Peripferal Nerve Regeneration in Response to Synthesized Nanofiber Scaffold Hydrogel

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Abstract: Background: earlier studies have demonstrated that Peptide RADA16-I (AcN-RADARADARADARADA-$CONH_2$) could repair spinal cord injury and optical pathway and restore visual function. The objective of the research is to investigate the role of RADA16-I in the regeneration of peripheral nerve injury in rats. Method: sciatic nerve injury was performed at female Sprague-Dawley rats. Two groups were conducted: crush group and sever group. There were RADA16-I treatment and blank control in each group. Sense Nerve Conduction (SNCV) parameters were EVALUATED on day 30 in the injured sciatic nerve by means of electrophysiological recording. The results indicates that SNCV in test groups shows a faster healing rate than blank control. Conclusion: self-assembling peptide plays a effective role in the regeneration of sciatic nerve in rats.

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INTRODUCTION

The synthetic self-assembling peptides (SAP), originally discovered by Zhang et al., can self-assemble into nanofiber *in situ* under physiological conditions, and surprisingly simulate thromboplastin-mediated fibrin blood blot process without any antigenicity 1,2(Zhao and Zhang 2006) (Caplan, Schwartzfarb et al. 2002). For example, RADARADARADARADA (RADA16-I) peptide., consisting of 16 alternating hydrophobic and hydrophilic (also alternating negative and positive charges) amino acids, forms extremely stable β-pleated sheet structure and then self-assembles into nanofibers to produce high-order interwoven nanofiber scaffold hydrogel. It has extremely high water content (＞99.5%(w/v) water) by changing to neutral pH or adding physiological salt solution. This nanofiber scaffold hydrogel was also similar to the extra-cellular matrix 3,4,5,6,7,8(Zhang, Holmes et al. 1995; Zhang and Rich 1997; Altman, Lee et al. 2000; Kisiday, Jin et al. 2002; Hong, Legge et al. 2003; Yokoi, Kinoshita et al. 2005).

This kind of Peptide can establish a nanofiber barrier to achieve complete hemostasis when applied directly to a wound in the site of injury 9,(Ellis-Behnke RG 2006;) and it has been demenstrated that this kind of Peptide could also repair spinal cord injury 10(Guo J 2007) and optical pathway and restore visual function11(Ellis-Behnke RG 2006). In this report, we estimated the healing rate of injured sciatic nerve in SAP treated group using Sense Nerve Conduction Velocity (SNCV) parameters. The purpose is to investagate whether the SAP (RADA16-I) can play a role in the peripheral nerve repair.

MATERIALS AND METHODS

Atomic force microscopy (AFM)

An aliquot of approximately 5 μL of wound dressing solution and different diluted solutions were evenly placed on the surface (10 mm×10 mm or 15 mm) of a freshly cleaved mica sheet. Each sample was left on the mica about 30 sec and then washed with aliquots of 100 μL of Milli-Q water to remove unattached material. The sample on the mica surface was then air-dried for AFM observation.

AFM imaging was performed at room temperature on SPI4000 Probe Station & SPA-400 SPM Unit (Seiko Instruments Inc., Chiba, Japan) using the dynamic force mode. The images utilized a 20 μm Scanner (400) and an Olympus Si-DF20 micro cantilever as well as a Si tip of radius 10 nm, rectangular base 200.00 μm, with tip height of 10.00 μm and spring constant Kz of 12.00 N/m. The main parameters of AFM observation were as follows: Amp. Ref. (Amplitude Reference): -0.15～-0.30, I Gain/ P

Gain (integral gain/proportional gain): 0.10 ～ 0.20/0.05～0.10, Amplitude: ～1V, Scan Speed: 0.5～ 1.5 Hz. Height images were recorded with 512 × 512 pixels resolution and the brightness of morphology increased as a function of height. All image data sets were subjected to first-order flattening, in order to correct tilt of selected area and cancel any distortion (reversal) of causeless drifting or vibration (creeping) of the scanner. The AFM observation of each sample was repeated at least four times.

