

A MnO₂ Nanoparticle-Dotted Hydrogel Promotes Spinal Cord Repair *via* Regulating Reactive Oxygen Species Microenvironment and Synergizing with Mesenchymal Stem Cells

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ABSTRACT. Spinal cord injury (SCI) is one of the most debilitating injuries and transplantation of stem cells in a scaffold is a promising strategy for the treatment. However, the stem cell treatment of SCI has been severely impaired by the increased generation of reactive oxygen species in the lesion microenvironment, which can lead to a high level of stem cell death and dysfunction. Herein, a MnO₂ nanoparticle (NP)-dotted hydrogel is prepared through dispersion of MnO₂ NPs in a PPFLMLLKSTR peptide modified hyaluronic acid hydrogel. The peptide modified hydrogel enables the adhesive growth of mesenchymal stem cells (MSCs) and nerve tissue bridging. The MnO₂ NPs alleviate the oxidative environment, thereby effectively improving the viability of MSCs. Transplantation of MSCs in the multifunctional gel generates a significant motor function restoration on a long-span rat spinal cord transection model and induces an *in vivo* integration as well as neural differentiation of the implanted MSCs, leading to

a highly efficient regeneration of central nervous spinal cord tissue. Therefore, the MnO₂ NP-dotted hydrogel represents a promising strategy for stem cell-based therapies of central nervous system diseases through the comprehensive regulation of pathological microenvironment complications.

KEYWORDS. manganese dioxide nanoparticle, reactive oxygen species, hydrogel scaffold, mesenchymal stem cells, spinal cord injury

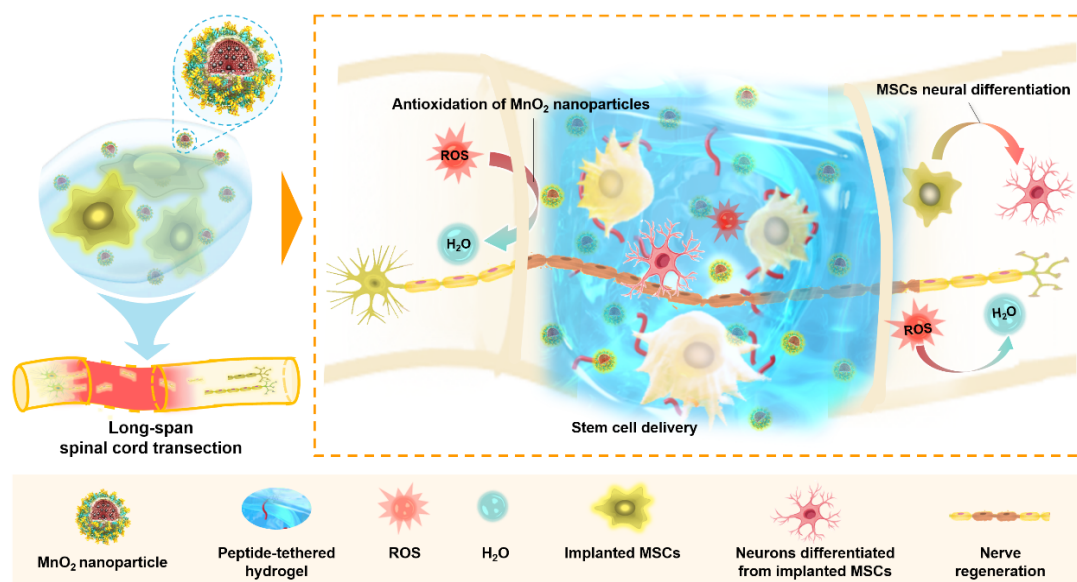
Traumatic spinal cord injury (SCI) is one of the most debilitating injuries with more than 27 million patients suffering with paralysis and depression after SCI across the world.¹⁻³ The highly limited self-recovery capacity and the extreme susceptibility of central nervous system (CNS) tissues present great challenges for nerve tissue repair.⁴ Currently available clinical therapies are largely palliative and unable to provide significant functional recovery.⁵ As a result, SCI patients usually suffer with permanent sensory and motor deficiency, and even fatal complications.

The transplantation of stem cells with biomaterial scaffolds has proven to be a promising strategy for SCI treatment, in which stem cells like mesenchymal stem cells (MSCs) compensate for the damaged neurons and neurotrophs, whilst scaffolds provide an extracellular matrix (ECM) for the implanted cells and guidance for tissue regrowth.⁶ The synergistic roles of MSCs and hydrogel scaffolds in nerve tissue regeneration after spinal cord transection has been demonstrated in our previous study.⁷ Especially, hyaluronic acid (HA), a polysaccharide derived from natural ECM, has been proved favorable for spinal cord tissue repair due to its inhibitory effect on glial scar formation.⁸ HA hydrogels could be fabricated with highly porous structures as well as viscoelasticity properties close to those of natural CNS tissue,^{7,9} and the HA scaffold has shown a neuroprotective effect after hemisection SCI.¹⁰ Since the lack of adhesive cues limits the bridging effect of HA, we have developed an adhesive HA hydrogel through one-pot gelation and peptide modification in our previous study. The laminin-derived peptide PPFLMLLKGSTR exhibited superior effect to promote stem cell adhesive growth and nerve tissue bridging.⁷

However, the efficacy of SCI treatment with stem cells in a hydrogel scaffold was severely reduced by the increased generation of reactive oxygen species (ROS) and severe inflammatory response in the SCI, which led to a high level of stem cell death and dysfunction.¹¹⁻¹² The

generation of ROS and severe inflammatory response occurred after the primary trauma, which not only affected the stem cells but also induced severe and continuous damage to the epicentral and surrounding nerve tissues.¹³⁻¹⁴ Thus, an effective method to eliminate ROS in the SCI in combination with treatment using stem cells in a hydrogel scaffold is highly desired to improve the SCI treatment. Recently, MnO₂ nanoparticles (NPs) were found able to catalyze the decomposition of H₂O₂ into H₂O and O₂ in a tumor microenvironment,¹⁵⁻¹⁶ and we reported a facile method to prepare biocompatible MnO₂ NPs through albumin-templated biomineralization.¹⁷

In this study, a MnO₂ NP-dotted HA hydrogel was constructed through dispersion of MnO₂ NPs in the hydrogel during gelation between HA-hydrazide chains and PPFLMLLKSTR peptide modified aldehyde-HA chains (HA-peptide). The peptide modified hydrogel could provide stem cell adhesive growth and nerve tissue bridging, and the MnO₂ NPs could alleviate the oxidative environment, thereby effectively improving the viability of MSCs (**Scheme 1**). The versatile MnO₂ NP-dotted hydrogel elicited significant restoration of motor function on a long-span rat spinal cord transection model and induced *in vivo* integration as well as neural differentiation of the implanted MSCs, leading to a highly efficient regeneration of central nervous spinal cord tissue. The MnO₂ NP-dotted hydrogel represents a promising strategy for stem cell-based therapies of CNS diseases through comprehensive regulation of pathological microenvironment complications.



Scheme 1. Concept illustration. The MnO₂ NP-dotted hydrogel is fabricated and dully with MSCs for implantation after a serious long-span spinal cord transection with a lesion gap of 4 ± 0.5 mm. The MnO₂ NP improves the survival, integration and neural differentiation of the transplanted MSCs and promotes nerve tissue regeneration *via* mitigating the oxidant microenvironment.

Results and Discussion

Construction of the MnO₂ NP-dotted hydrogel. The MnO₂ NP-dotted hydrogel was fabricated through facile crosslinking of HA-peptide and HA-adipic dihydrazide (HA-ADH) dissolved in a precursor solution containing homogeneously suspended MnO₂ NPs. Specifically, aldehyde and hydrazide groups were first introduced onto the HA chains through NaIO₄ oxidization and ADH modification,¹⁸⁻¹⁹ and the modified products were examined by Fourier transform infrared (FTIR) (**Figure 1a**) and ¹H NMR (**Figure 1b**), respectively. The HA-peptide was prepared by conjugating the adhesive peptide PPFLMLLKGSTR onto the aldehyde-modified HA chains (HA-CHO) (**Figure 1c**) at 1:64 (mole ratio, peptide to HA-CHO). The remaining -CHO groups

in HA-CHO were then crosslinked with the hydrazide groups in HA-ADH to form the peptide-modified hydrogel in the presence (MnO₂ NP-dotted hydrogel) or absence (blank hydrogel) of MnO₂ NPs (**Figure 1d**). The MnO₂ NP-dotted hydrogel showed much stronger magnetic resonance imaging (MRI) signals than the blank hydrogel because MnO₂ NPs could enhance the MRI through shorting the longitudinal relaxation time of water (**Figure 1e**). Both the blank (**Figure 1f**) and MnO₂ NP-dotted hydrogels (**Figure 1g**) exhibited three-dimensional (3D) highly porous inner structures. X-ray photoelectron spectroscopy (XPS) measurement verified the encapsulation of MnO₂ NPs in the hydrogel (**Figure 1h**).

