

# Effective repair of traumatically injured spinal cord by nanoscale block copolymer micelles

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**Spinal cord injury results in immediate disruption of neuronal membranes, followed by extensive secondary neurodegenerative processes. A key approach for repairing injured spinal cord is to seal the damaged membranes at an early stage. Here, we show that axonal membranes injured by compression can be effectively repaired using self-assembled monomethoxy poly(ethylene glycol)-poly(D,L-lactic acid) di-block copolymer micelles. Injured spinal tissue incubated with micelles (60 nm diameter) showed rapid restoration of compound action potential and reduced calcium influx into axons for micelle concentrations much lower than the concentrations of polyethylene glycol, a known sealing agent for early-stage spinal cord injury. Intravenously injected micelles effectively recovered locomotor function and reduced the volume and inflammatory response of the lesion in injured rats, without any adverse effects. Our results show that copolymer micelles can interrupt the spread of primary spinal cord injury damage with minimal toxicity.**

Most spinal cord injury (SCI) cases involve a primary injury and subsequent secondary damage<sup>1,2</sup>. During the primary injury, the acute mechanical stress to the spinal cord breaks neuronal membranes and causes  $\text{Ca}^{2+}$  influx into cells. The latter triggers a series of secondary biological events including inflammation, free radical release and apoptosis<sup>3,4</sup>, which further exacerbate the damage. Among various SCI treatments<sup>5</sup>, a key approach is to seal the damaged membrane at the early stage of SCI. Poly(ethylene glycol) (PEG) and Poloxamer 188 (P188), known as membrane sealing agents<sup>6,7</sup>, have been used to restore compound action potential (CAP) in *ex vivo* tissues<sup>8</sup> and recover behavioural functions *in vivo*<sup>9–13</sup>. Topical delivery of PEG ( $\geq 30\%$  w/w in water) to the site of injury was shown to be effective in alleviating secondary injuries, including oxidative stress and free radical production<sup>14,15</sup>. Systemic delivery methods including intravenous injection and subcutaneous injection were also used for the ease of clinical use. However, intravenous injection of 30% PEG only increased the locomotor rating score by 0.7 out of a 21-point scale when compared with the control group receiving an isovolumetric dose of saline solution<sup>16</sup>, whereas subcutaneously injected P188 has shown to be less effective than PEG in the improvement of cutaneous trunci muscle reflection<sup>12</sup>. The limited behavioural recovery is partly due to the difficulty in delivering a sufficient amount of agents to the injured site via the systemic circulation. For polymers, it has been shown that unimers have a circulation half-life of less than 10 min (ref. 17).

In this Article, we present a new approach that allows effective membrane repair and functional recovery in SCI animals. Instead of using individual polymers, we chose PEG–polyester micelles, which are spherical assemblies of di-block copolymers containing a hydrophilic PEG shell and a hydrophobic inner core<sup>18</sup>. These polymeric micelles, ranging in size from 10 to 100 nm, have unique properties such as biocompatibility and long blood residence time<sup>19</sup>, and have been widely investigated as nanocarriers of water-insoluble drugs<sup>20</sup>. By recognizing the high-density coronal

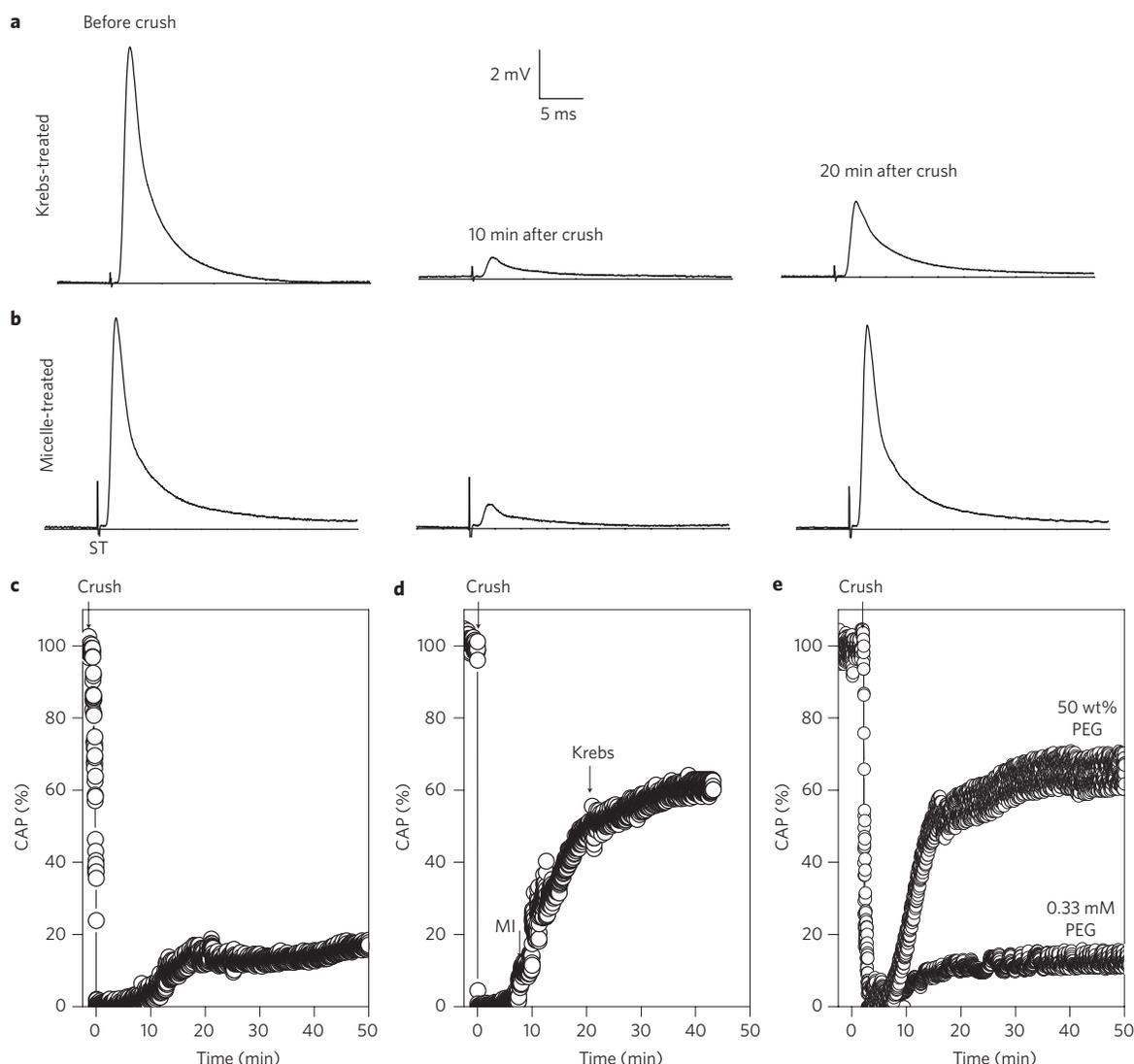
PEG and amphiphilic structure in a polymeric micelle, we demonstrate a new application of copolymer micelles as a membrane repair agent to treat SCI.

