Underwater-treadmill training attenuates blood-spinal cord barrier disruption by promoting angiogenesis and inhibiting MMP-2/9 expression following spinal cord injury

Xinwang Ying
The Second Affiliated Hospital and Yuying Children'Hospital of Wenzhou Medical University
Qingfeng Xie
The Second Affiliated Hospital and Yuying Children'Hospital of Wenzhou Medical University
Shengcun Li
The Second Affiliated Hospital and Yuying Children' Hospital of Wenzhou Medical University
Xiaolan Yu
The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University
Kecheng Zhou
The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University
Jingjing Yue
The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University
Xiaolong Chen
The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University
Wenzhan Tu
The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University
Guanhu Yang
The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University
Songhe Jiang (jiangsonghe@wmu.edu.cn)
The Second Affiliated Hospital and Yuying Children's Hospital of Wenzhou Medical University

Research

Keywords: Spinal cord injury, BSCB, Underwater-treadmill training, Vascular regeneration, MMP-2/9

Posted Date: June 22nd, 2020

DOI: https://doi.org/10.21203/rs.3.rs-37000/v1

Version of Record: A version of this preprint was published at Fluids and Barriers of the CNS on November 25th, 2020. See the published version at https://doi.org/10.1186/s12987-020-00232-1.

Abstract

Background

The blood-spinal cord barrier (BSCB) can be seriously damaged after SCI and is considered to be a therapeutic target. Exercise training is a recognized method for the treatment of SCI. The destruction of the BSCB mediated by matrix metalloproteinase (MMP) leads to inflammation, neurotoxin production, and apoptosis of neurons. The failure of effective regeneration of new blood vessels is also an important reason for the difficulty of recovery after SCI. We introduced underwater-treadmill training (TT) for the first time, which can help SCI rats successfully exercise, and we explored the role of TT in promoting the ability to exercise after SCI and its possible mechanism.

Methods

SCI models were established and randomly divided into three groups. Rats underwent TT after SCI. The degree of neurological deficit, water content, BSCB permeability, protein expression and ultrastructure of vascular endothelial cells were assessed by the BBB motor rating scale, haematoxylin-eosin (HE) staining, Evans blue (EB) staining, Western blot (WB) experiments, and immunofluorescence and transmission electron microscopy (TEM).

Results

Our experiments show that TT reduces the permeability of BSCB and decreased tissue structure damage and improved functional recovery after SCI; TT prevent the loss of TJ and AJ protein; TT promotes angiogenesis and inhibits the expression of MMP-2 and MMP-9 after SCI.

Conclusions

In this study, the results indicate that TT promotes functional recovery partly for the following reasons: (1) TT protects residual BSCB structure from further damage; (2) it promotes vascular regeneration; and (3) it inhibits the expression of MMP-2/9 to mitigate BSCB damage.

Background

Spinal cord injury (SCI) places a heavy burden on society and the economy [1]. Currently, it can only be relieved by surgery, but it cannot be cured [2]. The basement membrane, pericytes and the terminal foot process of astrocytes constitute the barrier of the BSCB, which protects and regulates the parenchyma of the spinal cord [3, 4]. After SCI, the barrier is destroyed, which is accompanied by the infiltration of immune cells and neurotoxic products, resulting in the death of nerve cells, and permanent neurological

dysfunction [5–7]. Therefore, it is crucial to identify an intervention that can effectively prevent the destruction of the BSCB after SCI.

MMP is a family of zinc-containing peptidases that degrade and reshape the extracellular matrix and other extracellular proteins, and it plays a key role in barrier function [7, 8]. Studies have shown that MMP plays a key role in the destruction of the BBB/BSCB under pathological conditions, including SCI. Two important members of the MMP superfamily are MMP-2 and − 9 [9]. MMP-9 can induce BSCB-related protein degradation, and upregulation of MMP-2 will lead to the initial opening of the BBB/BSCB [10, 11], while blocking MMP-9 activity will protect vascular permeability [12]. The expression of MMP-2/9 was still detected 7 d after SCI [13]. The failure of effective regeneration of new blood vessels is also an important reason for the difficulty of recovery after SCI.

Previous studies have mainly focused on drug treatment after SCI[14, 15]. However, this often has certain side effects, affecting the quality of life of patients. Therefore, it is very important for patients with SCI to find a safe, effective and healthy treatment. Exercise training is a non-traumatic rehabilitation method after SCI that can promote the functional recovery of paralyzed muscles [16–19]. Also, most studies have focused on the effect of drug therapy on the neurovascular system after SCI [20, 21], ignoring the protection and functional recovery of the vascular system by exercise training. Based on this fact, starting from the actual clinical practice and simulating the exercise rehabilitation of clinical patients, our experimental team designed and invented an underwater-treadmill for the first time which is suitable for rats to exercise after SCI. However, the protective effect of TT on SCI has not been reported in the literature; its effects on the BSCB are not clear.

The purpose of this study was to explore the protective effect of TT on the BSCB and its possible mechanism. As far as we know, this is the first time that we have applied TT to the treatment of SCI. We found that TT can enhance the expression of TJ and AJ proteins after SCI. We also found that TT could promote angiogenesis and inhibit the expression of MMP-2/9, which may be an important mechanism for TT to maintain the stability of BSCB. In addition, these experimental results will provide a better understanding of the possible mechanism of TT in the treatment of SCI and provide a reliable basis for TT application in the future.

Method

Antibodies

Anti-MMP-9, anti-Tubulin were purchased from Proteintech (Rosemont, IL, USA). Anti-VEGF and β-Actin were purchased from Abcam (330 Cambridge Science Park, Cambridge, UK). Anti-p120-Catenin, anti-β-Catenin, anti-ZO-1, anti-Occludin, and anti-Claudin were purchased from Affinity (OH, USA).

Animals

Adult male Sprague-Dawley rats (200–250g) were purchased from Shanghai Laboratory Animal Center. The protocols were approved by the Animal Research Committee of Wenzhou Medical University. All animals were housed in a controlled environment and regularly fed with food and water. Rats were randomly divided into the following three groups: Sham (group S); SCI (group M); SCI + TT (group TM).

SCI Model

Rats were anaesthetized with 2% pentobarbital sodium (30 mg/kg) and then were shaved to expose the T10 segment. The exposed site was impinged with a New York University(NYU) Impactor (10 g × 20 cm) in all groups except group S. The lower limb trembling contractions and tail wagging showed that SCI modeling was successful. Finally, the wound was sutured, disinfected with iodine volt, and the bladder was emptied every morning and evening.