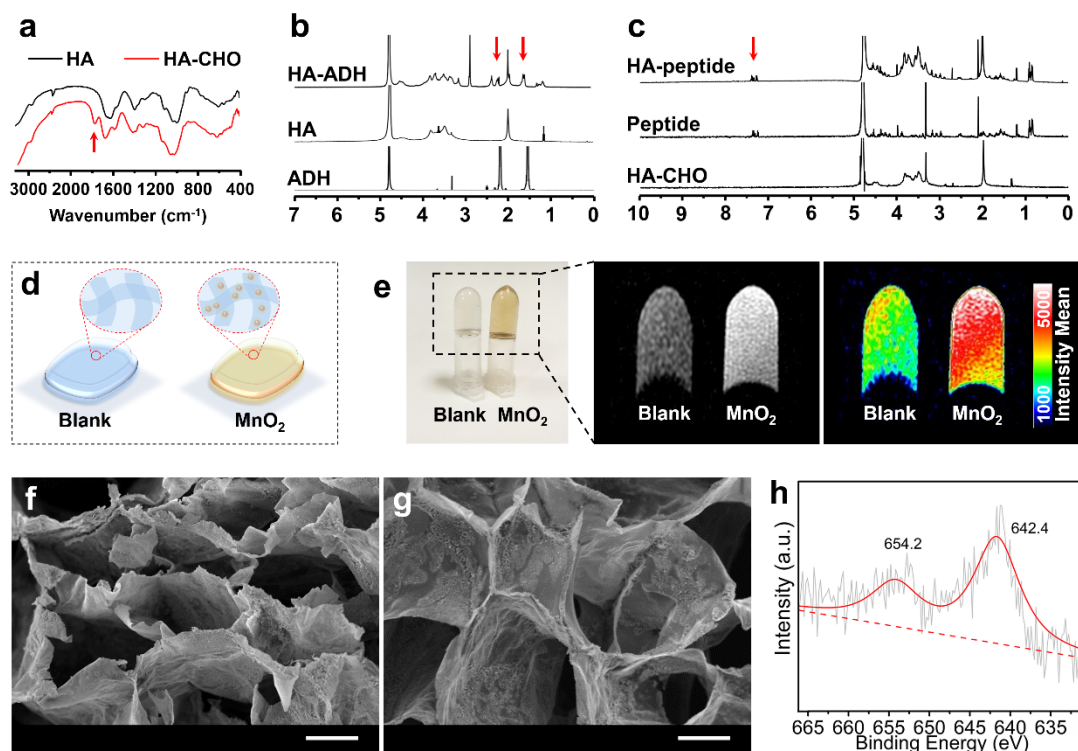


Figure 1. Characterization of materials and hydrogels. a) FTIR detection of aldehyde-modified HA chains (HA-CHO). b, c) ¹H NMR detection of HA-adipic dihydrazide (HA-ADH) (b) and peptide modified aldehyde-HA chains (HA-peptide) (c). Red arrows indicated characteristic peaks in the products. d) Schematic illustration of HA hydrogels in the presence or absence of

MnO₂ nanoparticles (NPs). e) The blank and MnO₂ NP-dotted hydrogels were imaged by MRI, which detected signals of MnO₂ NPs. f, g) Scanning electron microscopy (SEM) micrographs of blank (f) and MnO₂ NP-dotted (g) hydrogel scaffolds showing highly porous 3D structures. h) XPS examination of the MnO₂ NP-dotted hydrogel confirmed successful encapsulation of MnO₂ NPs in the hydrogel. Scale bar, 100 μ m.

Antioxidant and stem cell-protective effects of the MnO₂ NP-dotted hydrogel. To investigate the cytocompatibility of the hydrogels, the MSCs derived from human placenta amnion were cultured in the 3D hydrogels *in vitro*. The cells exhibited similar adhesive morphologies in the blank and MnO₂ NP-dotted hydrogels (**Figure 2a**), suggesting that encapsulation of MnO₂ NPs made no obvious influence on the hydrogel biocompatibility. The protective function of the hydrogels to MSCs against ROS microenvironment was then evaluated. To simulate the pathological oxidative microenvironment, H₂O₂ was added into cell culture medium at a concentration of 0.1 mM.²⁰ The hydrogels were immersed in the oxidative medium without the presence of cells and were detected for ROS neutralization effects (**Figure 2b**). Since H₂O₂ could react with the proteins in FBS from the cell culture medium, the H₂O₂ level also decreased in the cell culture medium with the presence of the blank hydrogel. Compared to the blank hydrogel, the MnO₂ NP-dotted hydrogel significantly reduced the H₂O₂ content both after 1 h and 2 h of incubation. MSCs were then cultured in the hydrogels under the simulated ROS condition (**Figure 2c**). Dichloro-dihydro-fluorescein diacetate (DCFH-DA) staining showed a significantly lower intracellular ROS level in the cells cultured in the MnO₂ NP-dotted hydrogel compared to the blank hydrogel (**Figure 2d**), indicating an efficient antioxidant function of the MnO₂ NP-dotted hydrogel. The MSC viability was examined by Live/Dead staining. While the blank hydrogel exhibited an extensive distribution of dead cells, only a small proportion of dead

cells were observed in the MnO₂ NP-dotted hydrogel, indicating that the existence of MnO₂ NPs could significantly decrease cell death under oxidative pressure (**Figure 2e**).

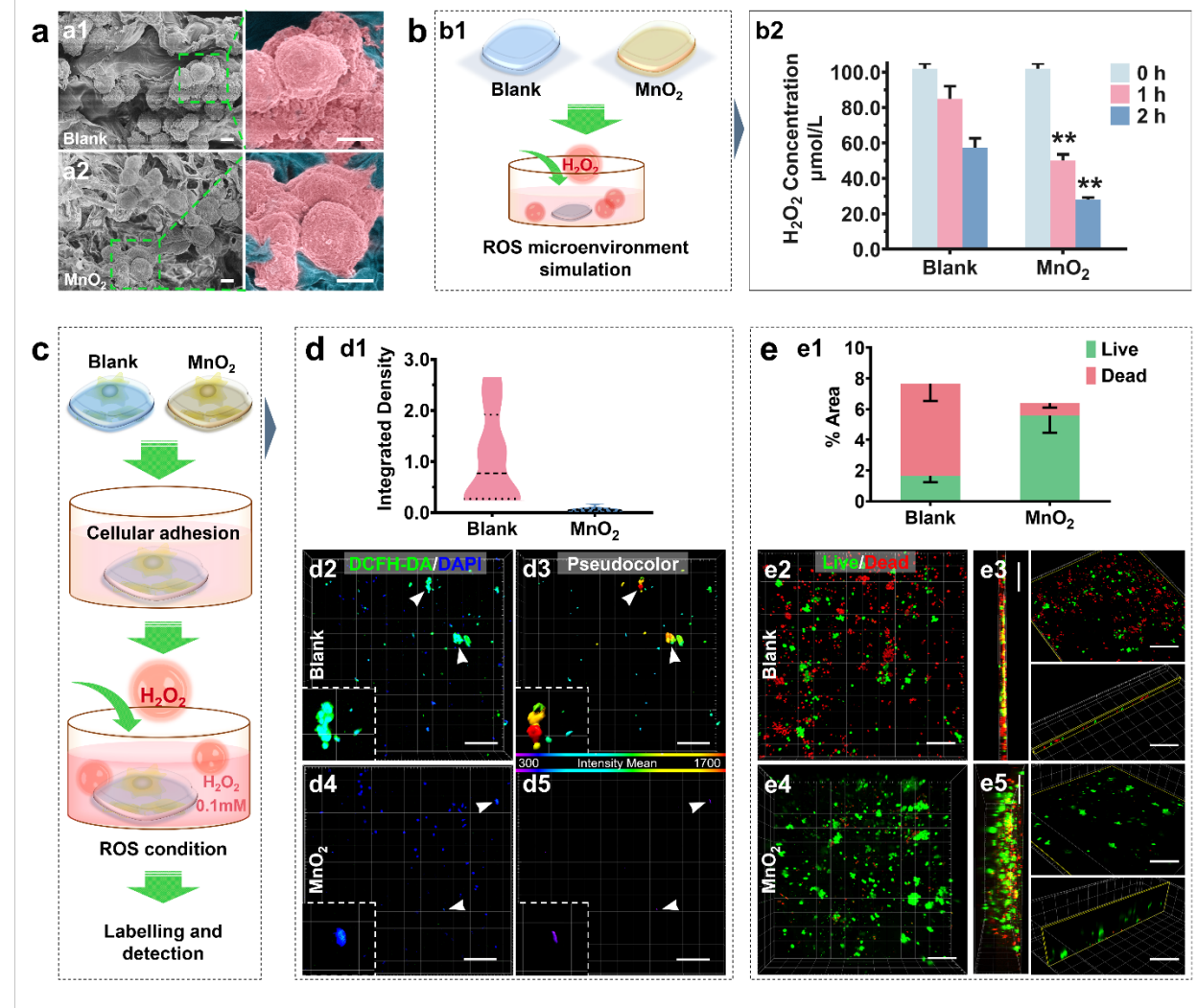


Figure 2. *In vitro* evaluation of antioxidant effects of the MnO₂ nanoparticle (NP)-dotted hydrogel. a) Scanning electron microscopy (SEM) micrographs showing similar adhesive morphologies of MSCs in the blank (a1) and MnO₂ NP-dotted (a2) hydrogels. False-color images showed more detailed views of the boxed areas. b) Evaluation of *in vitro* antioxidant effects of the blank and MnO₂ NP-dotted hydrogels through simulating pathological ROS microenvironment in the absence of cells. An initial concentration of H₂O₂ at 0.1 mM was used