## Restoration of CAP in *ex vivo* spinal tissue

We prepared monomethoxy PEG–poly(D,L-lactic acid) (mPEG–PDLLA) micelles (Supplementary Fig. S1) using dialysis, and applied them to compression-injured ventral white matter strips isolated from adult guinea pigs, a tissue model widely used in neuronal membrane repair studies<sup>8,14,15</sup>. The effect of mPEG–PDLLA micelles on compression-injured white matter was investigated by measuring the CAP amplitude, an indicator of the proportion of axons conducting action potential. The viability of the white matter was affirmed by initial CAP measurements using a double sucrose gap recording chamber (Supplementary Fig. S2). A constant displacement of white matter strips by 5–30 s compression with modified forceps with a 0.8-mm spacer was implemented to reduce the CAP amplitude to 0 mV (ref. 14). Without micelle treatment, the CAP self-recovered to  $18.5 \pm 5.1\%$  ( $n = 5$ ) of the pre-compression amplitude during the 10–20 min post-compression period (Fig. 1a). With application of a Krebs solution containing  $0.67 \text{ mg ml}^{-1}$  micelles, the white matter strips ( $n = 8$ ) recovered to  $66.5 \pm 14.8\%$  of the original CAP within 20 min after incubation with micelles (Fig. 1b). The time-dependent increase of CAP is shown in Fig. 1c–e. Without treatment, the CAP remained stable after self-recovery (Fig. 1c). For samples treated with micelles in the period 10–24 min after the compression injury, the CAP showed a continuous increase during the 40-min post-injury period (Fig. 1d). The difference in CAP recovery between the control and micelle-treated white matter was significant ( $P < 0.001$ , Student's *t*-test).

In the above experiment, the weight concentration of the mPEG–PDLLA block copolymer was  $2.0 \text{ mg ml}^{-1}$ , corresponding to  $0.33 \text{ mM}$  copolymer based on the average molecular weight of  $6,101 \text{ g mol}^{-1}$  ( $2,000$  from PEG and  $4,101$  from PDLLA).

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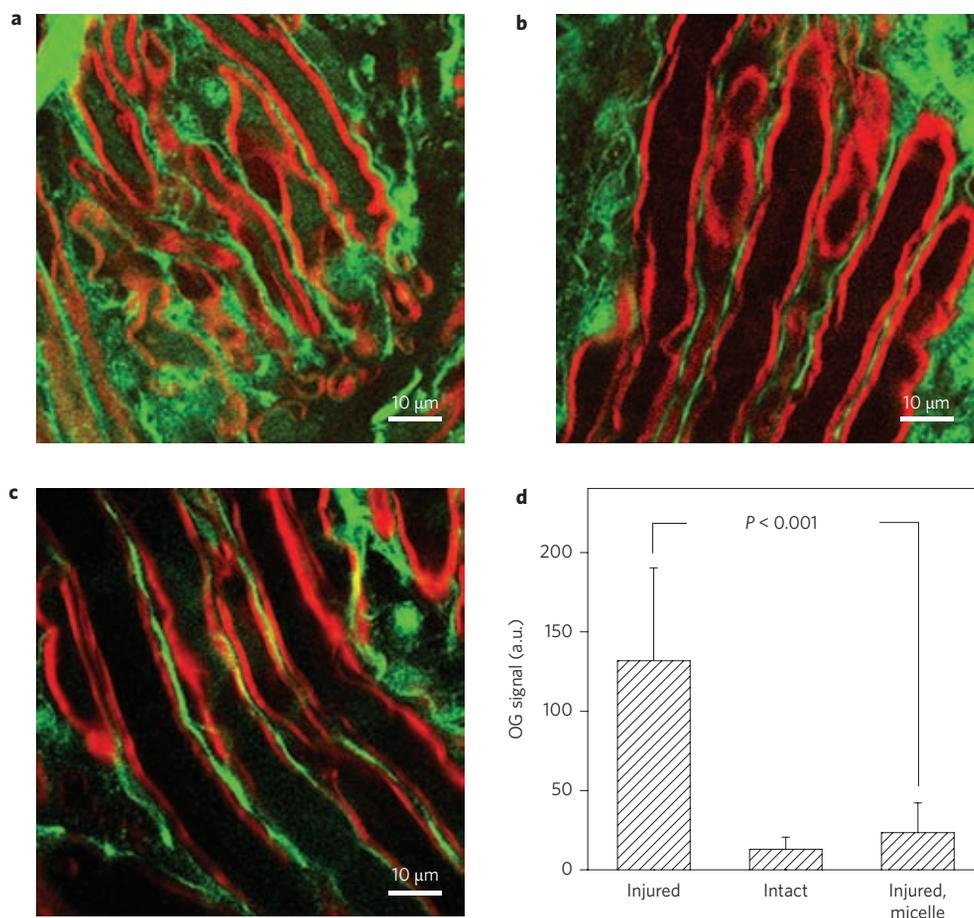
**Figure 1 | Immediate CAP restoration in compression-injured spinal cords after treatment with mPEG-PDLLA di-block copolymer micelles.**