Underwater-treadmill training

Our research group provided the initial designed of an underwater treadmill (Wenzhou Xinglong Stainless Steel Co., LTD, Zhejiang, China) and submitted it for a patent. Rats were given adaptive training for three days before spinal cord injury. One day after SCI, rats in the underwater-treadmill training group began training, which lasted for 7 d or 14 d (10-15 m/min, 5 min/round, 3 rounds in total, 5 min interval between rounds).

Behavioral tests

Two independent examiners who were blinded to the treatment groups conducted BBB scores on rats in an open field test [22]. To put it simply, the BBB score has a total of 21 points, and the higher the score is, the closer the animal is to normal.

Evaluation of BSCB permeability

Water content

At 7 d and 14 d after SCI, 2% sodium pentobarbital was intraperitoneally injected to anaesthetise the animals, and 0.5 cm of the T10 spinal cord was taken after perfusion with 0.9% normal saline. The degree of edema in this segment was measured by the dry and wet weight method.

Evans blue dye assays

According to previously reported methods [4, 8], rats were injected with EB dye (4 ml/kg) by tail vein at 7 d and 14 d after SCI, which was followed by 2% sodium pentobarbital anesthesia 2 hours late and 0.9% saline perfusion. Tissue containing T10 was soaked in N,N'-dimethylformamide at 50°C for 72 hours. The concentration of dyes in the samples were determined based on a standard curve (μg/g). Tissues were cut into 15 μm thick sections with a frozen microtome at 20°C, and then the sections were analysed. Quantitative analysis of data was performed with ImageJ software.

HE

Briefly, tissues were removed from rats at 7 and 14 d after spinal cord injury, and then they were stored in 4% paraformaldehyde for 24 hours (4°C). The spinal cord was immersed in a 0.1 m phosphate buffer solution and a 30% sucrose solution overnight (4°C). Successive sections (15 μm thick) were frozen and stored for subsequent use. HE staining experiments were carried out with the appropriate kits.

Western Blot Analysis

Tissues containing T10 segments were put into a collection tube containing a mixture of PMSF and RIPA (100:1) and then were microfuged at 12,000 rpm for 5 min at 4°C. We extracted the supernatant and calculated the protein concentration with a BCA kit. The mixed solution was heated to 100°C for 10 min. After electrophoretic transfer to membranes, primary and secondary antibodies were incubated successively. Then, the signal was digitally quantified.

Immunofluorescence Staining

After drying sections, they were washed 3 times for 15 minutes. After being treated with nonimmune goat serum for 1 hour, sections were incubated first with primary antibodies against rabbit anti-occludin antibody (1:100, Affinity, US), and rabbit anti-claudin antibody (1:100, Affinity, US) at 4°C, and then with Alexa Fluor 488 Affinipure goat anti-rabbit IgG (H+L) (1:200, Yeasen, China) for 50 min at room temperature. Phosphate-buffered saline (PBS) was used in place of the primary antibody in the negative control. Cells were incubated with DAPI for 10 minutes, and then washed with PBS. Observation of the fluorescence signal under laser confocal microscopy. Five fields on each of three slides per animal were randomly selected for visualization by light microscopy. Analysis was performed using ImageJ software (National Institutes of Health, Bethesda, MD, USA).

Transmission Electron Microscopy

Refer to previous research[23], fresh spinal cord tissue was removed after administering anaesthesia and performing perfusion. Tissue was quickly cut into 1 mm^3 pieces on ice and soaked in 2.5% glutaraldehyde. The tissue was fixed with a 1% oxidizing fixative for 1 h and stained with 1% uranyl acetate for 2 h, and then the tissue was embedded after dehydration in gradient acetone solution. After semithin sectioning and toluidine blue staining, ultrathin sections were cut and observed by Hitachi TEM.

Statistical Analysis

All experimental data are expressed as the mean ± standard deviation. When comparing the two groups, a t-test was used. One-way ANOVA and Dunnett's test were used to evaluate the data when comparing more than two groups of components. SPSS 16 statistical software for statistical analysis, and $p < 0.05$ was considered statistically significant.

Results

TT reduces the permeability of BSCB after SCI

We used an impactor to generate a model of SCI **(Fig. 1a)**. Meanwhile, Evan's Blue staining and statistical analysis of spinal cord water content were used to detect the function of BSCB. The results showed that the water content increased significantly after SCI, and TT could reduce the oedema caused by SCI **(Fig. 1b-c)**. The amount of EB exudation increased significantly after SCI compared with that of group S, suggesting BSCB leakage. After TT training, infiltration of the BSCB was significantly improved. **(Fig. 1d-e)**. The fluorescence intensity of EB after SCI was much higher than that in the S group. However, the fluorescence level of the TM group was significantly lower than that of the M group **(Fig. 1f)**. The quantitative analysis of EB also showed the same results **(Fig. 1g)**. All these data indicate that TT can inhibit BSCB disruption.

TT decreased tissue structure damage and improved functional recovery after SCI

At 7 and 14 d after injury, histomorphological differences in T9-T11 levels were observed by HE staining **(Fig. 2a)**. The arrangement of tissues in group S was normal, and the grey matter and white matter were destroyed to varying degrees in group M, which was accompanied by the death of multiple neurons; the results in group TM was significantly better than that in group M. The quantitative analysis of the cavity area showed the same results **(Fig. 2b)**. The functional recovery of the S, M and TM groups was evaluated by BBB scores at 6 h, 1 d, 3 d, 7 d, and 14 d after SCI **(Fig. 2c)**. The BBB scores of the TM group

were significantly higher than those of the M group at 7 d and 14 d after SCI. These results show that TT can significantly improve functional recovery and tissue preservation.

TT prevents the loss of TJ and AJ proteins

To further determine the effect of TT on BSCB protein, β-Catenin, p120-Catenin, ZO-1, Claudin-5, and Occludin were examined by Western blot. According to the results **(Fig. 3a)**, the expression of TJ and AJ protein decreased to some extent after SCI compared to the levels of the control. However, the rats treated with TT showed high TJ and AJ protein expression at 7 d **(Fig. 3b)**. Using Claudin-5/CD31/Hoechst **(Fig. 3c)** and Occludin/CD31/Hoechst **(Fig. 4)** staining to observe the distribution of BSCB proteins after SCI, we found that TT could reduce the degradation of Claudin-5/Occludin around the epicenter. These results suggest that TT can prevent the loss of TJ and AJ proteins after SCI.

