ROS scavenging ability

DCFH-DA, a dye sensitive to ROS, was used to assess oxidative stress in cells, with mean fluorescence intensity (MFI) reflecting ROS levels. As shown in Figure 7A and B, the control group had a DCFH-DA MFI of 78.374 ± 1.69 , indicating high baseline ROS. Across all treatment groups, MFI decreased, with the QUE-Mn₃O₄@PDA-GelMA group showing a notable drop to 35.358 ± 1.263 . This reduction suggests that QUE-Mn₃O₄@PDA-GelMA effectively scavenges ROS in cells. The Mn₃O₄ nanoparticles within the hydrogel play a central role in this process. Due to the coexistence of Mn²⁺ and Mn³⁺ oxidation states, Mn₃O₄ mimics superoxide dismutase (SOD) activity [15]. This SOD-like function allows it to catalyze the dismutation of superoxide anions, a major type of ROS, into less harmful molecules. PDA coating and quercetin further enhance the ROS-scavenging capacity by PDA stabilizes the Mn₃O₄ nanoparticles and may contribute additional redox-active sites, while QUE, a natural antioxidant, directly neutralizes ROS through its polyphenolic structure [10]. The GelMA hydrogel matrix provides a biocompatible scaffold that supports the sustained release and activity of these ROS-scavenging components, ensuring prolonged action within the cellular microenvironment [16]. In the context of SCI, excessive ROS production is a key driver of secondary damage, contributing to neuronal death and impaired tissue repair. By effectively reducing intracellular ROS levels, QUE-Mn₃O₄@PDA-GelMA addresses this critical pathological factor. The hydrogel's ability to modulate oxidative stress could create a more favorable microenvironment for neural cell survival, proliferation, and regeneration after SCI.



(A) Confocal fluorescence images of control, Mn_3O_4 , $\text{Mn}_3\text{O}_4@\text{PDA}$, QUE- $\text{Mn}_3\text{O}_4@\text{PDA}$, and [QUE- \$\text{Mn}_3\text{O}_4@\text{PDA}\$ -GelMA](#).



(B) MFI of DCFH-DA for each sample.

Figure 7. Confocal fluorescence of accumulating layers

3.5. QUE-Mn₃O₄@PDA-GelMA's anti-inflammation ability

To evaluate the immunomodulatory impact on macrophage polarization, flow cytometry was used to analyze CD86 (M1 marker) and CD206 (M2 marker) expression, as shown in Figure 8. In the Control group, a high M1 proportion (25.8%) and low M2 proportion (8.77%) indicated a pro-inflammatory microenvironment. GelMA showed a mild shift, with reduced M1 (20.9%) and increased M2 (20.5%), suggesting that GelMA's biocompatible matrix mildly modulates the immune response. The Mn₃O₄@PDA-GelMA group further decreased M1 to 24.3% and stabilized M2 in 19.9%, as the ROS-scavenging and anti-inflammatory properties of Mn₃O₄@PDA enhanced immunomodulation. Most significantly, the QUE-Mn₃O₄@PDA-GelMA group drastically reduced M1 (6.10%) and increased Q4 (82.1%), shifting toward an anti-inflammatory M2 or quiescent state—synergistically driven by QUE's anti-inflammatory action, Mn₃O₄@PDA's effects, and GelMA's sustained release. In SCI, this polarization shift could mitigate damage and promote repair, though molecular mechanisms [17]. Overall, QUE-Mn₃O₄@PDA-GelMA potently modulates macrophage polarization toward anti-inflammation *in vitro*, providing a favorable microenvironment for SCI repair.

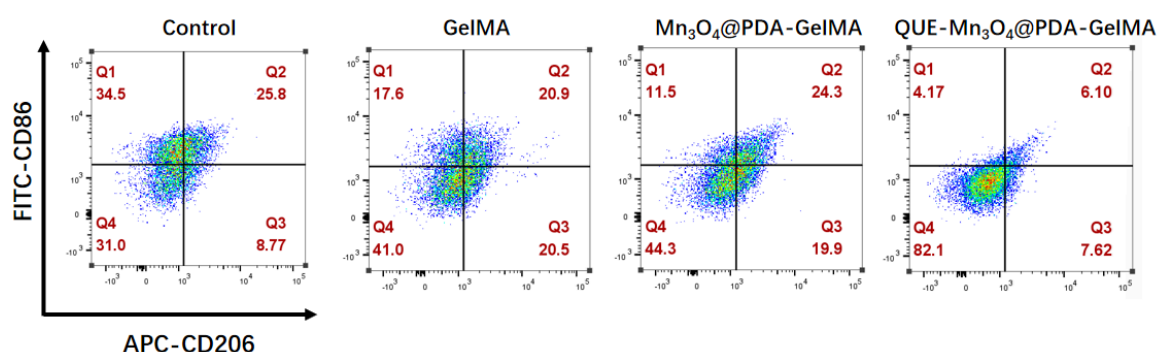
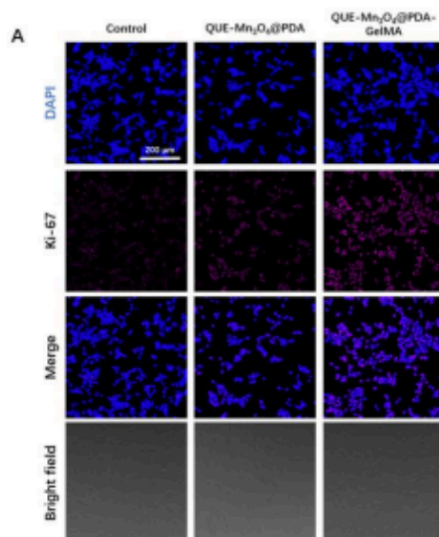