**a,b,** Electrophysiological recordings show typical responses to acute injury without (**a**) and with (**b**) micelle treatment. CAPs before compression injury (left), acute reduction of CAP by compression (middle) and self-recovery after injury (right) are shown. ST, stimulus. **c-e,** Three representative experiments demonstrate CAP restoration in control (**c**), micelle-treated (**d**,  $0.67 \text{ mg ml}^{-1}$ ), and poly(ethylene glycol) (PEG)-treated (**e**, 250 mM and 0.33 mM) spinal cords after compression injury. Arrows indicate the time of crushing, addition of micelles (MI) and Krebs washing. Without micelles (**c**), CAP self-recovered to 14.4% at 20 min post-compression and remained at the same level over the next 30 min. Adding  $500 \mu\text{l}$  of  $2.0 \text{ mg ml}^{-1}$  micelles (**d**) to the recording chamber increased CAP to 62.3% of the pre-compression amplitude within 30 min. Addition of 0.33 mM PEG (**e**) at 10 min post-compression increased (by up to 11.8%) CAP to the level of self-recovery. A 2-min administration of 50 wt% (250 mM) PEG(2000) significantly restored CAP.

In comparison, the 50 wt% PEG (2,000 molecular weight) used in previous studies has a molar concentration of 250 mM (refs 8,10). To evaluate the effectiveness of PEG in membrane repair, we performed the same CAP restoration experiment using a solution of PEG(2000) at 250 and 0.33 mM. The 0.33 mM PEG solution did not result in an additional increase in CAP (Fig. 1e) in comparison to the control group (Fig. 1c). With 0.33 mM PEG, the average recovery ( $n = 4$ ) was  $18.2 \pm 7.0\%$ , which was at the same level of self-recovery ( $P > 0.05$ , Student's *t*-test). Meanwhile, the 50% PEG produced the same CAP restoration as did application of the micelles (Fig. 1e). These results suggest that the self-assembled mPEG-PDLLA micelle could restore CAP in spinal cord tissues more efficiently and at a concentration much lower than free PEG.

Because high-concentration PEG is known to protect the intracellular environment by sealing cell membrane breaches<sup>21</sup>, we tested whether the mPEG-PDLLA micelles could reduce  $\text{Ca}^{2+}$

influx into axons in injured white matter. The  $\text{Ca}^{2+}$  concentration was evaluated by two-photon excited fluorescence (TPEF) imaging of a calcium indicator, Oregon Green 488 (OG). A coherent anti-Stokes Raman scattering (CARS) microscope (Supplementary Fig. S3) that permits label-free vibrational imaging of intact myelin sheath<sup>22</sup> was used to define the intra- and extra-axonal space in healthy, compression-injured and injured/micelle-treated white matter. Strong TPEF signals from OG were observed in the extra-axonal space in all cases, but the intra-axonal OG intensities were different, as shown in Fig. 2. Without any treatment, the OG intensity inside injured axons (shown in green) at 1 h after compression injury was 10 times higher ( $131.89 \pm 58.47 \text{ a.u.}$ , measured from 57 axons) than that in healthy axons ( $13.02 \pm 7.55 \text{ a.u.}$ , measured from 64 axons). In contrast, by incubating the white matter with  $0.67 \text{ mg ml}^{-1}$  micelles immediately after compression injury, the intra-axonal OG intensity ( $23.42 \pm 18.78 \text{ a.u.}$ , measured from 55 axons) was



**Figure 2 | Calcium influx into axons.** **a–c**, TPEF images of OG 488 (green) and coherent anti-Stokes Raman scattering images of myelin (red) show intraxonal free  $\text{Ca}^{2+}$  levels in compression-injured (**a**), healthy (**b**) and compression-injured and micelle-treated (**c**) spinal cords. Images were acquired 1 h after compression injury. **d**, Statistical analysis. Without micelle treatment, the TPEF intensity from OG inside the injured axons was 10 times greater than intact axons. The intensity was only twice that of intact axons when  $0.67 \text{ mg ml}^{-1}$  micelles were added immediately after compression injury.

only two times higher than that in healthy axons ( $P < 0.001$ , Student's *t*-test). These results demonstrate that calcium influx could be effectively diminished by micelle-mediated repair of axonal membrane.