TT promotes angiogenesis after SCI

We further detected the expression of VEGF protein 7 d after SCI. Compared with its expression in group S, the expression of VEGF decreased significantly after SCI. At the same time, TT significantly upregulated the expression of VEGF **(Fig. 5a-b)**. Blood vessels that colabeled with 5-bromo-2-deoxyuridine (BrdU) and laminin (Laminin) in spinal cord tissue were quantitatively analysed. As shown in **Fig. 5c-d**, angiogenesis in the M group was significantly higher than it was in the S group at 7 d after injury. Additionally, the neovascularization density of the TM group was increased compared with that in the M group, suggesting that TT can effectively promote angiogenesis.

TT inhibits the expression of MMP-2/9 after SCI

The expression level of MMP-2/9 protein was detected by Western blot. The results showed that TT could significantly inhibit the upregulation of MMP-2/9 after SCI **(Fig. 6a-c)**. In addition, to determine the defects of vascular endothelial cells, we analysed them by TEM. In the S group, the vascular endothelial cells of the spinal cord were closely connected to form a relatively closed vascular cavity, but the tight junction between cells was opened after SCI. Compared with the S group in the same period, the structure of the BSCB in group M was disordered, vascular endothelial cells were atrophied, and scar tissue had formed around blood vessels. In contrast, TT could significantly reduce the gap between cells **(Fig. 6d)**.

Discussion

In this study, our data indicate that TT can protect the integrity of BSCB and prevent further edema of spinal cord. After TT training, the organizational structure and motor function can also be significantly improved. Meanwhile, TT can promote angiogenesis and inhibit the expression of MMP-2/9. Therefore,

the vascular protection of the BSCB by TT may occur through the following mechanisms: (1) TT protects residual BSCB structure from further damage; (2) it promotes vascular regeneration; and (3) it inhibits the expression of MMP-2/9 to mitigate BSCB damage.

Emerging evidence indicates that exercise therapy can promote recovery after SCI [24–27]. It is well known that normal exercise training cannot be carried out in rats after SCI. We introduced underwater-treadmill training(TT), which simulates the clinical exercise treatment and can help rats successfully train on the treadmill in the early stage of SCI. TT is mainly used in rehabilitation training of motor system diseases [28, 29]. TT can reduce the resistance of forward movement, allowing patients to walk or run with a normal gait [30]. Additionally, the depth and speed of the TT can be adjusted, which facilitates the control of exercise intensity [30], and its related mechanism was studied for the first time in our experiment.

The integrity of the BSCB is essential for the spinal cord to maintain normal function [31]. However, the BSCB is destroyed after SCI, which leads to increased permeability, causing a series of secondary damage [14, 32]. In our study, through the horizontal comparison of data of each group in the same period, we found that the degree of edema in the M group increased significantly compared to that of S group. However, the water content of spinal cord decreased significantly after TT training, which indicated that the integrity of BSCB was significantly improved after exercise training in SCI rats. By longitudinal comparison of all the indexes 7 and 14 d after SCI in the M group, it was found that there was some amount of self-recovery. The recovery of these partial functions may be related to the preservation of BSCB function, and the formation of new blood vessels.

The motor function of rats was seriously damaged after SCI, while it was significantly recovered after TT training. In our experiment, through the BBB score, we can see that this trend increases over time, and it is most obvious at 14 d. After the impact of the percussion device, there were obvious cavities in the spinal cord tissue, disordered arrangement of cells, a large number of cell necrosis and nuclear pyknosis. There is such a phenomenon in both short-term and long-term. TT training can significantly improve the organizational structure. This can be explained by the fact that after the body is stimulated more than it can regulate, the cells will develop in a worse direction and eventually lead to death. However, if we can take appropriate measures in time, we can well curb the occurrence of this situation. In this experiment, TT has exactly this effect.

The BSCB protects the central nervous system by restricting the entry of plasma components and blood cells [12]. Following SCI, the destruction of the BSCB leads to increased microvascular permeability, inflammatory reaction, tissue edema, and neurotoxic products [33]. The BSCB is formed by a dense network of TJs and AJs, which is destroyed after SCI, resulting in a decrease in the expression of TJs and AJs proteins. Tight, adhesion, and gap junctions form the endothelial cells lining of microvessels in the spinal cord[3, 34]. Our results show that the expression of p120-Catenin, β-Catenin, ZO-1, Occludin, and Claudin-5 were greatly reduced after SCI. However, their expressions were significantly stabilized in the

TT-treated spinal cord. This shows that the residual BSCB structure is protected from being destroyed, which may be a mechanism by which TT plays a protective role.

In order to determine the protection mechanism of TT, we have carried out more in-depth research. Vascular endothelial growth factor (VEGF) is a highly specific vascular endothelial cell growth factor that promotes vascular permeability, proliferation, and angiogenesis [35]. It has been reported that VEGF reached a peak at 3 d, and there was still a large amount of expression at 7 d after SCI [36]. In addition, ischemia and injury can induce angiogenesis, which will provide oxygen and nutrition to the ischemic or diseased site, thus improving tissue repair and remodeling [37–39]. We used laminin as a vascular marker and BrdU as a proliferation marker [40]. The results showed that TT could promote angiogenesis after SCI, as shown by detecting the protein expression of VEGF and performing quantitative analysis of neovascularization 7 d after SCI with laminin and BrdU co-labelling. The new blood vessels form a closer connection on the original residual BSCB structure. This explains why the vascular permeability of early rising in rats with SCI is much more serious than that at 14 d.

The activation of MMP-2/9 after SCI plays an essential role in the destruction of the BBB/BSCB [9, 12]. The expression of MMP-2/9 will aggravate the damage of BSCB[41]. In this study, 7 d after SCI, the expressions of MMP-2/9 were up-regulated, which was due to physiological self-regulation after SCI. But this further aggravates the damage to BSCB. We were surprised to find that the expression of MMP-2/9 decreased significantly after TT training. Although we have not proved how TT down-regulates the expression of MMP-2/9 we believe that TT can effectively prevent the destruction of the BSCB, and part of its mechanism may be to inhibit the expression of MMP-2/9 after SCI.

We acknowledge the limitations of our studies. Due to the limitation of experimental conditions, we could not observe changes at the functional level through electrophysiological techniques to understand the role of TT. The specific mechanism by which TT inhibits the expression of MMP-2/9 remains to be further studied.

Conclusion

TT participates in protection of the BSCB after SCI by protects residual BSCB structure from further damage. Furthermore, TT promotes vascular regeneration. Additionally, TT Inhibits the expression of MMP-2/9 to mitigate BSCB damage (Fig. 7).

