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Effects of Hydrogel-Fiber on Cystic Cavity after Spinal Cord Injury

Xijie Zhou,

Department of Neurosurgery, University of Maryland School of Medicine, Baltimore, MD, USA 21201.

Jian Du,

Department of Neurosurgery, University of Maryland School of Medicine, Baltimore, MD, USA 21201.

Xiaofeng Jia [Member, IEEE EMBS]

Department of Neurosurgery, Anatomy Neurobiology, Orthopaedics, University of Maryland School of Medicine, Baltimore, MD, USA 2120, and Department of Biomedical Engineering, Anesthesiology and Critical Care Medicine Johns Hopkins University, Baltimore, MD, USA 21205

Abstract

Spinal cord injury (SCI) affects millions of people around the world, but, functional recovery is far from optimal. The continuous emergence of biomaterials provides a new idea for the repair of SCI. Hydrogels can mimic the extracellular matrix (ECM), however, the unstable hydrogel shape limits its application. In this study, we evaluated the effect of fibrous hydrogel fiber (Polycaprolactone, (PCL(Polycaprolactone) fibers were added to the hyaluronic acid HA (hyaluronic acidhydrogel) on the recovery after of SCI. 20 adult male Wistar rats were randomly divided into 4 groups, (N=5): SCI+hydrogel group (H), SCI+hydrogel + PCL fiber group (HF), SCI group (SCI) and SHAM group (SHAM) and (N=5). SCI contusion injury was induced by a MASCIS Impactor (20g weight, 50cm high) at the T9 level in rats. Hydrogels or PCL fibers were administered into the SCI site one week after surgery. Periodical Basso, Beattie, and Bresnahan (BBB) locomotor score, spinal cord hematoxylin and eosin stain (HE) staining, and immunofluorescence staining were performed 28 days after the operation. HE staining showed that the average cystic cavity area in the SCI ($20.78 \pm 2.93 \text{ mm}^2$) group was significantly higher than that in H group ($6.54 \pm 0.85 \text{ mm}^2$), HF group ($5.06 \pm 0.76 \text{ mm}^2$) and SHAM group ($1.76 \pm 0.27 \text{ mm}^2$) ($P < 0.001$). There was no significant difference in BBB motor score among the HF group (16.80 ± 1.10), SCI (14.20 ± 1.09) and H group (15.00 ± 1.23) ($P > 0.05$), except the sham group. Immunofluorescence showed higher neuronal nuclear (NeuN) positive cells in both the H group and the HF group. This preliminary result may indicate that PCL fiber optimized the strength of hydrogels, thus providing better support for the axon regeneration. Future investigation is needed to further characterize PCL fiber and elucidate related mechanisms.

I. INTRODUCTION

Spinal cord injury (SCI) affects millions of people around the world. Direct lifetime costs can range from \$1.1 million to \$4.6 million per patient [1]. Unlike peripheral nerve injury (PNI), which affects the peripheral nervous system, SCI, affects the central nervous system (CNS), and often leads to permanent neurological impairments, for which patients rarely recover fully [2]. Unfortunately, there is no effective treatment to ensure the recovery of neurological function. Pathophysiologically, primary mechanical trauma injury through neurons and glial cells results in extreme changes in the surrounding microenvironment, including lack of neurotrophic factors, glial scar formation, myelin-associated protein expression inhibition, and possible progressive cell death in weeks after SCI. Therefore, it is important to mitigate the factors that change the microenvironment surrounding the injury upon nerve tissue damage to minimize progressive cell death.

Biomaterials have become an exciting strategy to simulate the complex structure of the extracellular matrix (ECM). Hydrogel has been regarded as a bridge for repair after SCI [2], and different components can be added as needed, for example, to release growth factors, to add inflammatory inhibitors, and to use stem cell inoculation to improve survival. Acellular matrix hydrogels from tissues or organs have been used for tissue repair due to their biocompatibility, tunability and tissue-specific ECM components. They can transmit regulatory signals to guide the growth of cell axons and related regeneration processes. Hydrogels, on the other hand, produce a chemical barrier that reduces and controls inflammation [3]. The previous study has indicated that the direct application of hydrogel mixed with stem cells and injected into the injured spinal cord in rats, promoted the growth and recovery of spinal cord axons [4]. However, hydrogels do not have a stable shape due to their characteristics and easily flow out of the site of SCI, thus affecting the therapeutic effect. Therefore, hydrogels play a limited role as a biological container that stores growth factors or stem cells [5]. Unlike applications in PNI [6], hydrogels may be difficult to administer in the injured spinal cord in rats. Aliphatic polyesters, such as poly (ϵ -caprolactone), poly (lactide), poly (glycolide) and their copolymers, have been extensively investigated for biomedical and pharmaceutical use and easily to form the fibers [7]. Based on this, we aim to improve the strength of hydrogels by adding fiber, to evaluate the ability to support axon regeneration, and to promote the recovery after SCI [5].