Figure 8. Macrophage polarization analyzed by flow cytometry. Flow cytometry plots showing macrophage polarization using CD86 (M1 marker) and CD206 (M2 marker). Quadrants Q2 (M1), Q3 (M2) indicate polarization states, reflecting treatment effects on pro-inflammatory/anti-inflammatory shifts

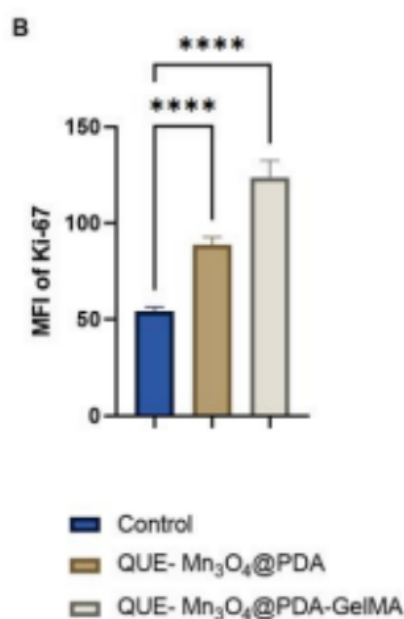
3.6. QUE-Mn₃O₄@PDA-GelMA's proliferation ability

Cell proliferation was assessed using Ki-67 immunofluorescence staining, where Ki-67 MFI reflects cell division activity [18]. As shown in Figures 9A and B, the Control group had a Ki-67 MFI of 54.423 ± 1.832 , indicating baseline proliferation. After treatment with QUE-Mn₃O₄@PDA, Ki-67 MFI significantly increased to 88.689 ± 4.186 , suggesting the nanocomposite promotes cell cycle progression by reducing oxidative stress. The QUE-Mn₃O₄@PDA-GelMA group showed an even higher MFI of 123.786 ± 8.751 , indicating that the GelMA hydrogel matrix enhances proliferation by providing structural support and facilitating cell-material interactions. GelMA hydrogel matrix likely synergizes with QUE-Mn₃O₄@PDA, as GelMA mimics the extracellular matrix (ECM), providing structural support, adhesion sites, and nutrient diffusion pathways, amplifying proliferation by enhancing cell-material interactions and sustained release of bioactive cues [13]. Mn₃O₄ nanozymes scavenge ROS to reduce oxidative damage that halts cell division, quercetin suppresses pro-inflammatory cytokines to prevent inflammation-mediated cell cycle inhibition, and the GelMA hydrogel provides a physiological niche for adhesion, spreading, and growth factor retention. These

effects push cells from a quiescent (G0) state into active cell cycle phases, as indicated by increased Ki-67 expression. In the context of SCI, this system could enhance endogenous repair by promoting neural stem cell proliferation and support cell-based therapies as a 3D scaffold.



(A) Confocal fluorescence of control, QUE-Mn₃O₄@PDA, and QUE-Mn₃O₄@PDA-GelMA.



(B) MFI of Ki-67 for each sample.

Figure 9. Confocal fluorescence of nanoparticles and hydrogel complex

4. Conclusion

This study developed the QUE-Mn₃O₄@PDA-GelMA hydrogel as a solution for SCI repair, addressing two major barriers to neural recovery. The hydrogel combines Mn₃O₄ nanozymes for

ROS scavenging, polydopamine for nanoparticle stabilization, quercetin for anti-inflammatory effects, and GelMA as a biocompatible scaffold. These components are integrated using a simple fabrication process involving dopamine polymerization and visible-light crosslinking. Physicochemical tests confirmed the hydrogel's suitability for SCI applications, showing mechanical properties similar to spinal soft tissue, controlled degradation to avoid collapse, and a porous structure that supports cell activity and nutrient transport. Biological assays demonstrated the hydrogel's safety for neural cells, its ability to reduce ROS, promote cell proliferation, and suppress pro-inflammatory macrophage polarization. By transforming the injury microenvironment into one that supports neural protection and regeneration, the QUE-Mn₃O₄@PDA-GelMA hydrogel shows strong potential for SCI therapy. Future studies will evaluate its in vivo performance to optimize its design and advance clinical translation.