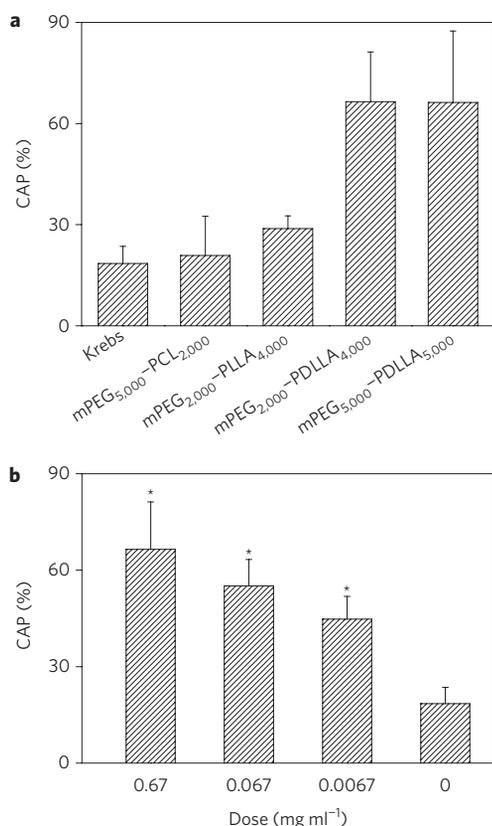
To determine the distinctive roles of the PEG shell and the hydrophobic core in micelle-mediated membrane repair, we prepared micelles with different polyester cores and varied the PEG chain lengths. Administration of mPEG–poly( $\epsilon$ -caprolactone) (PCL) micelles ( $20.9 \pm 11.7\%$ ,  $n = 3$ ) or mPEG–poly(L-lactic acid) (PLLA) micelles ( $28.8 \pm 3.8\%$ ,  $n = 4$ ) did not significantly increase the CAP compared with the control comprising Krebs solution only ( $18.5 \pm 5.1\%$ ,  $n = 5$ ; Fig. 3a). On the other hand, administration of mPEG–PDLLA micelles with two different PEG molecular weights (2,000 and 5,000) produced the same level of efficacy,  $66.5 \pm 14.8\%$  ( $n = 5$ ) and  $66.2 \pm 21.2\%$  ( $n = 3$ ), respectively. These results indicate a critical role of the hydrophobic chains in membrane repair. mPEG<sub>2,000</sub>–PDLLA<sub>4,000</sub> was selected to be used in the rest of our study.

Considering the challenge that membrane repair agents are significantly diluted during *in vivo* administration, we examined the CAP restoration efficiency at lower micelle concentrations. Krebs solutions containing 0, 0.0067, 0.067 and  $0.67 \text{ mg ml}^{-1}$  micelles were applied to separate white matter strips, which were compressed using the same procedure. As shown in Fig. 3b,  $0.67 \text{ mg ml}^{-1}$  micelle resulted in a CAP increase of  $66.5 \pm 14.8\%$ . This value was more than three times the CAP increase ( $18.5 \pm 5.1\%$ ) in the control group (no micelles). Micelles at  $0.067 \text{ mg ml}^{-1}$  and

$0.0067 \text{ mg ml}^{-1}$  caused a CAP increase of  $55.1 \pm 8.2\%$  ( $n = 5$ ) and  $44.8 \pm 7.1\%$  ( $n = 5$ ), respectively. These results indicate that higher concentrations of micelles resulted in a larger CAP increase, and the restoration remained efficient when the concentration was lowered by a factor of 100 from 0.67 to  $0.0067 \text{ mg ml}^{-1}$ . It is worth noting that  $0.0067 \text{ mg ml}^{-1}$  corresponds to  $1.1 \mu\text{M}$  PEG in the solution, which is a dilution by a factor of  $\sim 10^5$  of the PEG concentration (250 mM or 50 wt%) previously used for treatment of injured white matter<sup>8</sup>. The effective restoration of CAP by diluted micelles shows the great potential of micelles for *in vivo* repair of SCI.

### Behavioural recovery and tissue repair in SCI rats

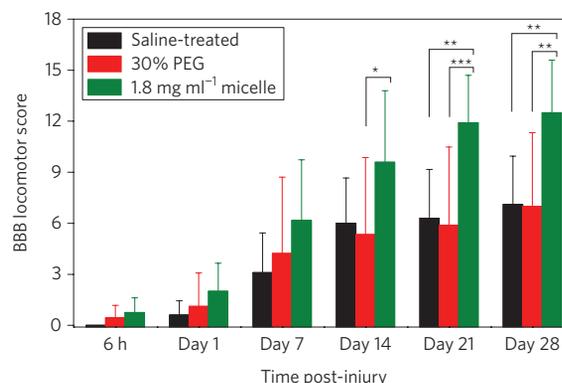
To determine whether micelles could improve functional outcomes in live animals, we performed a behavioural study using Long–Evans rats with compression-injured spinal cords and evaluated the hindlimb functional recovery using the Basso Beattie Bresnahan (BBB) locomotor rating scale. Within 10 min post-injury, we injected through the tail vein either 1.0 ml saline containing  $1.8 \text{ mg ml}^{-1}$  micelles, 1.0 ml saline containing 30 wt% PEG or 1.0 ml pure saline as control. The presence of the micelles at the injury site was demonstrated by confocal fluorescence signals of fluorescein isothiocyanate (FITC)-conjugated mPEG–PDLLA micelles (Supplementary Fig. S4). The first BBB test was carried out at 6 h post-injury when all the rats were able to ambulate with forelimbs. The BBB scores are shown in Fig. 4. At 6 h (day 0), day 1 and day 7 after treatment, we did not observe a



**Figure 3 | Efficiency of CAP restoration depends on micelle structure and concentration.** **a**, Application of 0.67 mg ml<sup>-1</sup> mPEG<sub>5,000</sub>-PCL<sub>2,000</sub> did not result in a significant increase in CAP amplitude compared with the Krebs control ( $P > 0.05$ , Student's *t*-test). Application of mPEG<sub>2,000</sub>-PLLA<sub>4,000</sub> micelles only slightly restored the CAP compared to the control. Application of mPEG<sub>2,000</sub>-PDLLA<sub>4,000</sub> and mPEG<sub>5,000</sub>-PDLLA<sub>5,000</sub> resulted in the same level of CAP restoration ( $P > 0.05$ , Student's *t*-test). **b**, Application of 0.0067, 0.067 and 0.67 mg ml<sup>-1</sup> mPEG-PDLLA micelles resulted in significant CAP restoration compared with the control group with no micelle supplement. \* $P < 0.001$ , ANOVA test.