II. METHODS

A. Preparation of hydrogel and PCL fiber

The hydrogels and fibers were provided by Dr. Hai-Quan Mao's lab from the Johns Hopkins University. 4% (w/v) PEGDA (poly(ethylene glycol) diacrylate) and 1.25% thiolated hyaluronic acid (HA-SH) were dissolved in PBS respectively, and gently shaken at room temperature for 30 min [5, 8, 9]. Finally, the solution was mixed according to the volume of 1:4 poly(ethylene glycol) diacrylate (PEGDA) solution: 4 HA-SH solution, adding another 1% of ECM and 5% Poly caprolactone(PCL electrospun fibers), in order to achieve better strength [10, 11].

B. Experimental Procedures

The animal study was approved by the University of Maryland School of Medicine Animal Care and Use Committee. A total of 20 male Wistar rats, (Charles River, Wilmington MA) weighing 200 ~ 250 g were housed according to the guidelines of the National Institutes of Health (NIH) and exposed to 12 hours of light: dark cycles.

All rats were randomly assigned into four groups (N=5): SCI+hydrogel group (H), SCI+hydrogel + PCL fiber group (HF), SCI group (SCI), and SHAM group (SHAM). SCI contusion injury was induced by MASCIS Impactor in the rat model [12]. In brief, after anesthesia, an approximately 2 cm long median incision of the back was made, the skin and subcutaneous tissue were cut in turn, and the vertical spinous muscle was cut close to the lamina and stripped to the lateral side of the transverse process to expose the spinous process and lamina of T8-T1. The total laminectomy of T9 and T10 was performed with a dissection microscope. The dura mater was exposed, and the bilateral vertical spinous muscles were properly trimmed to obtain a satisfactory exposure for surgery. The adjacent spinous process was fixed with a spinous process fixation clip and the exposed thoracic spinal cord was made horizontally. 20g impact needle was used to carry out a single strike at the height of 50cm. Immediately after the strike, the hind limbs of the rats twitched in varying degrees. Then the muscles and skin were sutured in turn. One week after surgery, the animal was re-anesthetized and the surgical wound was reopened to explore the strike area. We injected hydrogels into the center of the injured spinal cord site using a Hamilton microliter syringe. The needle stayed in place for at least 1 min to avoid efflux of the hydrogel or the hydrogel +PCL.

On the 28th day after the surgery, all rats were fixed on the operating table and anesthetized with 5% isoflurane. The heart was then exposed, perfused with saline, and then perfused with 4% paraformaldehyde. The injured segments of spinal cord specimens were immersed and fixed for 2 days and placed in 30% sucrose 0.1-MPBS solution for at least 48 h. After freezing the spinal cord specimens, 14 μ m thick cross-sectional serial sections were obtained at -20°C frozen slicer (Lycra, Germany), all of which were stored in -20°C cryoprotectant. Three specimens were randomly selected for quantitative immunofluorescence staining and histological HE staining.

C. Behavioral evaluation

Basso, Beattie, and Bresnahan (BBB) locomotor rating scale was recorded before surgery and on the 1st, 7th, 14th, 21st and 28th day after surgery. The range of BBB from 0 to 21 represents inactive to normal movement of the hind limbs. The animal was placed alone on the open field with free movement for 5 minutes. Multiple tests of the hind limbs of each rat were assessed for a BBB motor score as we previously described [13, 14].

D. Staining protocol

All fixed slices were washed three times in PBS solution (5mins per wash), incubated in 0.2% TritonX-100 solution (pH 7.4) at room temperature, and then . Then, washed in phosphate buffer on three times, with 5 washes each time. After further washing, slices were locked with bovine serum albumin (BSA) for 1 hour. Spinal cord sections were incubated

with the anti-NeuN antibody (mouse anti-Neuronal Nuclei diluted 1:500; Millipore, USA), then incubated at 4 °C for 24 h. After incubation with the first antibody, the specimens were washed three times in PBS (5min/ time) and incubated in secondary antiserum solution. The sections were observed at 37 °C with goat anti-mouse secondary antibody labeled with Alexa Fluor 594 (diluted 1:500; Abcam, United States). Under the microscope, three sections of each spinal cord were randomly selected for observation. The fluorescence images of SCI slices (fiber and anti-neuron) were analyzed by Leica DMI8 microimaging equipment (Leica Microsystems). The area of the spinal cord cystic cavity was recorded and calculated by using ImageJ software (National Institutes of Health) [15] (Version 1.52), as we previously published [16].

E. Statistics

The results of parametric data were presented as mean $\pm$ S.E.M. Univariate analysis was used to compare the BBB score, the ratio of the maximum injured area of the spinal cord, and the difference of quantitative immunofluorescence analysis among different groups. All the values were expressed as mean $\pm$ SEM. $P < 0.05$ was defined as statistically significant. All statistics were performed using SPSS (IBM SPSS Statistics, version 22).