Abbreviations

SCI, Spinal cord injury; TT, Underwater-treadmill training; BSCB, Blood-spinal cord barrier; MMP, Matrix metalloproteinase; BBB, Basso-Beattie-Bresnahan; HE, Haematoxylin-eosin; EB, Evans blue; WB, Western blot; TEM, Transmission electron microscopy; CD31
Platelet endothelial cell adhesion molecule-1.

Declarations

Ethics approval and consent to participate

The protocols were approved by the Animal Research Committee of Wenzhou Medical University

Consent for publication

Not applicable

Availability of data and materials

The datasets supporting the conclusions of this article are available from the corresponding author on reasonable request

Competing interests

The authors declare that they have no competing interests.

Funding

This research was partly supported by the National Natural Science Foundation of China (No.81873376, 81802243, 81603433, 81574074).

Authors' contributions

YXW designed the experiments and wrote the first manuscript; XQF and LSC was responsible for data acquisition and analysis; YXL, ZKC, YJJ and CXL conceived the experiments; TWZ and YGH provided guidance; JSH supervised the whole process of the experiment, and was a major contributor in writing the manuscript. All authors read and approved the final manuscript.

Acknowledgements

We would like to express our heartfelt thanks to all the technical personnel who have provided assistance in this work.

References

1. Cordaro M, et al. Fumaric Acid Esters Attenuate Secondary Degeneration after Spinal Cord Injury. J Neurotrauma. 2017;34(21):3027–40.
2. Rao J, Tiruchelvarayan R, Lee L. Palliative surgery for cervical spine metastasis. Singapore Med J. 2014;55(11):569–73.
3. Bartanusz V, et al. The blood-spinal cord barrier: morphology and clinical implications. Ann Neurol. 2011;70(2):194–206.

4. Zheng B, et al. Epidermal growth factor attenuates blood-spinal cord barrier disruption via PI3K/Akt/Rac1 pathway after acute spinal cord injury. J Cell Mol Med. 2016;20(6):1062–75.
5. Beck KD, et al. Quantitative analysis of cellular inflammation after traumatic spinal cord injury: evidence for a multiphasic inflammatory response in the acute to chronic environment. Brain. 2010;133(Pt 2):433–47.
6. Abbott NJ, Ronnback L, Hansson E. Astrocyte-endothelial interactions at the blood-brain barrier. Nat Rev Neurosci. 2006;7(1):41–53.
7. Yao Y, et al. Flufenamic acid inhibits secondary hemorrhage and BSCB disruption after spinal cord injury. Theranostics. 2018;8(15):4181–98.
8. Zhang D, et al., *Metformin ameliorates BSCB disruption by inhibiting neutrophil infiltration and MMP-9 expression but not direct TJ proteins expression regulation*. 2017. 21(12): p. 3322–3336.
9. Asahi M, et al. Effects of matrix metalloproteinase-9 gene knock-out on the proteolysis of blood-brain barrier and white matter components after cerebral ischemia. J Neurosci. 2001;21(19):7724–32.
10. Heo JH, et al. Matrix metalloproteinases increase very early during experimental focal cerebral ischemia. J Cereb Blood Flow Metab. 1999;19(6):624–33.
11. Chang DI, et al. Activation systems for latent matrix metalloproteinase-2 are upregulated immediately after focal cerebral ischemia. J Cereb Blood Flow Metab. 2003;23(12):1408–19.
12. Noble LJ, et al. Matrix metalloproteinases limit functional recovery after spinal cord injury by modulation of early vascular events. J Neurosci. 2002;22(17):7526–35.
13. Lee JY, et al., *17beta-estradiol inhibits MMP-9 and SUR1/TrpM4 expression and activation and thereby attenuates BSCB disruption/hemorrhage after spinal cord injury in male rats.* Endocrinology, 2015. **156**(5): p. 1838-50.
14. Lu Y, et al. Bone Mesenchymal Stem Cell-Derived Extracellular Vesicles Promote Recovery Following Spinal Cord Injury via Improvement of the Integrity of the Blood-Spinal Cord Barrier. Front Neurosci. 2019;13:209.
15. Park CS, et al. Protocatechuic acid improves functional recovery after spinal cord injury by attenuating blood-spinal cord barrier disruption and hemorrhage in rats. Neurochem Int. 2019;124:181–92.
16. Leech KA, Hornby TG. High-Intensity Locomotor Exercise Increases Brain-Derived Neurotrophic Factor in Individuals with Incomplete Spinal Cord Injury. J Neurotrauma. 2017;34(6):1240–8.
17. Ward PJ, et al., *Training-Induced Functional Gains following SCI.* Neural Plast, 2016. **2016**: p. 4307694.
18. Wang H, et al. Treadmill training induced lumbar motoneuron dendritic plasticity and behavior recovery in adult rats after a thoracic contusive spinal cord injury. Exp Neurol. 2015;271:368–78.
19. Hayashibe M, et al. Locomotor improvement of spinal cord-injured rats through treadmill training by forced plantar placement of hind paws. Spinal Cord. 2016;54(7):521–9.