significant difference in BBB scores between the micelle group and the PEG group. However, subsequent improvement in the locomotor function in the micelle-treated group was evident by a more rapid increase of BBB scores in the first 14 days and continuation of improvement over the following two weeks. Specifically, at 28 days post-injury, the BBB scores were  $12.5 \pm 3.1$ ,  $7.0 \pm 4.3$  and  $7.1 \pm 2.8$  for the micelle-treated, PEG-treated and saline-treated control animals, respectively, with the micelle-treated group having a significantly higher score than the other two groups ( $P < 0.01$ ) and no difference between the PEG-treated and control groups ( $P > 0.05$ ). Our result is consistent with an earlier comparison between 30% PEG and saline control<sup>16</sup>, neither of which showed axonal transduction through the lesion site. From a clinical perspective, an animal with a BBB score equal to or less than 11 lacks hindlimb and forelimb coordination, whereas a score of 12 to 13 corresponds to occasional to frequent forelimb and hindlimb coordination. Reaching a BBB score of 12 is significant in that it is a sign of axonal transduction through the lesion site<sup>23</sup>. In summary, the hindlimb function measurements demonstrate that mPEG-PDLLA micelles are significantly more effective than high-concentration PEG in the functional recovery of SCI.

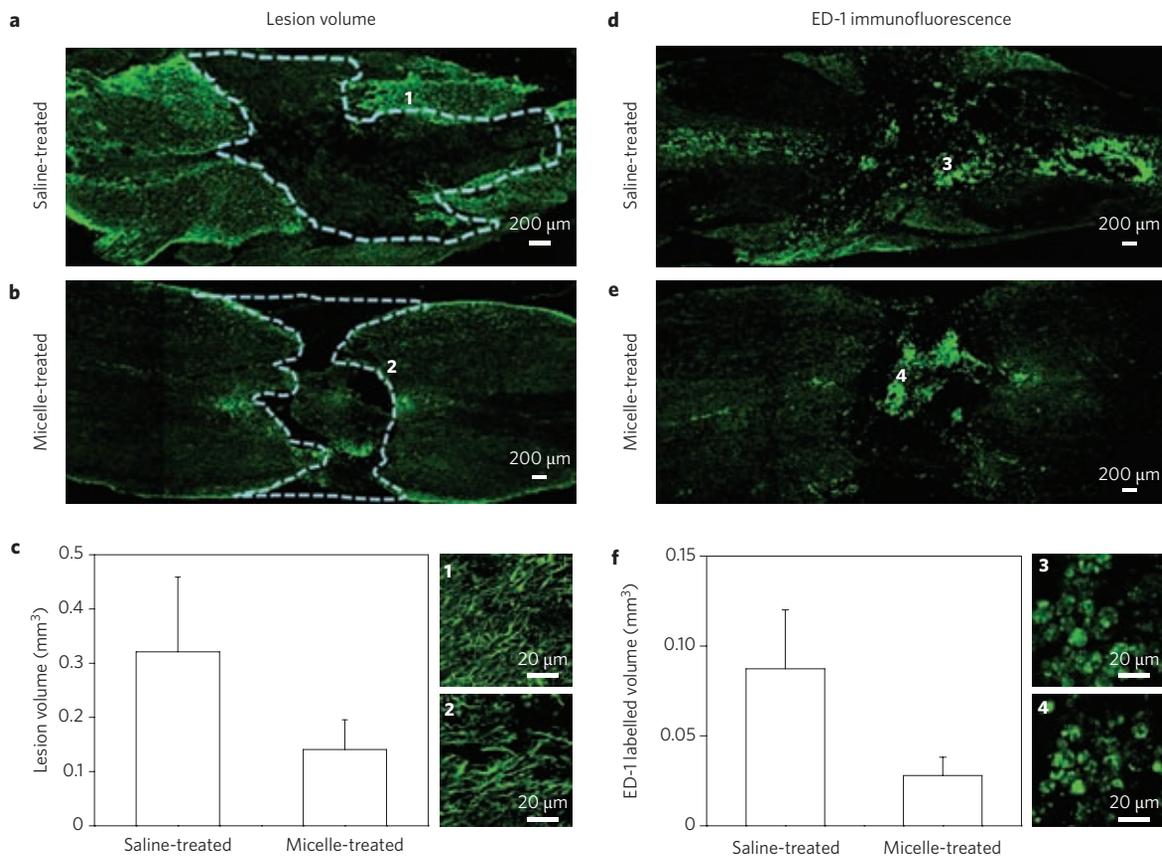
The effective recovery of locomotor functions by copolymer micelles is partly a result of the efficient systemic delivery of



**Figure 4 | Recovery of locomotor function in rats after compression injury.** After spinal cord injury, Long-Evans rats received saline, 30% poly(ethylene glycol) (PEG) or 1.0 ml of mPEG-PDLLA micelles (1.8 mg ml<sup>-1</sup>) by tail vein injection. Intravenous administration of mPEG-PDLLA micelles resulted in an average Basso Beattie Bresnahan score of  $12.5 \pm 3.1$  ( $n = 12$ ) at day 28, which was significantly higher than  $7.1 \pm 2.8$  with saline control ( $n = 10$ ) and  $7.0 \pm 4.3$  with 30% PEG ( $n = 9$ ) (ANOVA test, \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ).

