Supplementary methods

Modeling

Modeling preliminary experiment

In the preliminary experiment, six SD rats with a growth age of about 6 to 8 weeks and a weight of 200–220 g were selected to test the acuteness and feasibility of experimental modeling. The six rats were divided into three groups for modeling in the preliminary experiment: thread-tethering method modeling group A, B, and bio-adhesive model group C.

1) Thread-tying method for modeling:

After the rats in the suture-tethered model groups A and B were weighed, the rats were anesthetized by intraperitoneal injection of 1% sodium pentobarbital (0.35 ml/100 g, additional dose should not exceed 0.2 ml/100 g) in a sterile laboratory. Fix the prone position on the operating table, use depilatory agent to remove hair, expose the lumbosacral skin, perform routine iodine disinfection, lay a sterile drape, incise the waist skin and subcutaneous tissue under a microscope, peel off the muscles on both sides of the lamina, and use small elbow scissors Or in animal experiments, use a drill to make a small incision in the lamina to expose the end of the spinal cord and the filum terminale. Use a 5-0 non-invasive micro-suture to close to the root of the external filum terminale (the line connecting the iliac tubercles on both sides and the longitudinal axis of the spine). The intersection point is the center point (approximately the joint point of the sixth lumbar vertebra and the sacrum). Pass the filamentum terminale through the figure-eight suture, retain the two thread tails, and pass the two thread tails through needles respectively along the course of the filum terminale and lead them out from the skin of the tail root, about 0.5 cm to tie a knot, straighten and fix the filum terminale (appropriate tightness, no tension), and then fix the descending spinal cord on the paravertebral canal tissue to form a spinal cord tethering model. Observe the condition of the rat's lower limbs 1 day after surgery.

Pre-modeling results: The modeling operation of rat A using the thread-tethering method was about 40 min, and the postoperative Tarlov score of the lower limbs was 1 point. The activity of the rat mainly consisted of the forelimbs dragging the hind limbs. It is considered that excessive pulling after the sutures on the filum terminale of the spinal cord caused complete damage to the rat's spinal cord.

The thread-tethering method was used to create the model for rat B. The modeling operation lasted about 1 hour, and the skin was sutured and observed for 1 hour after the operation. The rat was dead. The reasons may be related to the long suturing time during the operation, excessive bleeding of the exposed spinal cord, excessive anesthesia during the operation, etc.

2) Bio-glue modeling:

Bioglue modeling method C: After the rats were weighed, they were anesthetized with 1% sodium pentobarbital (0.35 ml/100 g, additional dose should not exceed 0.2 ml/100 g) in a sterile laboratory, and then fixed in the prone position. On the operating table, use a depilatory agent to remove hair, expose the lumbosacral skin, perform routine iodine disinfection, lay a sterile drape, incise the lumbar skin and subcutaneous tissue under a microscope, peel off the muscles on both sides of the lamina, and use the same surgical operation position to Use small elbow scissors or a drill for animal experiments to cut a 2 mm circular opening in the lamina. Expose the end of the spinal cord and the filum terminale under a microscope. Use a 2 mm gelatin sponge to pad the opening to protect the conus tissue of the spinal cord. Use a 1 ml syringe to extract α – A compound of n-octyl cyanopropionate and α-n-butyl cyanopropionate (Fuale medical glue) and add 1 to 2 drops of medical glue to the opening of the spinal canal to straighten and fix the filum terminale (with moderate tightness, No tension), check that there is no obvious bleeding on the wound surface, suture the back wound, and observe the condition of the rat's lower limbs 1 day after surgery.

Preliminary experiment results: The bioglue modeling of rat C took about 20 min, and the Tarlov score of the lower limbs was 3 points 1 day after surgery, which met the criteria for successful modeling. This modeling method takes a short time, causes less damage to rats, and has high operation repeatability and uniformity.

TCS modeling method

The method of fixing the filum terminale of the spinal cord with biological glue is determined as the modeling method: weigh the rats, use 1% sodium pentobarbital (0.35 ml/100 g, additional dose does not exceed 0.2 ml/100 g) in the sterile animal operating room Anesthetize the rat by intraperitoneal injection, place it in the prone position and fix it on the operating table, expose the lumbosacral skin, disinfect it with

routine iodophor, lay a sterile drape, and place the spinal cord at the intersection of the iliac tubercle connection line on both sides and the longitudinal axis of the spine. Use tissue to cut off the skin and subcutaneous tissue on the lower back, peel off the muscles on both sides of the lamina, and fully stop bleeding. Use small elbow scissors or a drill for animal experiments to make a small incision in the lamina, and remove the end of the spinal cord and filum terminale under a microscope. When exposed, use 2 mm gelatin sponge to insert into the opening to protect the conus medullaris tissue. Use a 1ml syringe to drop 1 to 2 drops of medical glue into the opening of the spinal canal to straighten and fix the filum terminale (with moderate tightness and no tension).