20. Sharma HS, et al. Neuroprotective effects of a potent bradykinin B2 receptor antagonist HOE-140 on microvascular permeability, blood flow disturbances, edema formation, cell injury and nitric oxide synthase upregulation following trauma to the spinal cord. Int Rev Neurobiol. 2019;146:103–52.
21. Sahib S, et al. Potentiation of spinal cord conduction and neuroprotection following nanodelivery of DL-3-n-butylphthalide in titanium implanted nanomaterial in a focal spinal cord injury induced functional outcome, blood-spinal cord barrier breakdown and edema formation. Int Rev Neurobiol. 2019;146:153–88.
22. Ying X, et al. Hyperbaric oxygen therapy reduces apoptosis and dendritic/synaptic degeneration via the BDNF/TrkB signaling pathways in SCI rats. Life Sci. 2019;229:187–99.
23. Berliner J, et al. Abnormalities in spinal cord ultrastructure in a rat model of post-traumatic syringomyelia. Fluids Barriers CNS. 2020;17(1):11.
24. Dugan EA, Jergova S, Sagen J. Mutually beneficial effects of intensive exercise and GABAergic neural progenitor cell transplants in reducing neuropathic pain and spinal pathology in rats with spinal cord injury. Exp Neurol. 2020;327:113208.
25. Kobayakawa K, et al. Locomotor Training Increases Synaptic Structure With High NGL-2 Expression After Spinal Cord Hemisection. Neurorehabil Neural Repair. 2019;33(3):225–31.
26. Kissane RWP, et al., *Effects of treadmill training on microvascular remodeling in the rat after spinal cord injury*. 2019. 59(3): p. 370–379.
27. Ryu Y, et al. Effects of Treadmill Training Combined with Serotonergic Interventions on Spasticity after Contusive Spinal Cord Injury. J Neurotrauma. 2018;35(12):1358–66.
28. Ayan C, et al. Treadmill training combined with water and land-based exercise programs: Effects on Parkinson's disease patients. NeuroRehabilitation. 2016;39(2):295–9.
29. Narasaki-Jara M, et al. Aquatic treadmill walking at three depths of water in people with traumatic brain injury. Physiother Res Int. 2020;25(2):e1817.
30. Munoz A, et al. The Use of the Water Treadmill for the Rehabilitation of Musculoskeletal Injuries in the Sport Horse. J Vet Res. 2019;63(3):439–45.
31. Zheng B, et al. Dl-3-n-butylphthalide prevents the disruption of blood-spinal cord barrier via inhibiting endoplasmic reticulum stress following spinal cord injury. Int J Biol Sci. 2017;13(12):1520–31.
32. He Z, et al., *Inhibition of Endoplasmic Reticulum Stress Preserves the Integrity of Blood-Spinal Cord Barrier in Diabetic Rats Subjected to Spinal Cord Injury*. 2017. 7(1): p. 7661.
33. Yang Y, et al. Matrix metalloproteinase-mediated disruption of tight junction proteins in cerebral vessels is reversed by synthetic matrix metalloproteinase inhibitor in focal ischemia in rat. J Cereb Blood Flow Metab. 2007;27(4):697–709.
34. Joshi HP, et al. CORM-2-Solid Lipid Nanoparticles Maintain Integrity of Blood-Spinal Cord Barrier After Spinal Cord Injury in Rats. Mol Neurobiol. 2020;57(6):2671–89.
35. Xie Q, et al. Treadmill exercise ameliorates focal cerebral ischemia/reperfusion-induced neurological deficit by promoting dendritic modification and synaptic plasticity via upregulating caveolin-1/VEGF

signaling pathways. Exp Neurol. 2019;313:60–78.

36. Yu D, et al., *[Effects of bone marrow mesenchymal stem cells transplantation on expression of vascular endothelial growth factor gene and angiogenesis after spinal cord injury in rats].* Zhongguo Xiu Fu Chong Jian Wai Ke Za Zhi, 2011. **25**(7): p. 837 – 41.
37. Loh KP, et al. TRPM4 inhibition promotes angiogenesis after ischemic stroke. Pflugers Arch. 2014;466(3):563–76.
38. Zhu W, et al. Insulin growth factor-1 gene transfer enhances neurovascular remodeling and improves long-term stroke outcome in mice. Stroke. 2008;39(4):1254–61.
39. Huang XT, et al. Intracerebroventricular transplantation of ex vivo expanded endothelial colony-forming cells restores blood-brain barrier integrity and promotes angiogenesis of mice with traumatic brain injury. J Neurotrauma. 2013;30(24):2080–8.
40. Pang Q, et al. Role of caveolin-1/vascular endothelial growth factor pathway in basic fibroblast growth factor-induced angiogenesis and neurogenesis after treadmill training following focal cerebral ischemia in rats. Brain Res. 2017;1663:9–19.
41. Jing N, et al. Exogenous activation of cannabinoid-2 receptor modulates TLR4/MMP9 expression in a spinal cord ischemia reperfusion rat model. J Neuroinflammation. 2020;17(1):101.

Figures

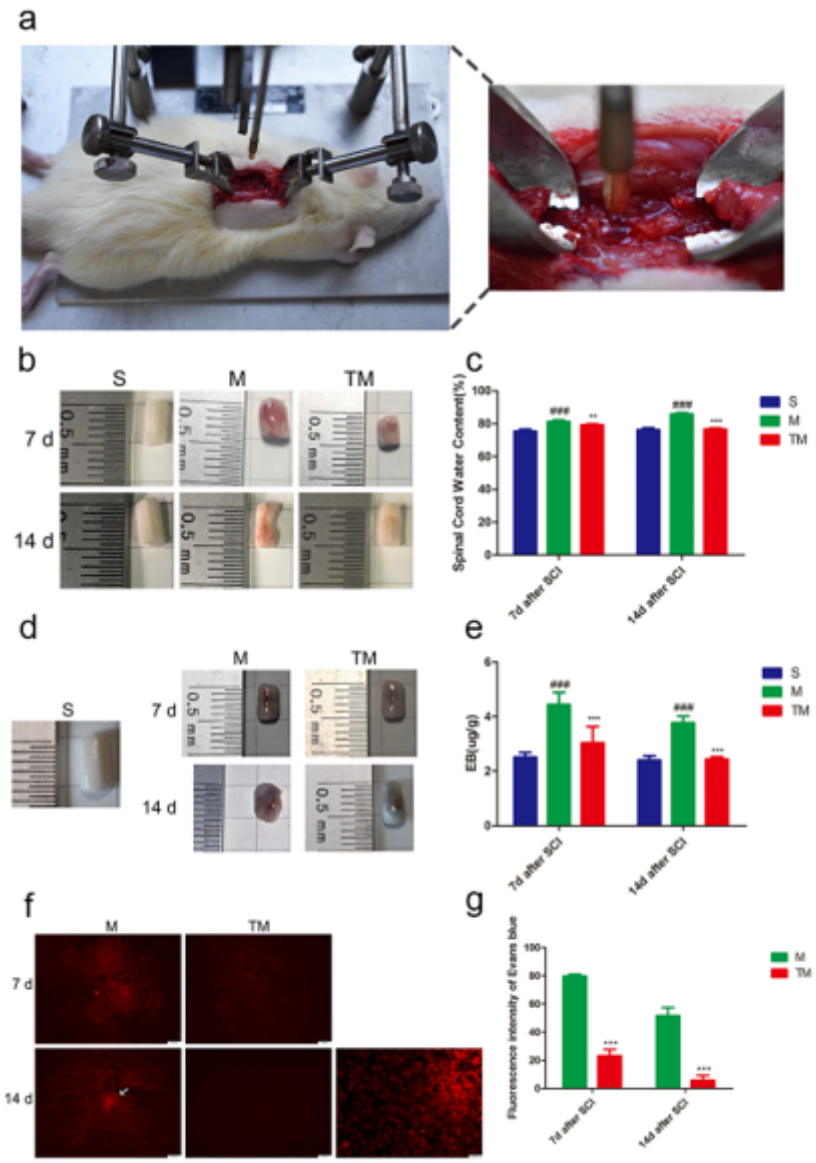

Figure 1

TT reduces the permeability of BSCB after SCI. (a) Spinal cord injury(SCI) was performed by NYU Impactor (10g × 20cm). (b, c) Representative quantification data of spinal cord water content in S M TM groups, columns represent mean ± SD, n=5. (d, e) Represent Evan's Blue dye permeabilized into spinal cord after SCI and quantification of the amount of Evan's Blue (ug/g), n =5. (f-g) Representative fluorescent images of Evans Blue Dye extravasation and quantification of the fluorescence intensity, n=5. #p < 0.05 as M group versus S group, *p < 0.05 as TM group versus M group. (#p, *p < 0.05; ##p, **p < 0.01; ###p, ***p < 0.001)