in the culture medium without the presence of cells (b1) and the H₂O₂ levels were detected after incubation with the hydrogels for 1 h and 2 h (b2). Data in graph is presented as average $\pm$ SD (n =5). The significance between Blank and MnO₂ groups at each time point is assessed by unpaired t-test (two-tailed value), and the double asterisk symbol (**) denotes to $p < 0.001$. c-e) Protective effects of the hydrogels to 3D cultured MSCs were investigated in a simulated *in vitro* ROS microenvironment. c) MSCs were encapsulated in the hydrogels and allowed to adhere overnight before being exposed to the ROS microenvironment simulating medium containing 0.1 mM H₂O₂. d) After 24 h, the intracellular ROS levels of MSCs were quantified (d1) in the blank (d2, d3) and MnO₂ NP-dotted (d4, d5) hydrogels after Dichloro-dihydro-fluorescein diacetate (DCFH-DA) labelling (n=3), demonstrating effective antioxidant impact of the MnO₂ NP-dotted hydrogel for the MSCs. Green, DCFH-DA; blue, DAPI. Fluorescence of DCFH-DA in (d2, d4) were analyzed using pseudocolor (d3, d5) according to mean intensities to further show more detailed differences. e) Cytoviability of the MSCs after 24 h of incubation was analyzed by Live/Dead assay. Numbers of live cells (green) and dead cells (red) were quantified in terms of stained areas (e1). Data in graph is presented as average $\pm$ SD (n= 9 views). Representative images of the cells in the blank (e2, e3) and MnO₂ NP-dotted (e4, e5) hydrogels were presented, with n= 3 hydrogels for each group. Scale bar, 4 μ m (a), 100 μ m (d and e).

The antioxidant function of the MnO₂ NP-dotted hydrogel was further investigated *in vivo* after 7 days of implantation of the MSC-encapsulated hydrogels. The intracellular ROS level was significantly diminished in the spinal cord tissue after implantation of the MnO₂ NP-dotted hydrogel, as indicated by dihydroethidium (DHE) staining (**Figure 3a**). The peroxidation products of the spinal cord tissue were detected in the lesion site, showing that both the lipid peroxidation product 4-hydroxynonenal (4-HNE) (**Figure 3b**) and the oxidative DNA damage

marker 8-hydroxy-2'- deoxyguanosine (8-OHdG) (**Figure 3c**) were effectively reduced in the spinal cord tissues as a result of implantation of the MnO₂ NP-dotted hydrogel. Since the peroxidation damage is a dominant factor resulting in secondary nerve damage and hindering tissue recovery, the demonstrated antioxidant performance indicated the promising application of the MnO₂ NP-dotted hydrogel in nerve tissue repair after SCI.

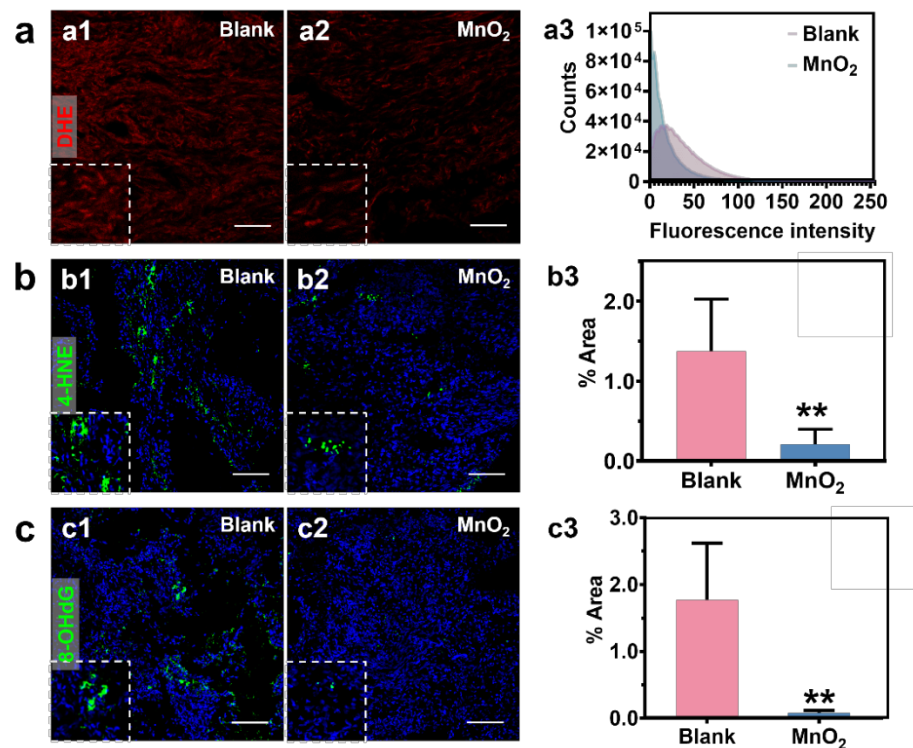


Figure 3. MSC-encapsulated hydrogels were implanted into rat spinal cord tissue after a long-span transection and antioxidant effects were detected after 7 days of implantation. a) dihydroethidium (DHE) staining was applied to directly reflect the ROS levels in the tissues. The corresponding representative micrographs (a1, a2) and histogram of the fluorescence intensity (a3) showed an obvious decrease of DHE distribution in the MnO₂ group. b, c) Products of lipid peroxidation 4-hydroxynonenal (4-HNE) (b) and oxidative DNA damage 8-hydroxy-2'-deoxyguanosine (8-OHdG) (c) in the spinal cord tissues were detected, which confirmed the

effective antioxidant and protective functions of the MnO₂ nanoparticle (NP)-dotted hydrogel *in vivo*. Extent of oxidative damage was analyzed in terms of the percentage of the stained area with $n = 6$ micrographs ($20 \times$ objective magnification) of 3 animals for each group (b3, c3). Data represents mean $\pm$ SD. Statistical analysis among groups was preformed using two-tailed unpaired t-test. The double asterisk symbol (**) denotes to $p < 0.001$. Details were shown in boxes with dashed borders in representative images. Scale bar, 100 μ m.

Nerve regenerative function and synergism with MSCs. The effects of the MnO₂ NP-dotted hydrogel on nerve tissue regeneration and stem cell reinforcement were evaluated through implantation in a rat long-span transection SCI model with a lesion gap of 4 ± 0.5 mm. The MnO₂ NP-dotted hydrogel encapsulated with MSCs (MnO₂ group) was implanted into the lesion gap immediately after surgery, with merely PBS implantation (SCI group) and implantation of the MSC-encapsulated blank hydrogel (Blank group) conducted in parallel as controls (**Figure 4a**).

The rat transection SCI models have been typically reported with a lesion gap of 1-2 mm.²¹⁻²³ However, due to the higher self-recovery capacity of rat nerve tissues, partial motor function recovery has been reported even without any treatment in these rat models.^{7, 24-26} Neurological outcomes of therapy could be confusing and overestimated because of a certain degree of self-recovery. In this study, the severe long-span rat transection SCI model was established to mimic the extremely limited self-recovery capacity of motor function in clinical cases. The spinal cord transection gap in this study was 4 ± 0.5 mm, about 1 mm longer than the width of the spinal cords (SC) (**Figure 4b**) and 2.5 mm longer than that of the widely reported transection models with lesion gaps of 1-2 mm.

After the serious SCI, rats exhibited almost total paralysis without treatment (SCI group), while function restoration was effectively achieved in the MnO₂ group, with significantly superior Basso, Beattie, Bresnahan (BBB) scores (9.75 ± 0.66) compared to the Blank group (7 ± 1.22) and the SCI group (1.75 ± 0.66) on Day 28 post-surgery (**Figure 4c**). Notably, compared to the recovery in Blank group, there was a more rapid restoration of motor functions in MnO₂ group (**Figure 4c and d, Video S1-S10**). A rapid recovery was elicited by the MnO₂ NP-dotted hydrogel within the first two weeks, with 75% of the rats obtaining BBB scores over 8 on Day 14 post-surgery (Figure 4d). In contrast, no animal could get 8 points without treatment over 28 days, and in the Blank group, there were only 12.5% on Day 14 and 37.5% on Day 28 post-surgery. This was in good agreement with the cumulative BBB scores (**Figure S1a**) and more sensitive 7-point scale BBB subscores (**Figure S1b**). In comparison to the sweeping hindlimb without weight support after 28 days of treatment with the MSC-encapsulated blank hydrogel (**Figure 4e, Video S5**), the MnO₂ NP-dotted hydrogel containing MSCs induced frequent weight-supported plantar steps and occasional coordination of hindlimb with forelimb (**Figure 4f, Video S10**). The consecutive walking patterns of the rats in the two groups were recorded and presented in supplementary videos. The therapeutic result in the MnO₂ group was significantly better than that in the Blank group and the previously reported SCI treatment results,^{7, 23-27} even though the 4 ± 0.5 mm long-span transection in this study was much broader than 1.5 mm transection in previous studies, indicating the notable regenerative impact of the MnO₂ NPs.