PEGylated nanostructures. It has been shown that the PEG chains on the nanoparticle surface create a highly water bound barrier that blocks the adhesion of opsonins<sup>24</sup>. Moreover, the nanoscale particle size is known to prevent glomerular filtration, which further extends the retention of micelles in the blood<sup>24</sup>. These two factors allow prolonged blood residence times for the mPEG-PDLLA micelles ( $t_{1/2} = 2.66$  h; ref. 18). In contrast, it has been shown that unimers have a blood half-time of only  $\sim 10$  min (ref. 17). Note that the micelles can be gradually destabilized by lipophilic agents *in vivo*, as has been shown recently<sup>25,26</sup>. However, it is possible that a fraction of polymer micelles remain intact and circulate for hours, resulting in an effective delivery of the agents to the injured site.

We further examined whether intravital micelle treatment would result in tissue protection. By immunofluorescence imaging of sliced spinal tissues, we measured the total lesion volume and immunoreactivity in micelle- and saline-treated rats killed at week 4 after compression injury. As shown in Fig. 5a,b, the compression injury created a cavity (outlined by dashed lines), an area depleted of both neurons and astrocytes, in the spinal cord tissue. The lesion volume was calculated as the volume of the cavity. It was found that micelle treatment resulted in a significantly decreased lesion volume of  $0.14 \pm 0.03$  mm<sup>3</sup> compared to that with saline treatment ( $0.32 \pm 0.08$  mm<sup>3</sup>) at 4 weeks post-injury (Fig. 5c). Using tissues from the same animals, we also mapped macrophages and/or activated microglia by ED-1 immunofluorescence. Representative distributions of ED-1<sup>+</sup> cells in the injured spinal cord following treatment with saline and micelles are shown in Fig. 5d and e, respectively. The ED-1<sup>+</sup> cells in the micelle-treated group were more confined than those in the control group. The volume that ED-1<sup>+</sup> cells spread was found to be significantly reduced in the micelle-treated group ( $0.03 \pm 0.01$  mm<sup>3</sup>) compared with the control group ( $0.09 \pm 0.02$  mm<sup>3</sup>; Fig. 5f). The significant reduction in both lesion volume and inflammatory response at week 4 after injury could be considered a consequence of membrane repair and inhibition of Ca<sup>2+</sup> influx results from the micelles administered in the acute phase. To determine whether micelles could repair the cell membrane *in vivo*, we injected a cell-impermeable dye into the cerebral spinal fluid before SCI, and quantified the number of cell bodies taking up the dye using confocal fluorescence microscopy. We found that micelle treatment significantly



**Figure 5 | Lesion volume and immunoreactivity analysis of rat tissue.** **a, b**, Immunostaining for glial fibrillary acidic protein highlights the lesion area 28 days post-compression injury in a saline-treated (**a**) and micelle-treated (**b**) spinal cord. The images represent longitudinal sections at 350  $\mu\text{m}$  from the dorsal surface. Images of high magnification highlight the branched astrocytes at the border of the scar (insets to **c**). **c**, Quantitative comparison of the lesion volumes in micelle-treated and saline-treated groups, showing a smaller lesion volume in the micelle-treated spinal tissue. **d, e**, ED-1 immunofluorescence showing the distribution of macrophages/reactive microglia in the saline-treated (**d**) and micelle-treated (**e**) spinal cords. The representative images of the longitudinal sections at 500  $\mu\text{m}$  from the dorsal surface show a more confined area of immunoreactivity in the micelle-treated spinal tissue. Images of high magnification (insets to **f**) highlight individual ED-1<sup>+</sup> cells inside the lesion area. **f**, Quantitative comparison of the ED-1<sup>+</sup> volumes in the micelle- and saline-treated groups.

reduced the number of damaged cells in both grey matter and white matter (Supplementary Fig. S5).