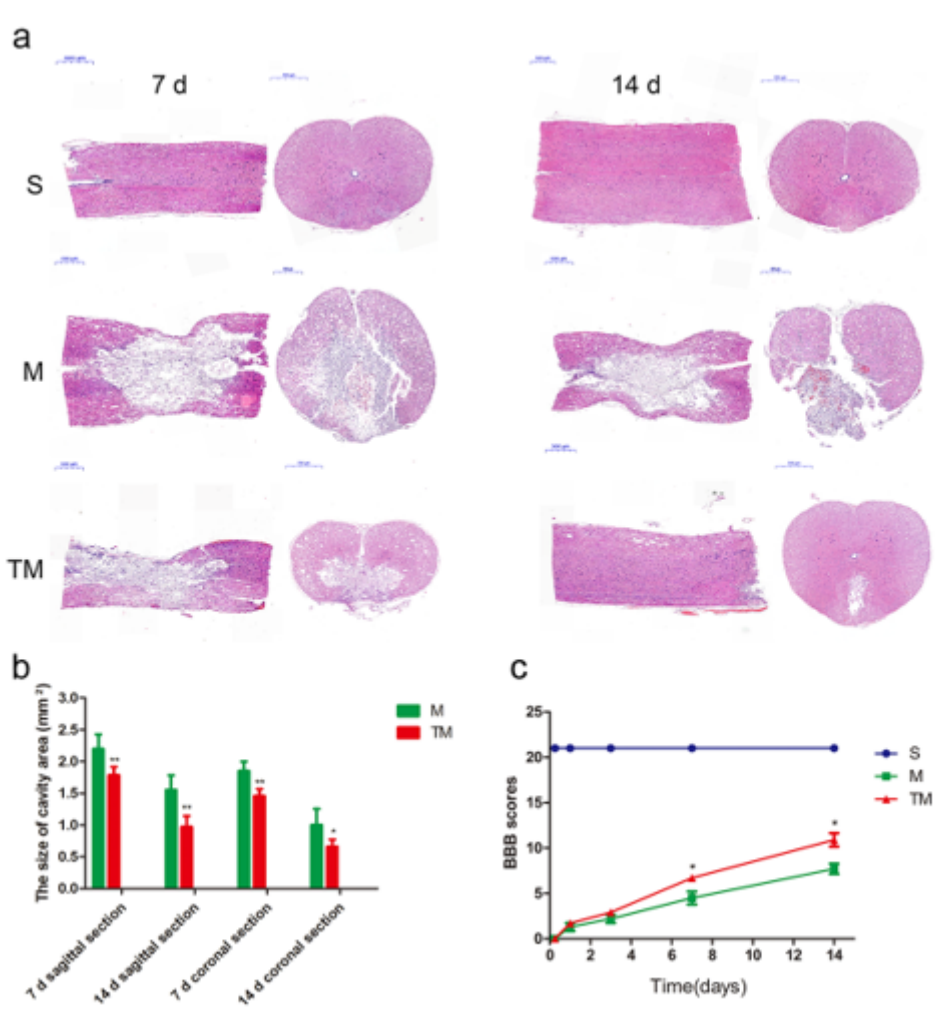

Figure 2

TT decreased tissue structure damage and improved functional recovery after SCI. (a) HE staining at 7 d and 14 d after SCI. Scale bars are 500 μm. (b) Quantification of the size of cavity area, columns represent mean ± SD, n=5. (c) BBB scores in S, M, TM groups. #p < 0.05 as M group versus S group, *p < 0.05 as TM group versus M group. (#p, *p < 0.05; ##p, **p < 0.01; ###p, ***p < 0.001)