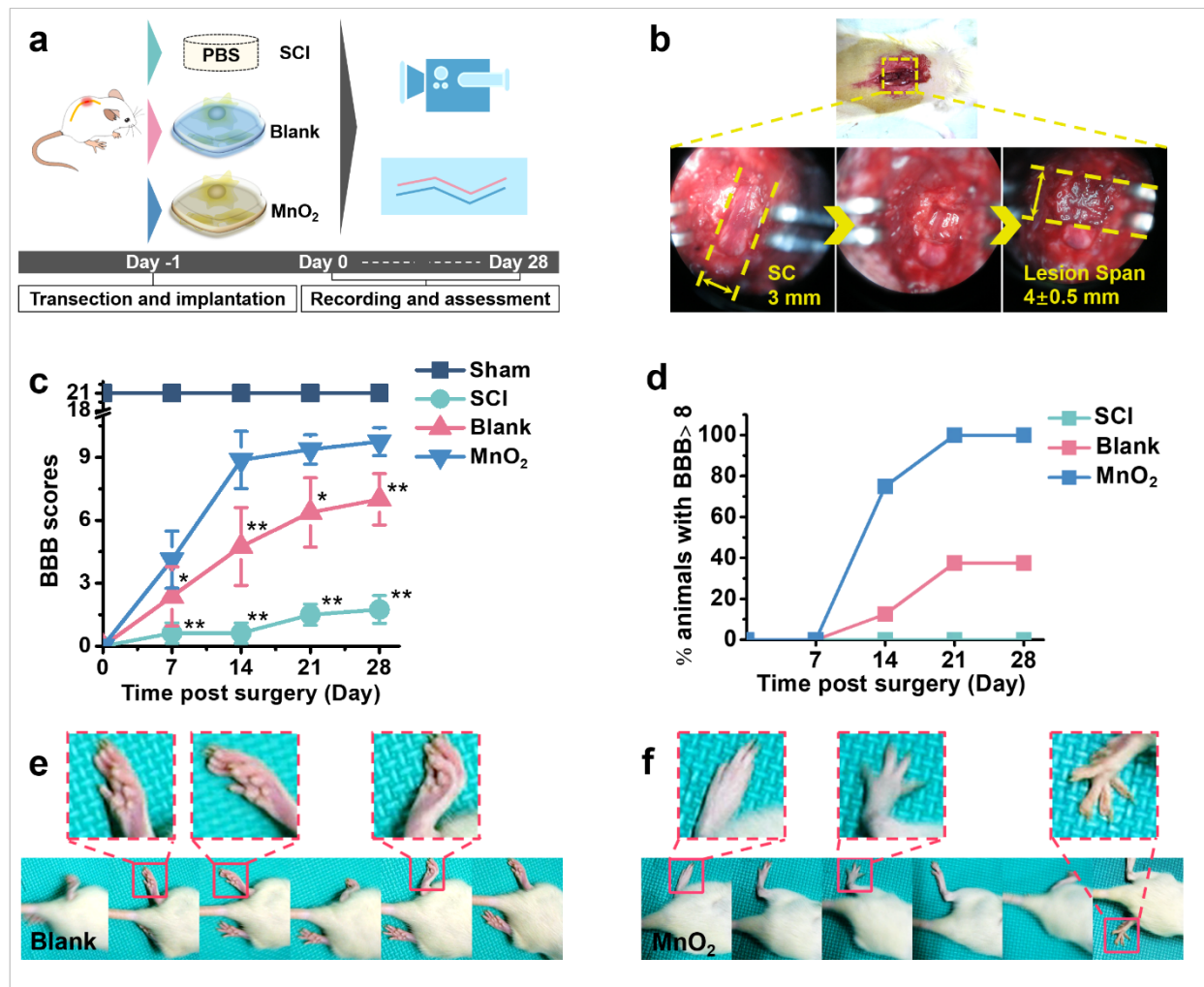


Figure 4. Motor functional recovery of rats after treatment of the hydrogels encapsulated with MSCs. a) Schematic illustration of the therapeutic experiment. b) Surgical procedure of long-span spinal cord transection and implantation of the MSCs-encapsulated hydrogels. The span of spinal cord transection was larger than the width of spinal cord tissue, with a length of 4 ± 0.5 mm compared to the width of 3 mm. c) Function recovery of animals on Day 28 post-surgery was evaluated through BBB scores. d) Analysis of % animal of BBB scores > 8 further indicated the function restoration in the presence of the MnO₂ nanoparticle (NP)-dotted hydrogel containing MSCs. e, f) Typical records of animal walking gaits on Day 28 post-surgery showed direct evidence of the hindlimb walking patterns of the Blank group e) and the MnO₂ group f).

Significance assessment between two groups (Blank and MnO₂ group, SCI and MnO₂ group) was conducted using Mann–Whitney U test for data with normal distribution as well as variance homogeneity and two-tailed unpaired t-test for data with non-normal distribution. * $p < 0.05$, ** $p < 0.001$.

To further understand the anatomical basis of the motor function recovery elicited in the groups with and without MnO₂ NPs, immunostaining of spinal cord sagittal sections was applied to observe the nerve tissue regeneration. Glial scar formation that encompasses the lesion and isolates a noxious cavity is a leading inhibitor for nerve tissue regrowth after SCI. The main contributor of glial scars is the activated astrocytes, of which the recognized marker is glial fibrillary acidic protein (GFAP). The density and distribution of neurofilaments (NF) across the gathered astrocytes, an indicator of mature neurons, have been proven to be negatively correlated with that of GFAP and suggest the extent of nerve tissue regeneration.²⁸ In the 1.5 mm transection cases previously reported, the implantation of MSC-encapsulated HA hydrogel was able to suppress the glial scar obstacle. While in the current long-span transected spinal cord treated with the MSC-encapsulated blank hydrogel, excessive density of GFAP positive astrocytes was observed in the lesion edge on Day 28 post-surgery, suggesting the glial scar surrounding the lesion site (**Figure 5 a1**). In contrast, incorporation of MnO₂ NPs effectively arrested the over reactivity and gathering of astrocytes during the secondary injury and triggered extensive redistribution of longer nerve fibers as indicated by the NF fluorescence surmounting the GFAP⁺ astrocytes (**Figure 5 a2**). The density ratios of NF and GFAP in the lesion edge was reversed by the MnO₂ NPs (**Figure 5b**). As the lesion site was determined by the morphology of the injury edge lines, tissues within and over approximately 1.5 mm distance from the lesion edges were defined as the adjacent and distant regions, respectively. Tissues of the two groups

were analyzed for nerve fiber regeneration throughout not only the lesion and adjacent tissue but also distant segments of injured spinal cord (**Figure 5c**), showing the significant regenerative effect of the MnO₂ NP-dotted hydrogel. Consecutive NF distribution (**Figure 5d**) in representative full-field views (**Figure 5e and 5f**) was further quantified. For almost all of the visualized segments of the injured spinal cord tissue, the density of NF exhibited an obvious superiority in the MnO₂ group compared to the Blank group, which was consistently presented by the merged (**Figure 5 e1 and f1**) and single NF channel (**Figure 5 e2 and f2**) views. In both the adjacent (**Figure 5 e3 and f3**) and distant regions (**Figure 5 e4 and f4**), the incorporation of MnO₂ NPs induced longer and larger amounts of nerve fibers with the mitigated GFAP⁺ astrocyte accumulation. The mature NF⁺ fibers surmounting the lesion edge in MnO₂ group mostly exceeded 5 μm in length with a maximum of 50 μm, significantly different from the punctate fibers in Blank group (**Figure S2**). In addition, CD31 labelling was used to indicate the angiogenesis in lesion sites of the injured spinal cords on Day 28 post-surgery and showed a superior angiogenesis effect in the MnO₂ group with a higher density and larger aggregation of the CD31 positive structures (**Figure S3**). These results presented consistent evidence with the locomotor function outcomes, suggesting that the MnO₂ NP-dotted hydrogel encapsulated with MSCs contributed to functional nerve recovery through the inhibitory effect on glial scar formation and the facilitation for nerve fiber regeneration across the lesion.