### Toxicity analysis of micelles in rats

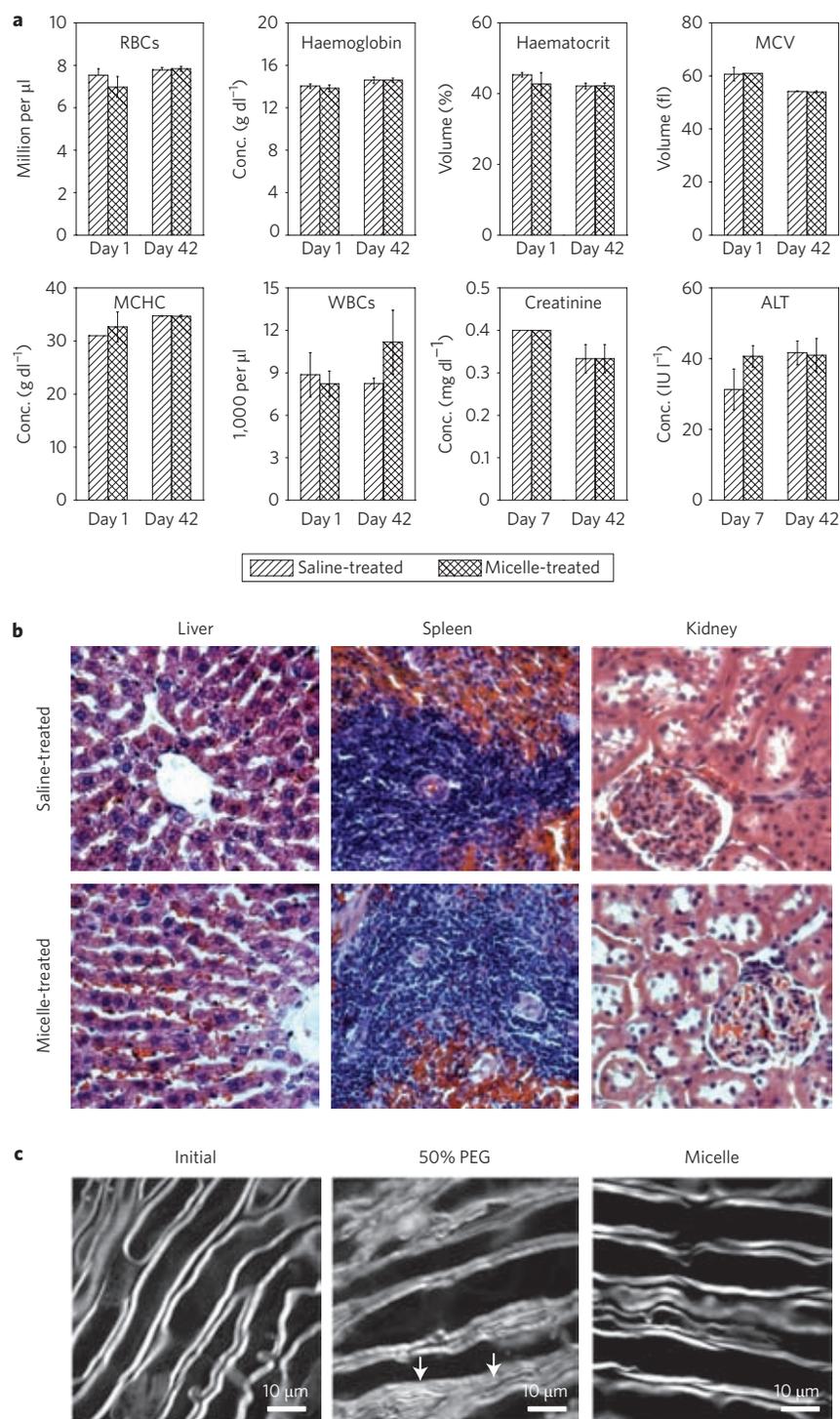
We examined both the acute and chronic toxicity of the micelles administered to Long-Evans male rats. The animals were randomized into a micelle-treated group ( $n = 3$ ) or a saline-treated group ( $n = 3$ ). Each animal received 1 ml saline containing either 1.8  $\text{mg ml}^{-1}$  mPEG-PDLLA micelles or 1 ml saline through tail vein injection. After the treatment, blood samples were drawn through the jugular vein at day 1 and day 7 for acute toxicity analysis, and at day 42 for chronic toxicity analysis. The results are shown in Fig. 6a. There was no significant difference between the micelle group and the saline group in terms of red blood cell (RBC) counts, haemoglobin, haematocrit, mean corpuscular volume (MCV) or mean corpuscular haemoglobin concentration (MCHC). The total number of white blood cells (WBCs) in the micelle group at day 42 was within the normal range, as well as the saline group. In particular, the levels of creatinine and alanine transaminase (ALT) in serum were not increased, indicating no damage to either the kidneys or the livers. Morphologically, linear hepatic cords were observed in both the micelle and saline groups (Fig. 6b). No sign of splenic changes such as hematopoietic cell proliferation or hemosiderin deposition occurred in either group. Moreover, the kidney tubules were

regularly oriented in good shape and the glomerulus was not attached to the surroundings in both micelle- and saline-treated animals. To summarize, the above study revealed no adverse effect in healthy animals after systemic administration of mPEG-PDLLA micelles.

Using CARS microscopy, we further examined the morphological changes of myelinated axons induced by PEG and micelle treatments (Fig. 6c). After incubating a non-compressed white matter strip with 50% PEG for 17 min, the adjacent parallel axons appear to become attached to one another, implying the occurrence of axonal fusion. In contrast, the axons maintained normal morphology after incubation with 0.67  $\text{mg ml}^{-1}$  micelles for 3 h. We also evaluated functional toxicity (Supplementary Fig. S6) by circulating Krebs solution containing 0.67  $\text{mg ml}^{-1}$  micelles through the white matter strip for 45 min, and found that the CAP remained at the same level. In contrast, by incubating the white matter with 50% PEG, the CAP exhibited a decrease to 40% of the initial value in 45 min. These observations indicate that micelles can effectively restore CAP in injured white matter with minimal toxicity.

### Conclusions

The current study demonstrates that polymeric micelles can effectively repair a traumatically injured spinal cord. Administration of mPEG-PDLLA micelles immediately after traumatic injury leads



**Figure 6 | Toxicity analysis.** **a**, Following injection of 1 ml mPEG-PDLLA micelle or saline solution, neither complete blood count of Long-Evans rats at day 1 and day 42 or serum analysis at day 7 and day 42 shows adverse effects in the two groups. Error bars,  $\pm$ s.d. **b**, Histological analysis of explanted liver, spleen and kidney with haematoxylin and eosin staining of the control group and the micelle-treated group indicates no signs of cellular or tissue damage. Magnification,  $\times 400$ . **c**, Morphological analysis. After incubating a healthy spinal cord strip (left) in 50% PEG in Krebs solution for 17 min, the axons became attached to each other (middle). The white arrows indicate possible fusion of adjacent axonal myelin. In contrast, after incubation with  $0.67 \text{ mg ml}^{-1}$  mPEG-PDLLA micelles for 3 h (right), the spinal cord strip displays no obvious morphological changes in the myelin. RBC, red blood cell; MCV, mean corpuscular volume; MCHC, mean corpuscular haemoglobin concentration; WBC, white blood cell; ALT, alanine transaminase.

to significant electrophysiological recovery in spinal tissue and functional recovery in live animals without noticeable toxicity. Because of the small number of animals used and the fixed dosage of mPEG-PDLLA micelles, our work should be considered a pilot study. Further studies using a larger pool of

animals are to be carried out to determine how dosage, administration frequency and timing of micelle treatment affect clinical outcome. Moreover, follow-up studies will aim to elucidate how the micelles interact with a damaged membrane. In summary, our work provides an exciting example of how the unique

properties of nanomaterials can be used for the treatment of traumatic injuries.