III. RESULTS

During the 28-day follow-up period, the motor function of all groups recovered to varying degrees. according to BBB score, the motor function of the four groups recovered was shown in Figure 1. Compared with SHAM group, the BBB function scores of the other three groups were significantly decreased ($p < 0.001$), but aggregate BBB data showed there was no significant difference among the HF group (16.80 ± 1.10), SCI (14.20 ± 1.09) and H group (15.00 ± 1.23) ($p > 0.05$).

HE staining showed that both H group and HF group had different degrees of regeneration with decreased cystic cavity area (Figure 2), while the cystic cavity areas in SCI group ($20.78 \pm 2.93 \text{ mm}^2$) was significantly larger than that in the H group ($6.54 \pm 0.85 \text{ mm}^2$), HF group ($5.06 \pm 0.76 \text{ mm}^2$) and SHAM group ($1.76 \pm 0.27 \text{ mm}^2$) ($P < 0.001$) (Figure 3).

Finally, immunofluorescence results showed that there were more NeuN positive cells around fiber in the HF group ($9.12 \pm 0.86\%$) than the H group ($3.32 \pm 0.73\%$) and the SCI group ($1.52 \pm 0.39\%$) ($P < 0.001$) (Figure 4).

IV. DISCUSSION

SCI is a major disabling disease currently with no satisfying treatment options. After the occurrence of SCI, due to the influence of many factors in different stages, recovery can be difficult, with serious potential consequences [17]. At present, the main treatment methods are surgical treatment and drug treatment, but the effectiveness is difficult to determine [18]. With the discovery of new biomaterials, more studies are focusing on the use of a variety of biomaterials to promote spinal cord regeneration, with the main purpose being to promote axonal growth and reduce the formation of glial scars [19–21]. Shenglian Yao et al. used many aligned fibrin coated with hydrogel to guide spinal cord regeneration and showed

certain improvement [3]. In our study, we used hydrogels mixed with PCL fiber to optimize the hydrogel strength [20] and lessened the cystic cavity formation after a T9 contusion SCI in rats.

Although the results of the BBB score showed that there was no significant difference among the three groups except the sham operation group, the motor function usually recovered slowly. HE staining showed that PCL fiber optimized hydrogel alleviated the damaged cystic cavity to a certain extent compared with SCI group. It may reduce the formation of glial scars, induce the proliferation of endogenous cells, and promote neuronal regeneration. The immunofluorescence results showed that there were more NeuN positive cells in the HF group, compared to the H group and the SCI group, dominantly around PCL fiber. We believe that the fibers in hydrogel could guide neuronal regeneration and promote axonal myelin formation. In addition, the fiber improved the strength of hydrogel and enhanced the beneficial effect of the hydrogel.

While the animal numbers in this preliminary study might not have the sufficient power to detect potentially significant differences in BBB scores, the following reasons may contribute to the inconsistent results between the staining results and behavioral score. First, it takes a long time for behavioral functions to recover after SCI. In general, the recovery time is at least 8–12 weeks [3]. The full recovery course might be longer than 4 weeks in our preliminary experiments. The neurons in the injured site may better regenerate and differentiate before a further lessening of the cystic cavity during the full recovery. Second, it may take additional time for the new regeneration pathways to be established and become functional. Third, the axon regeneration is affected by changes in the surrounding microenvironment, such as inflammatory factors, cell growth factors, etc. The mechanism of post-traumatic repair and recovery of limb movement needs to be further studied.

V. CONCLUSION

After the occurrence of SCI in rats, the addition of PCL fibers into hydrogel has a therapeutic effect on promoting regeneration in the injured spinal cord. This preliminary result may indicate PCL fibers optimized the strength of hydrogel, thus providing better support to promote the regeneration.

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Fig 1.

Compared with the SHAM group, the BBB function scores of the HF group (16.80 ± 1.10), SCI (14.20 ± 1.09) and H group (15.00 ± 1.23) were significantly decreased ($P < 0.001$). Although the scores of the HF group and H group were slightly higher than those of the SCI group at 28 days, there was no significant difference among the three groups ($P > 0.05$).

Fig 2.

Histological examination of the spinal cord showed the formation of the cystic cavity and tissue necrosis in the center of the injured area in the strike side. There were more microcapsule cavities around the central aqueduct and the neurons on the injured side were necrotic in varying degrees. HF group and H group had different degrees of tissue regeneration, while SCI group showed larger cystic cavity, accompanied by different degrees of tissue necrosis. HE staining, Magnification 5X and 20X.

Fig 3.

The quantitative area of spinal cord histology showed that the cystic cavity in the central lesion area of SCI group ($20.78 \pm 2.93 \text{ mm}^2$) was significantly larger than that in the H group ($6.54 \pm 0.85 \text{ mm}^2$), HF group ($5.06 \pm 0.76 \text{ mm}^2$) and SHAM group ($1.76 \pm 0.27 \text{ mm}^2$) ($P < 0.001$). There was no significant difference between the H group and the HF group ($P > 0.05$).

Fig 4.

Representative immunofluorescence images from the center of the injured area in the strike side in the spinal cords. Neuron staining (NeuN) was positive in red, while fiber showed its own color in green. Magnification 400X.