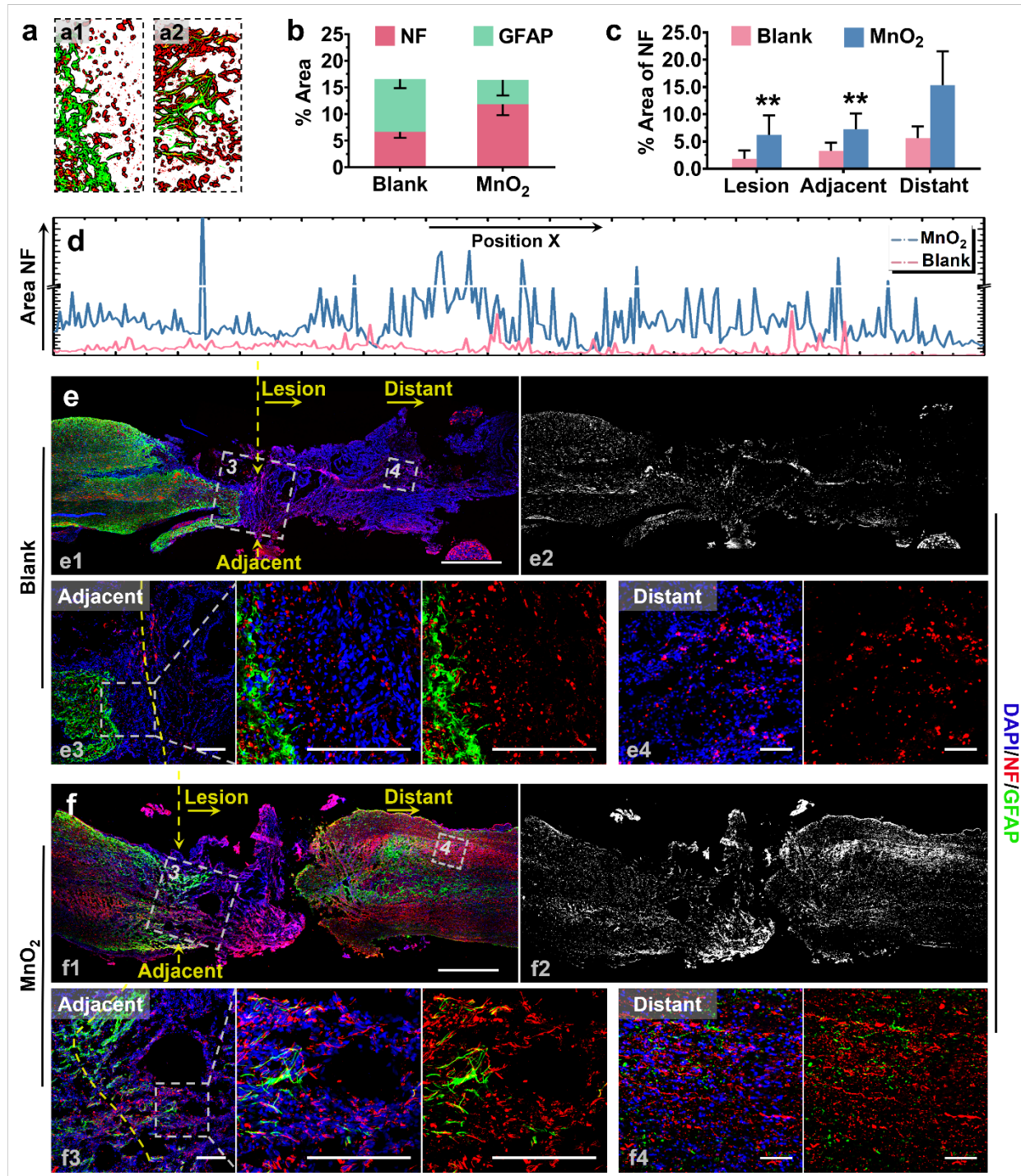


Figure 5. Investigation of nerve fiber regeneration across the lesion cavity on Day 28 post-surgery by labelling of neurofilament (NF, stained to red) and glial fibrillary acidic protein (GFAP, stained to green). a, b) Analysis of NF redistribution and GFAP⁺ astrocyte gathering in

the areas adjacent to the lesion site. a) Glial scar formation was indicated by the gathered GFAP⁺ astrocytes in the spinal cord implanted with the blank hydrogel (a1), whereas implantation with the MnO₂ nanoparticle (NP)-dotted hydrogel (a2) restrained the excessive gathering of the activated astrocytes and significantly induced NF regeneration across the lesion border. b) Redistribution of NF across the GFAP bordering was quantified in terms of % area of positive staining. c, d) Nerve fiber regeneration was analyzed by quantification of NF in sagittal sections. Redistribution of NF was analyzed in the lesion site, adjacent tissue and distant areas respectively of n = 10 micrographs for each group (c) and in representative sections for continuous distribution in the whole area as presented by continuous curves (d). e, f) Representative micrographs of the Blank (e) and MnO₂ (f) groups showed the full-field situations of NF and GFAP expression (e1, f1) and the separated channel of NF distribution (e2, f2) in the spinal cord tissues, with detailed images magnified from the boxed adjacent (e3, f3) and distant (e4, f4) regions. Dashed lines denote to the lesion edges of interest. More and longer nerve fibers were observed in not only the lesion site and the adjacent tissue, but also the distant segments of the injured tissue in the MnO₂ group than that in Blank group. Significance in (c) was assessed by Mann–Whitney U test, and the asterisk symbols (**) denotes to p< 0.001. Scale bar, 1 mm (e1, f1), 200 μm (e3, f3), 100 μm (e4, f4).

The demonstrated nerve tissue regeneration ability of the MnO₂ NPs dotted in the HA hydrogel could be reasonably attributed to the roles of the MnO₂ NPs in antioxidant microenvironment mitigation and in the consequent protection for the implanted MSCs. The survival and integration of MSCs in the spinal cord tissues were investigated on Day 28 post-surgery, and the two groups with or without MnO₂ NPs were compared (**Figure 6**). In both groups, the implanted cells in hydrogels integrated into the host spinal cord tissues. The MSCs implanted with the

MnO₂ NP-dotted hydrogel turned out to be in larger amounts and more clustered both in the adjacent and in the distant areas of the injured spinal cord (**Figure 6a, Figure S4**). Moreover, the MnO₂ group exhibited a significantly higher degree of colocalization of MSCs and neurotransmitter choline acetyl transferase (ChAT), which indicated an extent of differentiation to cholinergic neurons. A more extensive distribution of ChAT in the MnO₂ group was also in agreement with the superior performance of motor functional restoration. The co-labelling of NF and GFP consolidated the result of NF regeneration shown in Figure 3 and provided understanding of the integration and differentiation of implanted MSCs (**Figure 6b and c**). The implanted MSCs effectively integrated into distant spinal cord tissues and exhibited neuronal differentiation especially in the distant (**Figure 6 b2, b5, and b6**) and adjacent (**Figure 6 b4 and b8**) regions. These activities were greatly attenuated in the absence of MnO₂ NPs (Figure 4c). Injury of spinal cord leads into neurogenic bladder, which is a fatal complication for SCI patients. The enhanced repair for the spinal cord nerve tissue in MnO₂ group was found to further protect the rat bladders after spinal cord transection (**Figure S5**). Pathological damage of the bladder tissue was effectively restrained after treatment in MnO₂ group, with reduced extents of bladder endometriosis, cell vacuolation, and bladder wall fibrosis compared to Blank group. These results showed effective functions of the MnO₂ hydrogel in spinal cord recovery in combination with hMSCs. In addition, effects of the MnO₂ NP-dotted HA hydrogel in the absence of MSCs were also evaluated and exhibited relatively limited facilitation on functional recovery and nerve regrowth (**Figure S6**). Taking account of the the essential roles of hMSCs demonstrated in previous report⁷, nerve recovery after SCI in the current strategy requires synergism of the ROS mitigation by the functional NPs and the cellular functions of the stem cells.

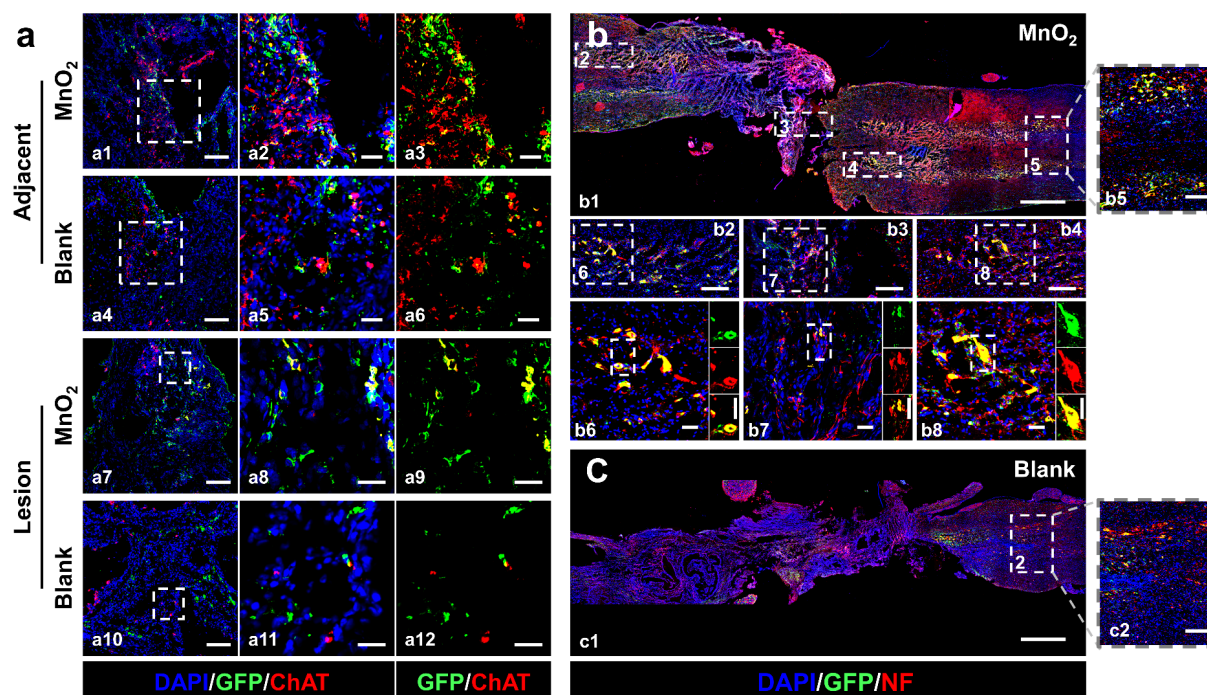


Figure 6. Integration of MSCs delivered in different hydrogels and neural restoration on Day 28 post-surgery. a) A notably larger amounts of implanted green fluorescent protein (GFP)-labelled stem cells (green) survived and integrated in both the adjacent (a1-a6) and lesion (a7-a12) areas of the host spinal cord tissue in the MnO₂ group (a1-a3, a7-a9), in comparison to the Blank group (a4-a6, a10-a11), accompanied by an extensive redistribution of neurotransmitter choline acetyl transferase (ChAT) (red) and its co-localization with the implanted stem cells. Boxed fields in (a1, a4, a7, a10) were magnified in (a2, a5, a8, a11) in the middle column, respectively, with specially separated channels of GFP-MSCs and ChAT on the right. b, c) Neuronal differentiation of integrated MSCs and the nerve regenerative effect in different regions of the injured spinal cord tissues were investigated in the MnO₂ (b) and Blank (c) groups. Nerve fibers were significantly regenerated by the MnO₂ nanoparticle (NP)-dotted hydrogel across the whole tissue (b1) with integrated MSCs (green) colocalizing with NF (red), the marker of mature neurons. MSCs integrating into the distant (b2) and adjacent (b4) regions exhibited larger total amount as