## Methods

**Synthesis and characterization of mPEG–PDLLA micelles.** The mPEG(2000)–PDLLA block copolymer (Supplementary Fig. S1) was synthesized by ring opening polymerization of  $\text{-D,L-lactide}$ <sup>27</sup>. mPEG–PDLLA micelles with a weight concentration of  $2.0 \text{ mg ml}^{-1}$  were prepared by dialysis. Details can be found in the Supplementary Information.

**Spinal sample preparation and CAP recording.** The procedure for isolation of the spinal cord white matter has been described previously<sup>8</sup> and can be found in the Supplementary Information. CAPs were recorded using the double sucrose gap recording chamber shown in Supplementary Fig. S2.

**Compression injury and local micelle treatment.** Compression injury was inflicted by a constant displacement of 5–30 s compression of the spinal cord using modified forceps with a spacer until the CAP dropped to 0 mV (ref. 14). For local application of micelles, immediately after injury, the spinal cord white matter strips were kept in perfusing Krebs solution at a speed of  $2.0 \text{ ml min}^{-1}$ , controlled by a peristaltic pump for 10 min. The perfusion was then stopped and  $500 \mu\text{l}$  of  $2.0 \text{ mg ml}^{-1}$  micelle solution added gently to the 1.0 ml Krebs solution in the central compartment, leading to a final concentration of  $0.67 \text{ mg ml}^{-1}$ . Following micelle treatment, the spinal cord strips were thoroughly rinsed with Krebs solution. All solutions were bubbled with 95%  $\text{O}_2$ /5%  $\text{CO}_2$  throughout the experiment.

**CARS and TPEF imaging of spinal tissues.** CARS and TPEF imaging were carried out on a multimodal nonlinear optical microscope as shown in Supplementary Fig. S3. The CARS signal was generated by two synchronized 2.5-ps lasers. The TPEF signal was generated by a mode-locked 200-fs Ti:sapphire oscillator (Mira 900, Coherent). Details can be found in the Supplementary Information.

**In vivo spinal cord injury and micelle administration.** All protocols for this experiment were approved by the Purdue Animal Care and Use Committee. Adult male Long–Evans rats (Hilltop Lab Animals) weighing 300–350 g were used. The tenth thoracic (T10) spine was removed by laminectomy and the T10 spinal cord segment was constantly compressed for 15 s by the modified forceps under aseptic condition as described previously<sup>9</sup>. Before the compression injury, all rats were anaesthetized deeply with a mixture of  $90 \text{ mg kg}^{-1}$  ketamine and  $5 \text{ mg kg}^{-1}$  xylazine. After the compression injury, the laminectomy was closed by suturing the muscle with 3-0 prolene followed by the use of 7.5-mm Michel wound clips (Fine Science Tools) to close the skin incision.

Rats were randomized into three groups: 1 ml micelle ( $1.8 \text{ mg ml}^{-1}$  in saline;  $n = 14$ ), 1 ml PEG (30% in saline  $n = 12$ ), and isovolumetric doses of saline ( $n = 12$ ). Treatments were given within 10 min post-injury by intravenous tail vein injection. Animals were caged individually 1 week before the surgery and then 4 weeks post-surgery. For post-operation pain management, the analgesic buprenorphine ( $0.05$ – $0.10 \text{ mg kg}^{-1}$ ) was given every 12 h through subcutaneous injection during anaesthesia recovery and for the first 3 days post-surgery. One rat in the micelle treated group and one rat in the PEG group were excluded from the study due to the abnormally high BBB score on day 0. Two rats in the PEG group, one rat in the micelle group and two rats in the control group suffered from kidney failure during the first week post-surgery and were killed.

**Behavioural testing.** The locomotor recovery of the animals was determined using the 21-point BBB open-field locomotor scale<sup>23</sup>. The test was conducted by two observers, with one observer blind to the treatments. Two observers scored independently and made an agreement on the score before the scores were finalized. Locomotor scoring was conducted on day 0 at 6 h post-injury, day 1 and subsequently once a week for 4 weeks.

**Histological assessment.** Three micelle-treated and three saline-treated rats were used for histology. Samples were immuno-processed with glial fibrillary acidic protein (GFAP) to detect reactive astrocytes and ED-1 to detect activated microglial cells and macrophages using standard procedures<sup>28,29</sup>. Details can be found in the Supplementary Information.

**Toxicity assessment.** Long–Evans adult male rats were randomized into the micelle-treated group ( $n = 3$ ) or the saline-treated group ( $n = 3$ ). Each animal received 1 ml micelle ( $1.8 \text{ mg ml}^{-1}$  suspended in saline) or saline solution through tail vein injection. Blood samples (1 ml) were drawn through the jugular vein at day 1, day 7 and day 42 post-treatment. Haematology study and serum analysis were performed by the Purdue University Veterinary Clinical Pathology Lab in a blind manner. The rats were then killed and tissues including liver, kidney and spleen were fixed in 10% neutral buffered formalin for at least 48 h, embedded into paraffin. Sections of 5- $\mu\text{m}$  thickness were stained with haematoxylin and eosin in Purdue University Histopathology Lab. The slides were then examined with a Nikon microscope equipped with a charge-coupled device camera.

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### Author contributions

J.X.C., R.S., R.B.B. and K.P. equally contributed to the concept, experimental design, materials, equipment and conduct of the study. Y.S., S.K. and T.B.H. performed the experiments. Y.S. analyzed the data. Y.S. and J.X.C. co-wrote the paper. All authors discussed the results and commented on the manuscript.

### Additional information

The authors declare no competing financial interests. Supplementary information accompanies this paper at [www.nature.com/naturenanotechnology](http://www.nature.com/naturenanotechnology). Reprints and permission information is available online at <http://npg.nature.com/reprintsandpermissions/>. Correspondence and requests for materials should be addressed to J.X.C.