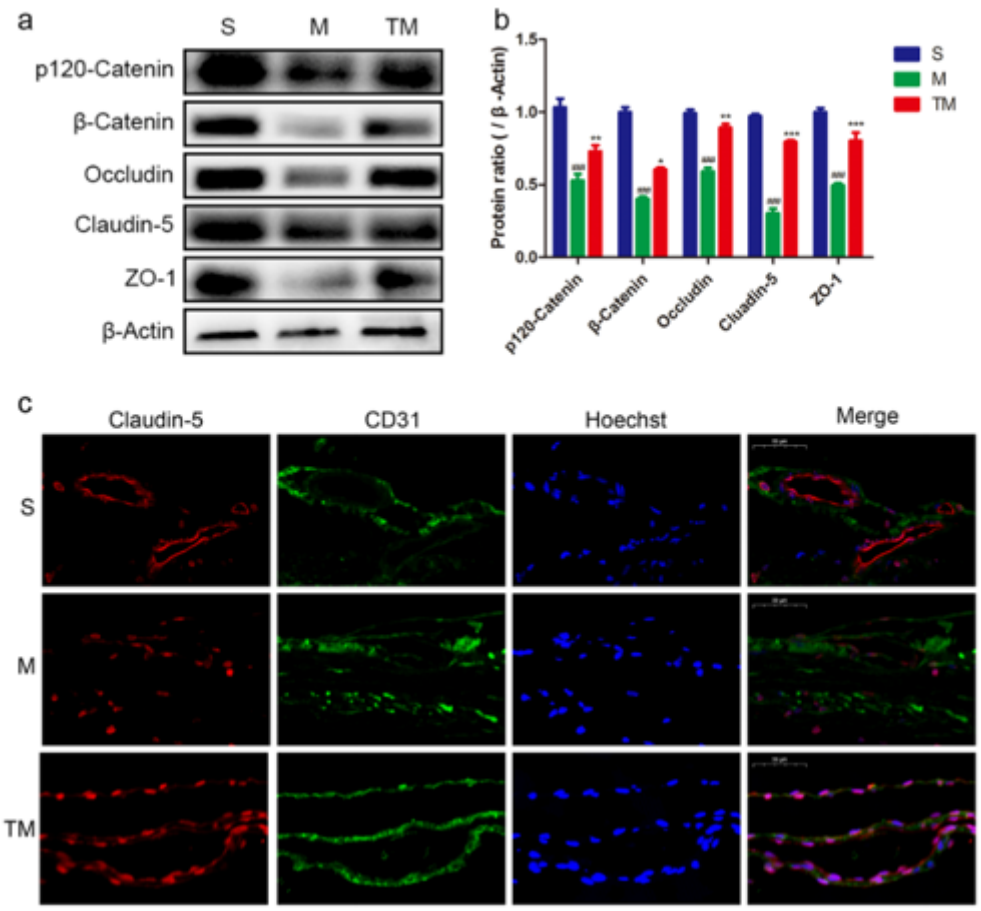

Figure 3

TT prevent the loss of TJ and AJ protein. (a, b) Represent western blots and quantification data of TJ and AJ protein in each group, columns represent mean ± SD, n=5. #p < 0.05 as M group versus S group, *p < 0.05 as TM group versus M group. (c)Double staining of Claudin-5/CD31/Hoechst. Red: Claudin-5; Green: CD31; Blue: Hoechst. Scale bar, 50 μm. #p < 0.05 as M group versus S group, *p < 0.05 as TM group versus M group. (#p, *p < 0.05; ##p, **p < 0.01; ###p, ***p < 0.001)

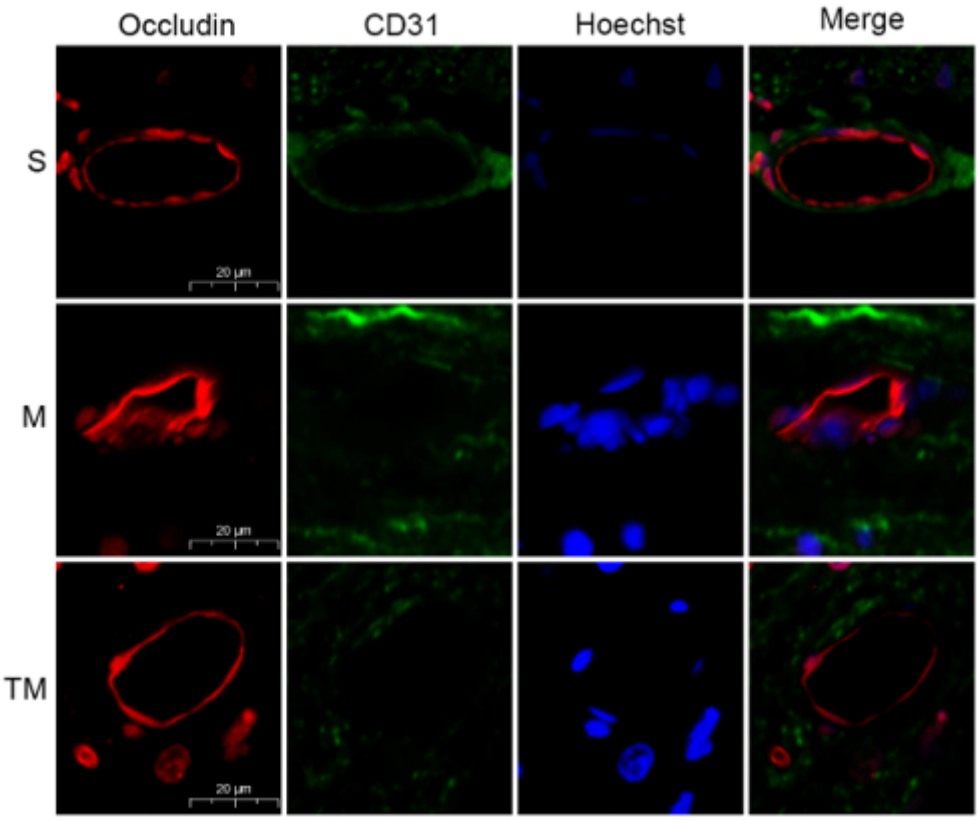

Figure 4

Double staining of Occludin/CD31/Hoechst. Red: Occludin; Green: CD31; Blue: Hoechst. Scale bar, 50 μm. The color overlap indicates the tight junction of vascular endothelium. Scale bar, 20 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article)

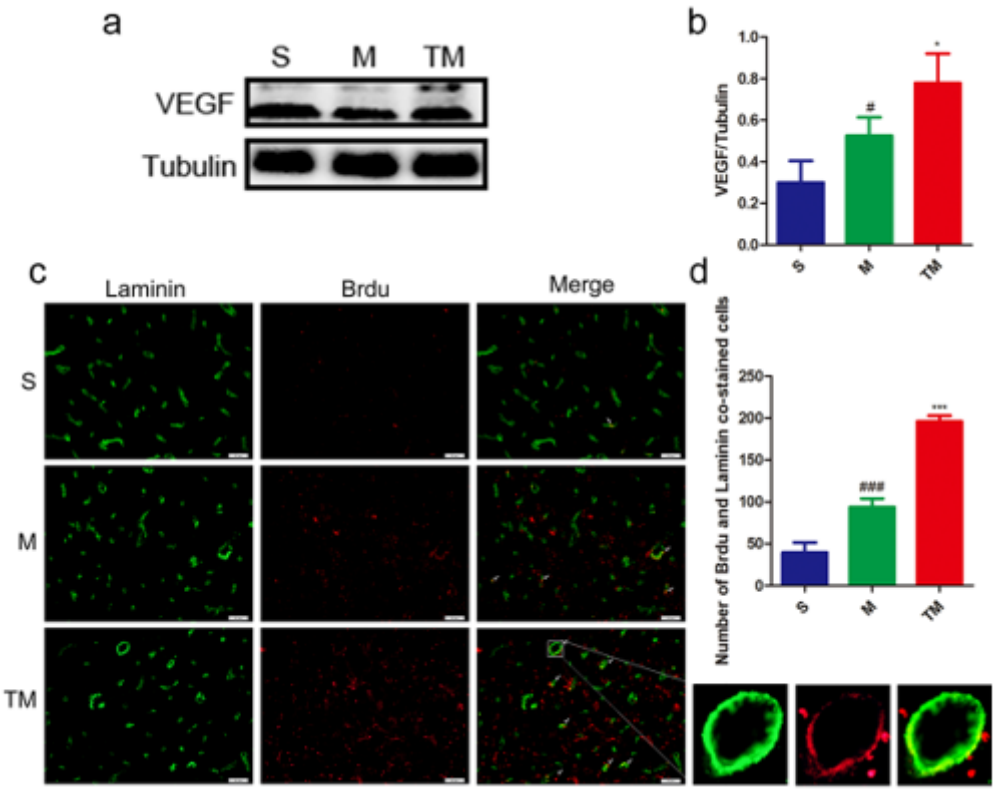

Figure 5

TT promotes angiogenesis after SCI. (a, b) Representative western blots and quantification data of VEGF/Tubulin, columns represent mean ± SD, n=5. (c) Double staining of Laminin(green)/Brdu (red) of sections from the spinal cord in each group rats. Scale bars are 20 μm. (d) Quantification data of number of Brdu and Laminin co-stained cells, columns represent mean ± SD, n=5. #p < 0.05 as M group versus S group, *p < 0.05 as TM group versus M group. (#p, *p < 0.05; ##p, **p < 0.01; ###p, ***p < 0.001)