well as higher proportion of neural differentiation compared to those sustained in the lesion site (b3). Caudal distant segments of the tissue (b5) showed the same result as that in the rostral side (b2). Micrographs in (b6-b8) show the magnified views of the boxed fields in (b2-b4). Separated NF and GFP-MSCs channels of the boxed cells in (b6-b8) were shown in detail and presented on the right of each micrograph. Image in (c2) showed a detailed view of the boxed distant area of the Blank group in (c1), confirming the significant difference from the MnO₂ group in MSC survival, integration and differentiation, as well as in nerve fiber regeneration. Scale bar, 1 mm (b1, c1), 200 μ m (b2-b5, c2), 100 μ m (a1, a4, a7, a10), 50 μ m (b6-b8), 30 μ m (a2, a3, a5, a6), 20 μ m (a8, a9, a11, a12).

***In vivo* elimination of Mn and safety evaluation.** Elimination of Mn in the spinal cord tissues during 28 days after implantation was investigated (**Figure S7**). Rostral and caudal regions exhibited similar levels of Mn content, both significantly lower than that of the lesion site implanted with the MnO₂ NP-dotted hydrogel, which suggested a partial elimination of Mn in the lesion site during 28 days. Further comparison of the initial Mn content in the MnO₂ NP-dotted hydrogel and the Mn content in the implanted spinal cord tissue on Day 28 post-surgery showed that the amount of Mn decreased by about 87 % in the implanted lesion site, indicating the rate of Mn elimination *in vivo* during 28 days after implantation. In addition, tissues of the rat heart, liver, spleen, lung and kidney showed no obvious difference after the 28-day treatment by the MnO₂ NP-dotted hydrogel compared with those in the Blank and Sham groups (**Figure S8**). Also, the MnO₂ NPs were demonstrated previously biocompatible and safe in systemic application.¹⁷

Conclusions

In conclusion, an MnO₂ NP-dotted HA hydrogel was fabricated by dispersion of MnO₂ NPs in a PPFLMLLKWSTR peptide-modified hydrogel. The MnO₂ NP-dotted hydrogel could significantly improve the stem cell adhesive growth and nerve tissue bridging, and effectively alleviate the oxidative environment thereby effectively improving the viability of the MSCs. This, as a result, induced an *in vivo* integration as well as neural differentiation of the implanted MSCs, leading to a highly efficient regeneration of central nervous spinal cord tissue. The implantation of MSC in the multifunctional gel provided significant motor function restoration on a long-span rat spinal cord transection model. In addition, as the peptide PPFLMLLKWSTR modification of HA hydrogel has proved effective in the previous study as well as in the current strategy, other adhesive peptides, like RGD and IKVAV, may also be capable for this therapeutic strategy with proper design and optimization of the hydrogel modification in further studies. Therefore, the MnO₂ NP-dotted hydrogel represents a promising strategy for stem cell-based therapies of CNS diseases through the comprehensive regulation of pathological microenvironment complications.

Methods

Reagents and Animals. Hyaluronic acid was obtained from Novozymes (1.3 MDa for HA-ADH preparation and 2.3 MDa for HA-CHO preparation, Beijing, China). Potassium permanganate (KMnO₄) was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Bovine serum albumin (BSA) was obtained from Sangon Biotech Co., Ltd. (Shanghai, China). Dopamine hydrochloride (DA) were purchased from Tansoole Co., Ltd. (Shanghai, China). Low-sugar Dulbecco's modified Eagle's medium (DMEM), fetal bovine serum (FBS), penicillin, streptomycin, L-glutamine, and 0.25 wt.% trypsin with 0.02 wt.% Ethylene Diamine Tetraacetic Acid (EDTA) were purchased from Gibco BRL (Gaithersburg, MD). For use of MSCs derived

from amniotic membrane of human placenta, the informed signed consent was obtained from the patient or from next of kin.

Female SD rats of 220-250 g for animal model of SCI were purchased from Slac Laboratory Animal Co. Ltd. (Shanghai, China). All animal procedures and experiments were approved by the Institutional Animal Care and Use Committee at Zhejiang University.

Fabrication of MnO₂ NPs. The MnO₂ NPs were prepared according to our previous report with some modification.¹⁷ BSA (200 mg) was dissolved in an aqueous solution containing DA (100 mL, 100 mg) at room temperature. Then, KMnO₄ (2 mL, 30 mg) was added immediately to the above mixture. After stirring for 2 h, the obtained MnO₂ NPs were collected by dialysis and ultrafiltration.

Construction of Hydrogel Scaffolds. Gelation and peptide modification of HA hydrogels were based on crosslinking between -CHO and -NH₂ modified HA chains. HA of 2.3 MDa molecular weight (MW) was oxidized by NaIO₄ to introduce -CHO at -OH sites. In detail, HA (500 mg, 1.25 mmol) was dissolved in 150 mL ultrapure water, after which NaIO₄ solution (10 mg/mL) was added dropwise at a mole ratio of 0.5:1 (NaIO₄: HA). The oxidizing reaction was allowed for 2 days in the dark before ethylene glycol (600 μ L) was added and stirred for another 1 h to end the reaction. The product was dialyzed for 5 days and lyophilized.

The adhesive peptide PPFLMLLKGSTR was tethered on HA-CHO. HA-CHO (40 mg) was dissolved in PBS (pH = 7.4, 4 mL), and of the peptide (2 mg) was dissolved in DMSO (1.6 mL). The solutions were mixed thoroughly and kept stirred for 2 h to produce HA-peptide. After dialysis for 2 days in PBS, the product was lyophilized.

ADH was used for the introduction of -NH_2 in lower MW HA chains (1.3 MDa) through crosslinking of -NH_2 in ADH and -COOH in HA under catalyzing by 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) and 1-Hydroxybenzotriazole (HOBt).^{18, 29} Briefly, HA (270 mg, 0.675 mmol) was dissolved in ultrapure water (150 mL) and added with 30-fold mole ADH (20.25 mmol, 4.64 g). After complete dissolving and mixing, the pH was adjusted to 6.8. EDC (0.96 g) and HOBt (0.675 g) (both of 5 mmol) was dissolved in the solvent consisting of ultrapure water and DMSO at a volume ratio of 1:1 (5 mL each) and added into the reaction system. The pH of the mixture was adjusted and maintained to 6.8 for 4 h at room temperature, after which the reaction was allowed to continue overnight. Finally, the pH was adjusted to 7.0, and the product was exhaustively dialyzed against H_2O for 5 days.

HA-peptide and HA-ADH was diluted in PBS at $20 \text{ mg}\cdot\text{mL}^{-1}$ and $15 \text{ mg}\cdot\text{mL}^{-1}$, respectively. The blank hydrogel was formed after intensive mixing of HA-peptide solution (30 μL) and HA-ADH solution (30 μL). For construction of MnO_2 hydrogel, MnO_2 NPs were suspended in HA-peptide and HA-ADH solutions at a Mn content of $60 \text{ ng}\cdot\mu\text{L}^{-1}$ before the gelation. The Mn content in each hydrogel (60 μL) was 3.6 μg . The gelation was finally allowed at 4 $^\circ\text{C}$ overnight in 384-well plates to form the cylindrical-shaped hydrogels. The hydrogels were lyophilized and swelled again in PBS before further experiments.

Characterization of Hydrogel Scaffolds. The MnO_2 NPs was observed by a transmission electron microscopy (TEM, JEM-1230, acceleration voltage = 200 kV). The MR images of MnO_2 NP-dotted hydrogel were taken on a GE signa HDxt MRI 3-T system. The XPS measurements of MnO_2 NP-dotted hydrogel was performed by an X-ray photoelectron spectroscopy (XPS, ESCALAB 250Xi, Thermo Scientific) with the radiation from an Al $\text{K}\alpha$ (1486.6 eV) X-ray source.

Internal three-dimensional structures of the hydrogels were examined by Scanning Electron Microscopy (SEM) (SU-8010, Hitachi, Japan). The lyophilized blank and MnO₂ scaffolds were fractured for observation. Samples were coated with gold in vacuum before examination.