References

- [1] Hu X, Xu W, Ren Y, et al. Spinal cord injury: molecular mechanisms and therapeutic interventions [J]. *Signal transduction and targeted therapy*, 2023, 8(1): 245.
- [2] Fan B, Wei Z, Feng S. Progression in translational research on spinal cord injury based on microenvironment imbalance [J]. *Bone research*, 2022, 10(1): 35.
- [3] Zheng J, Chen T, Wang K, et al. Engineered multifunctional zinc–organic framework-based aggregation-induced emission nanozyme for accelerating spinal cord injury recovery [J]. *ACS nano*, 2024, 18(3): 2355-69.
- [4] Gao W, He J, Chen L, et al. Deciphering the catalytic mechanism of superoxide dismutase activity of carbon dot nanozyme [J]. *Nature communications*, 2023, 14(1): 160.
- [5] Tang M, Zhang Z, et al. Manganese-based nanozymes: preparation, catalytic mechanisms, and biomedical applications [J]. *Advanced Healthcare Materials*, 2022, 11(21): 2201733.
- [6] Fan R, Chuan D, Liu Z, et al. Antioxidant MnO₂ nanozymes-encapsulated hydrogel synergistically regulate the spinal ROS microenvironment and promote spinal cord repair [J]. *Chemical Engineering Journal*, 2023, 478: 147148.
- [7] Sun Y, Zhang J, Gu Y, et al. Biomineralized MnO₂ nanoparticle-constituted hydrogels promote spinal cord injury repair by modulating redox microenvironment and inhibiting ferroptosis [J]. *Pharmaceutics*, 2024, 16(8): 1057.
- [8] Li H, Yin D, et al. Polydopamine-based nanomaterials and their potentials in advanced drug delivery and therapy [J]. *Colloids and Surfaces B: Biointerfaces*, 2021, 199: 111502.
- [9] Dong Z, et al. Polydopamine nanoparticles as a versatile molecular loading platform to enable imaging-guided cancer combination therapy [J]. *Theranostics*, 2016, 6(7): 1031.
- [10] Li Y, Yao J, et al. Quercetin, inflammation and immunity [J]. *Nutrients*, 2016, 8(3): 167.
- [11] Jiang M, Lv Z, Huang Y, et al. Quercetin alleviates lipopolysaccharide-induced inflammatory response in bovine mammary epithelial cells by suppressing TLR4/NF-κB signaling pathway [J]. *Frontiers in Veterinary Science*, 2022, 9: 915726.
- [12] Ahmed E M. Hydrogel: Preparation, characterization, and applications: A review [J]. *Journal of advanced research*, 2015, 6(2): 105-21.
- [13] Lv B, Lu L, et al. Recent advances in GelMA hydrogel transplantation for musculoskeletal disorders and related disease treatment [J]. *Theranostics*, 2023, 13(6): 2015.
- [14] Zhang Y, Zhuo G, Yu F, et al. The role of Genipin crosslinked extracellular matrix loaded bFGF in the repair of peripheral nerve injury [J]. *BMC biotechnology*, 2025, 25(1): 76.
- [15] Yao J, Cheng Y, Zhou M, et al. ROS scavenging Mn₃O₄ nanozymes for in vivo anti-inflammation [J]. *Chemical science*, 2018, 9(11): 2927-33.
- [16] Yue K, Trujillo-de Santiago G, Alvarez M M, et al. Synthesis, properties, and biomedical applications of gelatin methacryloyl hydrogels [J]. *Biomaterials*, 2015, 73: 254-71.
- [17] Zheng J, Li C, Zhang Q, et al. Bioinspired porous core-shell microspheres with spatiotemporal delivery coordinate immunomodulatory-osteogenic coupling via NF-κB/P-STAT6 and Rho/MAPK signaling for enhanced calvarial regeneration [J]. *Bioactive Materials*, 2025, 54: 103-25.
- [18] Inwald E, Klinkhammer-Schalke M, Hofstädter F, et al. Ki-67 is a prognostic parameter in breast cancer patients: results of a large population-based cohort of a cancer registry [J]. *Breast cancer research and treatment*, 2013, 139(2): 539-52.