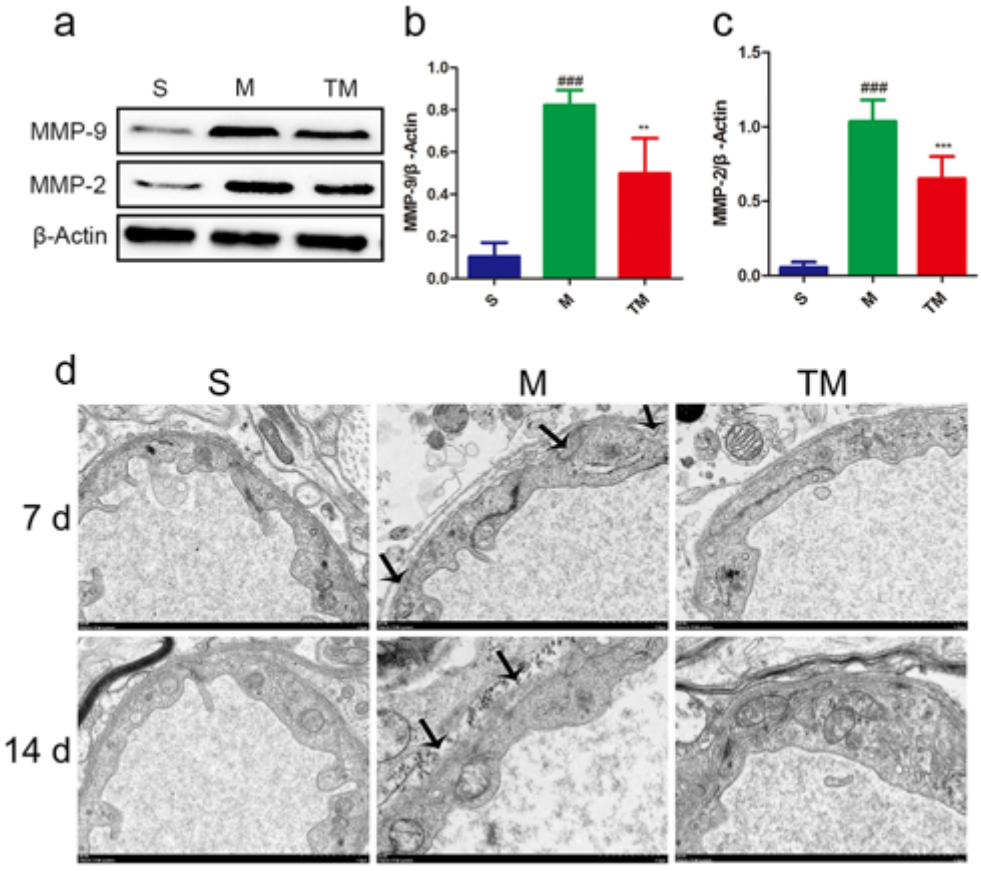

Figure 6

TT inhibits the expression of MMP-2 and MMP-9 after SCI. (a-c) Represent western blots and quantification data of MMP-2/9 in each group, columns represent mean ± SD, n=5. #p < 0.05 as M group versus S group, *p < 0.05 as TM group versus M group. (d) Transmission electron microscopy showed the vascular endothelial cell junction in S, M, TM. Arrows indicate an open tight junction and the scale bars are 1.0 μm. (#p, *p < 0.05; ##p, **p < 0.01; ###p, ***p < 0.001)

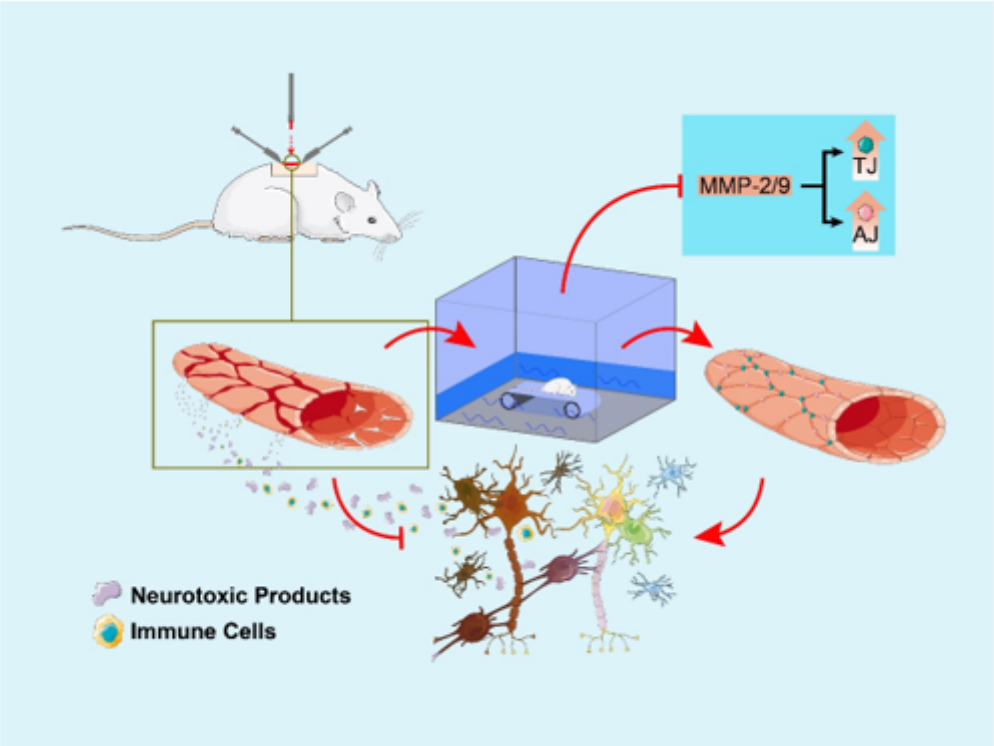

Figure 7

Proposed mechanism for the BSCB protection of underwater-treadmill training after SCI.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- videoS1.mp4