Isolation of MSCs. Human-placenta-derived MSCs were obtained according to previously reported protocols.³⁰ GFP-labelled MSCs were obtained through lentivirus (Gene Pharma, Shanghai, China) transfection of GFP-encoding gene into MSCs. MSCs were cultured in MesenCult Human Basal Medium with 10% of MesenCult Stimulatory Supplements at 37 °C in 5% CO₂ atmosphere. All protocols for handling of human tissues and cells were approved by the Research Ethics Committee of First Affiliated Hospital, School of Medicine, Zhejiang University (Reference number 2013–272).

***In vitro* 3D culture of MSCs.** MSCs derived from amniotic membrane of human placenta was three-dimensionally cultured in the hydrogels to evaluate the bio-adhesive properties and protective effects against ROS of the blank and MnO₂ NP-dotted hydrogel. A total of 1×10⁵ MSCs suspended in 40 μL PBS were slowly injected into the hydrogel, with repetitive injection at different sites of the hydrogel surfaces. Fresh culture medium added before the hydrogels were incubated in cell culture environment at 37 °C and 5 % CO₂ atmosphere. After 3 days of culture, MSCs in the hydrogels were examined for adhesive cellular morphologies by SEM. Hydrogels encapsulated with MSCs were washed by PBS and fixed in glutaraldehyde for 2 h and then in osmium tetroxide for 1 h sequentially. After the fixative was washed off, samples were dehydrated with graded ethanol. Fractures of the samples were examined.

To evaluate the protective function of the blank and MnO₂ hydrogels for MSCs against ROS, the hydrogels were encapsulated with MSCs as described above. The culture medium was replaced

by peroxidative medium containing 100 μM H_2O_2 at 24 h after cell encapsulation. After the peroxidative culture for another 24 hours, cells in the different hydrogels were detected with Live/Dead staining kit (Yeasen, Shanghai, China) and DCFH-DA staining kit (Beyotime, Shanghai, China) according to the provided protocols. Samples were observed using laser scanning confocal microscopy (A1R, Nikon, Japan), and the results were analyzed by Image J 1.52n and GraphPad prism 8.0.

Surgery for Spinal Cord Transection and Implantation. Complete transection SCI model was prepared with long-span lesion gap. After weighing and anesthesia, hair near the T10 spinous process on the back of the rats was removed. Then the skin was cut along the midline of the back, and the muscle was separated under aseptic condition. After laminectomy, the dorsal surface of the T9-10 segment was exposed. The spinal cord was transected to make a 4.0 ± 0.5 mm gap. After exact hemostasis, the hydrogel was implanted into the gap of spinal cord and was ensured to fit the lesion. GFP-MSCs at a concentration of 1×10^5 in 30 μL PBS were then implanted into the hydrogel using a micropipettor. Finally, the muscle and skin were sutured. For postsurgical care, penicillin was given to prevent infection within 7 days after operation, and the rats received manual massage of bladder twice daily until reflexive bladder control returned.

Locomotor Function Investigation. Hind limb motor functions of the rats were evaluated weekly after the surgery. Animals were allowed to walk freely in an open field, and the 21-point BBB locomotion testing was conducted by blinded observations within 5 min for each animal. To improve the sensitivity and reliability of the assessment, a BBB subscore test of a 7-point scale was applied at the same time. Results were analyzed using OriginLab 8.0 and GraphPad prism 8.0.

Tissue Processing. Animals were sacrificed at Day 7 for detection of DHE, 4-HNE and OHdG levels in the spinal cord tissues and at Day 28 for other examinations. The rats were perfused with isotonic physiological saline followed by 4% paraformaldehyde under deep anesthesia. Spinal cords were harvested and dissected with a total length of 1.5 cm encompassing the lesion site. The spinal cord samples were then processed according to the requirements of corresponding experiments.

Immunohistochemistry. For immunofluorescence labeling, spinal cord samples were embedded and frozen in optimal cutting temperature compound and then cryosectioned for 20 μm slices. After fixation in 4 °C acetone, primary antibodies of GFP, neurofilament (NF) or glial fibrillary acidic protein (GFAP) were incubated with the sections overnight at 4 °C. Alexa Fluor 488- and 594-conjugated secondary antibodies were incubated with the sections at 37 °C for 1 h. Nuclei were labeled using DAPI (Sigma, St. Louis, USA). The samples were observed using laser scanning confocal microscopy (LSM710NLO, Zeiss, Germany). ImageJ 1.52n was used for the quantitative analysis.

Examination of Mn elimination in spinal cord tissues. Contents of Mn were detected both at the initial time before implantation and at Day 28 after implantation. Spinal cord tissues embedded with MnO_2 hydrogels were harvested at Day 28 and samples of the rostral, lesion and caudal tissues were nitrated with HNO_3 and each diluted to 2 mL. For the initial content of Mn before implantation, MnO_2 hydrogels were nitrated with HNO_3 and each diluted to 2 mL. Finally, the Mn contents in the MnO_2 hydrogels and in the treated tissues were determined by an inductively coupled plasma-mass spectroscopy (ICP-MS, PerkinElmer).

Statistical Analysis. The results of quantitative data were showed as mean $\pm$ standard deviation (SD) values. Statistical analysis among groups was preformed using Mann–Whitney U test and t-test with IBM SPSS Statistics 22. When the P value of the t-test is less than 0.05, it is defined as a significant difference. Other analysis was conducted by OriginLab 8.0 and GraphPad prism 8.0.

ASSOCIATED CONTENT

Supporting Information.

The following files of supporting information are available free of charge on the ACS Publications website.

Supporting data, including cumulative BBB scores and BBB subscores on Day 28, quantification of the length of NF⁺ fibers across the spinal cord lesion edges in the Blank group and MnO₂ group, angiogenesis evaluation, quantification of the ChAT staining in the lesion and adjacent tissues of the Blank group and MnO₂ group, analysis of bladders in Sham, Blank and MnO₂ groups, therapeutic effect of the MnO₂ NPs-dotted hydrogel in absence of hMSCs, *in vivo* elimination of the MnO₂ nanoparticles, HE staining of systemic tissues in Sham, Blank and MnO₂ groups (PDF)

Video S1. Recording of rat hindlimb motor functions in Blank group at Day 1 (MP4)

Video S2. Recording of rat hindlimb motor functions in Blank group at Day 7 (MP4)

Video S3. Recording of rat hindlimb motor functions in Blank group at Day 14 (MP4)

Video S4. Recording of rat hindlimb motor functions in Blank group at Day 21 (MP4)

Video S5. Recording of rat hindlimb motor functions in Blank group at Day 28 (MP4)

Video S6. Recording of rat hindlimb motor functions in MnO₂ group at Day 1 (MP4)

Video S7. Recording of rat hindlimb motor functions in MnO₂ group at Day 7 (MP4)

Video S8. Recording of rat hindlimb motor functions in MnO₂ group at Day 14 (MP4)

Video S9. Recording of rat hindlimb motor functions in MnO₂ group at Day 21 (MP4)

Video S10. Recording of rat hindlimb motor functions in MnO₂ group at Day 28 (MP4)

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript. #These authors contributed equally.

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REFERENCES

- (1) Rubiano, A. M.; Carney, N.; Chesnut, R.; Puyana, J. C. Global Neurotrauma Research Challenges and Opportunities. *Nature*. **2015**, 527, S193-197.