After checking that there was no obvious bleeding on the wound surface, a tethered model was formed. The muscles and skin are sutured layer by layer, and penicillin is injected to prevent infection 1 to 3 days after the operation. The Tarlov score of the nerve function of the lower limbs of rats is used as the evaluation criterion for the success of TCS modeling. After the rats wake up from anesthesia and undergo a 1-day pain adaptation period, the spinal cord function Tarlov score (0 to 5 points) is evaluated 24 h later and is between 2 and 4 points, which belongs to the success criteria of TCS modeling. A score of 0 to 1 considers postoperative spinal cord injury, while a score of 5 considers that the spinal cord was not stretched or fixed during the operation.

Supplements

Tarlov score and BBB score

Tarlov rating

As a preliminary screening for the degree of hormone damage in rodents, it can be used as a scoring standard for the success of modeling:

0 points: No active movement of hind limbs;

1 point: The hind limbs can show very little movement but cannot bear weight;

2 points: The hind limbs move frequently or powerfully, and cannot bear weight or walk;

3 points: The hind limbs can support the body weight and can take 1-2 steps, but no correct gait;

4 points: Can bear weight and walk, with only mild impairment and obvious weakness;

5 points: Walking normally.

BBB rating

The BBB (Basso Beattie Bresnahan) score divides rat hindlimb movements into 22 levels, which covers almost all behavioral changes during the recovery process of rat hindlimbs after spinal cord injury.

Grading:

0 points: No visible hind limb movement;

1 point: Slight movement of one or two joints, usually the hip and/or knee;

2 points: Large movement of one joint or large movement of one joint with slight movement of another joint;

3 points: substantial movement of both joints;

4 points: All three joints of the hind limbs are slightly mobile;

5 points: Two joints can move slightly, and the third joint can move widely;

6 points: substantial movement of two joints, slight movement of the third joint;

7 points: All three joints of the hind limbs can move widely;

8 points: Can touch the ground on the paws without bearing any weight;

9 points: The sole of the foot is only in the weight-bearing position, or walking occasionally/frequently/continuously with weight-bearing on the top of the foot, without walking with

weight-bearing on the sole of the foot. Weight-bearing: The HL extensor muscles contract when the foot is in the weight-bearing position or only when the rear trunk is elevated;

10 points: Occasionally, weight-bearing movement of the paw surface is seen, and there is no coordinated movement of the front and rear limbs;

11 points: More weight-bearing movement of the palm surface can be seen, but there is no coordinated movement of the front and rear limbs;

12 points: More weight-bearing movement of the palm surface can be seen, and coordinated movements of the front and rear limbs can be seen occasionally;

13 points: Weight-bearing movement of the palm surface is common, and coordinated movements of the front and rear limbs are common;

14 points: There is sustained weight-bearing movement of the palm surface and coordinated movements of the front and rear limbs, or common movement of the palm surface, sustained coordinated movement of the front and rear limbs, and occasionally dorsal movement of the claws;

15 points: Continuous movement of the palm and sustained coordinated movements of the front and rear limbs, no or occasional gripping of the ground during the advancement of the forelimbs, and the position of the active claw at the initial contact is parallel to the body;

16 points: Continuous movement of the palm and sustained coordinated movements of the forelimbs and forelimbs during gait. The forelimbs often grip the ground during advancement. The active claws are positioned parallel to the body during initial contact and rotate after the weight is transferred;

17 points: Continuous movement of the palm and sustained coordinated movements of the forelimbs and forelimbs can be seen in the gait. The claws are often gripping the ground during the advancement of the forelimbs. The position of the active claws is parallel to the body during initial contact and after weight transfer;

18 points: Continuous movement of the palm and sustained coordinated movements of the forelimbs and forelimbs can be seen in the gait. The forelimbs can sustainably grip the ground during advancement. The position of the active paws is parallel to the body during initial contact, and they rotate after the weight is transferred;

19 points: Visible sustained volar movement and sustained coordinated movements of the forelimbs and forelimbs during the gait, sustainable claw gripping of the ground during the advancement of the forelimbs, the position of the active claws is parallel to the body during initial contact and after load transfer, the tail is sometimes or always sagging;

20 points: Continuous palm movement, sustained coordinated gait, toes continue to grip the ground, the position of the active claws is parallel to the body during initial contact and after weight transfer, the trunk is unstable, and the tail continues to be raised;

21 points: Continuous movement of the palm, continuous coordinated gait, continuous gripping of the ground with the toes, active claw position always parallel to the body during the activity, continuous stability of the trunk, and continuous raising of the tail.