- (2) Gabbe, B. J.; Simpson, P. M.; Cameron, P. A.; Ponsford, J. Lyons, R. A.; Collie, A.; Fitzgerald, M.; Judson, R.; Teague, W. J.; Braaf, S.; Nunn, A.; Ameratunga, S.; Harrison, J. E. Long-Term Health Status and Trajectories of Seriously Injured Patients: A Population-Based Longitudinal Study. *PLoS Med.* **2017**, *14*, e1002322.
- (3) Badhiwala, J. H.; Wilson, J. R.; Fehlings, M. G. Global, Regional, and National Burden of Traumatic Brain Injury and Spinal Cord Injury, 1990-2016: A Systematic Analysis for the Global Burden of Disease Study 2016. *Lancet Neurol.* **2019**, *18*, 56-87.
- (4) Sofroniew, M. V. Dissecting Spinal Cord Regeneration. *Nature.* **2018**, *557*, 343-350.
- (5) Dietz, V.; Fouad, K. Restoration of Sensorimotor Functions after Spinal Cord Injury. *Brain.* **2014**, *137*, 654-667.
- (6) Assinck, P.; Duncan, G. J.; Hilton, B. J.; Plemel, J. R.; Tetzlaff, W. Cell Transplantation Therapy for Spinal Cord Injury. *Nat Neurosci.* **2017**, *20*, 637-647.
- (7) Li, L. M.; Han, M.; Jiang, X. C.; Yin, X. Z.; Chen, F.; Zhang, T. Y.; Ren, H.; Zhang, J. W.; Hou, T. J.; Chen, Z.; Ou-Yang, H. W.; Tabata, Y.; Shen, Y. Q.; Gao, J. Q. Peptide-Tethered Hydrogel Scaffold Promotes Recovery from Spinal Cord Transection *via* Synergism with Mesenchymal Stem Cells. *ACS Appl Mater Interfaces.* **2017**, *9*, 3330-3342.
- (8) Khaing, Z. Z.; Milman, B. D.; Vanscoy, J. E.; Seidlits, S. K.; Grill, R. J.; Schmidt, C. E. High Molecular Weight Hyaluronic Acid Limits Astrocyte Activation and Scar Formation after Spinal Cord Injury. *J Neural Eng.* **2011**, *8*, 046033.
- (9) Fuhrmann, T.; Obermeyer, J.; Tator, C. H.; Shoichet, M. S. Click-Crosslinked Injectable Hyaluronic Acid Hydrogel Is Safe and Biocompatible in the Intrathecal Space for Ultimate Use in Regenerative Strategies of the Injured Spinal Cord. *Methods.* **2015**, *84*, 60-69.
- (10) Kushchayev, S. V.; Giers, M. B.; Hom Eng, D.; Martirosyan, N. L.; Eschbacher, J. M.; Mortazavi, M. M.; Theodore, N.; Panitch, A.; Preul, M. C. Hyaluronic Acid Scaffold Has a Neuroprotective Effect in Hemisection Spinal Cord Injury. *J Neurosurg Spine.* **2016**, *25*, 114-124.
- (11) Xu, W.; Chi, L.; Xu, R.; Ke, Y.; Luo, C.; Cai, J.; Qiu, M.; Gozal, D.; Liu, R. Increased Production of Reactive Oxygen Species Contributes to Motor Neuron Death in a Compression Mouse Model of Spinal Cord Injury. *Spinal Cord.* **2005**, *43*, 204-213.
- (12) Neirinckx, V.; Cantinieaux, D.; Coste, C.; Rogister, B.; Franzen, R.; Wislet-Gendebien, S. Concise Review: Spinal Cord Injuries: How Could Adult Mesenchymal and Neural Crest Stem Cells Take up the Challenge? *Stem Cells.* **2014**, *32*, 829-843.
- (13) Visavadiya, N. P.; Patel, S. P.; VanRooyen, J. L.; Sullivan, P. G.; Rabchevsky, A. G. Cellular and Subcellular Oxidative Stress Parameters Following Severe Spinal Cord Injury. *Redox Biol.* **2016**, *8*, 59-67.
- (14) Naziroglu, M. Molecular Role of Catalase on Oxidative Stress-Induced Ca(2+) Signaling and Trp Cation Channel Activation in Nervous System. *J Recept Signal Transduct Res.* **2012**, *32*, 134-141.
- (15) Chen, Q.; Feng, L. Z.; Liu, J. J.; Zhu, W. W.; Dong, Z. L.; Wu, Y. F.; Liu, Z. Intelligent Albumin-MnO₂ Nanoparticles as Ph-/H₂O₂-Responsive Dissociable Nanocarriers to Modulate Tumor Hypoxia for Effective Combination Therapy. *Adv Mater.* **2016**, *28*, 7129-7136.
- (16) Prasad, P.; Gordijo, C. R.; Abbasi, A. Z.; Maeda, A.; Ip, A.; Rauth, A. M.; DaCosta, R. S.; Wu, X. Y. Multifunctional Albumin-MnO(2) Nanoparticles Modulate Solid Tumor Microenvironment by Attenuating Hypoxia, Acidosis, Vascular Endothelial Growth Factor and Enhance Radiation Response. *ACS Nano.* **2014**, *8*, 3202-3312.

- (17) Xiao, B.; Zhou, X.; Xu, H.; Wu, B.; Hu, D.; Hu, H.; Pu, K.; Zhou, Z.; Liu, X.; Tang, J.; Shen, Y. Integration of Polymerization and Biomineralization as a Strategy to Facilely Synthesize Nanotheranostic Agents. *ACS Nano*. **2018**, *12*, 12682-12691.
- (18) Yeo, Y.; Highley, C. B.; Bellas, E.; Ito, T.; Marini, R.; Langer, R.; Kohane, D. S. *In Situ* Cross-Linkable Hyaluronic Acid Hydrogels Prevent Post-Operative Abdominal Adhesions in a Rabbit Model. *Biomaterials*. **2006**, *27*, 4698-4705.
- (19) Ito, T.; Yeo, Y.; Highley, C. B.; Bellas, E.; Benitez, C. A.; Kohane, D. S. The Prevention of Peritoneal Adhesions by *In Situ* Cross-Linking Hydrogels of Hyaluronic Acid and Cellulose Derivatives. *Biomaterials*. **2007**, *28*, 975-983.
- (20) Gupta, M. K.; Martin, J. R.; Werfel, T. A.; Shen, T.; Page, J. M.; Duvall, C. L. Cell Protective, Abc Triblock Polymer-Based Thermoresponsive Hydrogels with Ros-Triggered Degradation and Drug Release. *J Am Chem Soc*. **2014**, *136*, 14896-14902.
- (21) Lu, P.; Wang, Y.; Graham, L.; McHale, K.; Gao, M.; Wu, D.; Brock, J.; Blesch, A.; Rosenzweig, E. S.; Havton, L. A.; Zheng, B.; Conner, J. M.; Marsala, M.; Tuszynski, M. H. Long-Distance Growth and Connectivity of Neural Stem Cells after Severe Spinal Cord Injury. *Cell*. **2012**, *150*, 1264-1273.
- (22) Sakai, K.; Yamamoto, A.; Matsubara, K.; Nakamura, S.; Naruse, M.; Yamagata, M.; Sakamoto, K.; Tauchi, R.; Wakao, N.; Imagama, S.; Hibi, H.; Kadomatsu, K.; Ishiguro, N.; Ueda, M. Human Dental Pulp-Derived Stem Cells Promote Locomotor Recovery after Complete Transection of the Rat Spinal Cord by Multiple Neuro-Regenerative Mechanisms. *J Clin Invest*. **2012**, *122*, 80-90.
- (23) Zeng, X.; Qiu, X. C.; Ma, Y. H.; Duan, J. J.; Chen, Y. F.; Gu, H. Y.; Wang, J. M.; Ling, E. A.; Wu, J. L.; Wu, W.; Zeng, Y. S. Integration of Donor Mesenchymal Stem Cell-Derived Neuron-Like Cells into Host Neural Network after Rat Spinal Cord Transection. *Biomaterials*. **2015**, *53*, 184-201.
- (24) Hong, L. T. A.; Kim, Y. M.; Park, H. H.; Hwang, D. H.; Cui, Y.; Lee, E. M.; Yahn, S.; Lee, J. K.; Song, S. C.; Kim, B. G. An Injectable Hydrogel Enhances Tissue Repair after Spinal Cord Injury by Promoting Extracellular Matrix Remodeling. *Nat Commun*. **2017**, *8*, 533.
- (25) Asboth, L.; Friedli, L.; Beauparlant, J.; Martinez-Gonzalez, C.; Anil, S.; Rey, E.; Baud, L.; Pidpruzhnykova, G.; Anderson, M. A.; Shkorbatova, P.; Batti, L.; Pages, S.; Kreider, J.; Schneider, B. L.; Barraud, Q.; Courtine, G. Cortico-Reticulo-Spinal Circuit Reorganization Enables Functional Recovery after Severe Spinal Cord Contusion. *Nat Neurosci*. **2018**, *21*, 576-588.
- (26) Liu, D.; Chen, J.; Jiang, T.; Li, W.; Huang, Y.; Lu, X.; Liu, Z.; Zhang, W.; Zhou, Z.; Ding, Q.; Santos, H. A.; Yin, G.; Fan, J. Biodegradable Spheres Protect Traumatically Injured Spinal Cord by Alleviating the Glutamate-Induced Excitotoxicity. *Adv Mater*. **2018**, *30*, e1706032.
- (27) Lai, B. Q.; Che, M. T.; Du, B. L.; Zeng, X.; Ma, Y. H.; Feng, B.; Qiu, X. C.; Zhang, K.; Liu, S.; Shen, H. Y.; Wu, J. L.; Ling, E. A.; Zeng, Y. S. Transplantation of Tissue Engineering Neural Network and Formation of Neuronal Relay into the Transected Rat Spinal Cord. *Biomaterials*. **2016**, *109*, 40-54.
- (28) Su, W.; Chen, H. B.; Li, S. H.; Wu, D. Y. Correlational Study of the Serum Levels of the Glial Fibrillary Acidic Protein and Neurofilament Proteins in Parkinson's Disease Patients. *Clin Neurol Neurosurg*. **2012**, *114*, 372-375.
- (29) Jia, X.; Colombo, G.; Padera, R.; Langer, R.; Kohane, D. S. Prolongation of Sciatic Nerve Blockade by *In Situ* Cross-Linked Hyaluronic Acid. *Biomaterials*. **2004**, *25*, 4797-4804.

(30) Zhang, T. Y.; Li, F. Y.; Xu, Q. H.; Wang, Q. Y.; Jiang, X. C.; Liang, Z. Y.; Liao, H. W.; Kong, X. L.; Liu, J. A.; Wu, H. H.; Zhang, D. P.; An, C. H.; Dong, L.; Lu, Y.; Cao, H. C.; Kim, D.; Sun, J. H.; Hyeon, T.; Gao, J. Q.; Ling, D. S. Ferrimagnetic Nanochains-Based Mesenchymal Stem Cell Engineering for Highly Efficient Post-Stroke Recovery. *Adv Funct Mater.* **2019**, *29*, 1900603.

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