To quantitatively analyze the data, a large number of images have been examined in order to find the surface coverage of nanofibers, and further calculate the length of nanofibers. In higher magnification images, the width and height of nanofibers can be determined using SPI3800N-offline image analysis program for win2000 software, also called as "SPIWin".

Animals

50 female Sprague–Dawley (SD) rats, weighing from 250 g to 290 g, from the Center of Laboratory Animals of Clinical Medicine School of Sichuan University were used in this study. The animals were fed a standard diet ad libitum and housed individually at controlled temperature (22-23^{o}C) and lighting (12 hours light and 12 hours darkness).

Experiment Grouping

Fifty rats were divided into five paralleled groups: normal group, crush blank control group, crush test group (SAP treated), suture blank control group and suture test group (SAP treated). 10 rats in each group.

Surgical procedures

All procedures were conducted in accordance with the policy on the Use of Animals in Neuroscience. Adult female Sprague-Dawley ras(180-220g) were deeply anesthetized with sodium pentobarbital(30mg/kg i.p.). After an incision had been in the skin, the sciatic nerve was exposed by making a muscle splitting incision among the muscle tendon of the biceps femoris,semitendinosus and semimembranosus.

In the crush group: The nerve was crushed with a smooth-tipped (2mm) locking suturing needle holder that was clamped on the nerve for 2 minutes. Then 20ul of RADA16-I (1%,w/v)was injected into perineurium of the injured nerve segment using microinjection and in the control group the RADA16-I was replaced by saline.

In the suture groups, the nerve was transected with a microscissors and repaired using 10-0 sutures. RADA16-I was daubed on the two ends (RADA16-I was replaced by saline in blank control).

In the normal group (n=10) the animals were submitted to sham operation. The insicion site was closed by layers and the rats were allowed to recover 12 (RobertoS.Martins 2005).

Electrophysiological recording

One month after surgery, electrophysiological recording was done under anesthesia with sodium pentobarbital(30mg/kg i.p.). After the initial surgical exposure, the animals were submitted to a first electrophysiological evaluation consisting of Sense Nerve Conduction Velocity (SNCV) to measure the degree of regeneration in surgically repaired sciatic nerves in rats: comparison of the size of nerve responses evoked by stimulation distal to the anastomosis. The stimulation electrode was placed on the nerve stem and 2 monopolar straight needle electrodes were installed on the posterior limb at a distance of 3mm from one another the other ground electrode was installed on the tail. Using the exported current flow evoked by electrical stimulator (electromyogram evoked potential machine, Varytech. Co. Ltd. Japan) to stimulate the peripheral nerve of the posterior limb. The recording electrode record the sensory nerves action potential (SANP). The latent time (T) of the SANP was measured using dividers and represented by "S". Sense Nerve Conduction Velocity (SNCV) was expressed as formula: SNCV = S/T. Five different repair procedures were evaluated. 12, (Kazuya Matsumoto 2000; RobertoS. Martins 2005).

Statistical Analysis

All quantitative data were shown as x ± s (Mean ± SD). The mean was analyzed for significance using the two-side t test; the confidence level is 95%. The groups were compared using one factor analysis of variance(ANOVA) followed by Turkey posthoc test for normally distributed data,otherwise by one-way analysis of variance on ranks followed by Dunn's posthoc test.

RESULTS

Electrophysiological recording

Fig. 1 shows the mean values of Sense Nerve Conduction Velocity (SNCV) parameters 30 days after operation. There was statistically difference among the groups for the parameters evaluated. SNCV in test groups shows a faster healing rate than blank control. The four experimental groups all showed significant differences compared with normal group (P = 0.00). In both gourps (crush group and suture group，see figure 2), significant differences between RADA16-I treatment and blank treatment can be observed (P = 0.013, P = 0.036). Figure 3 indicate data of SNCV represented by electrophysiological recording graph.

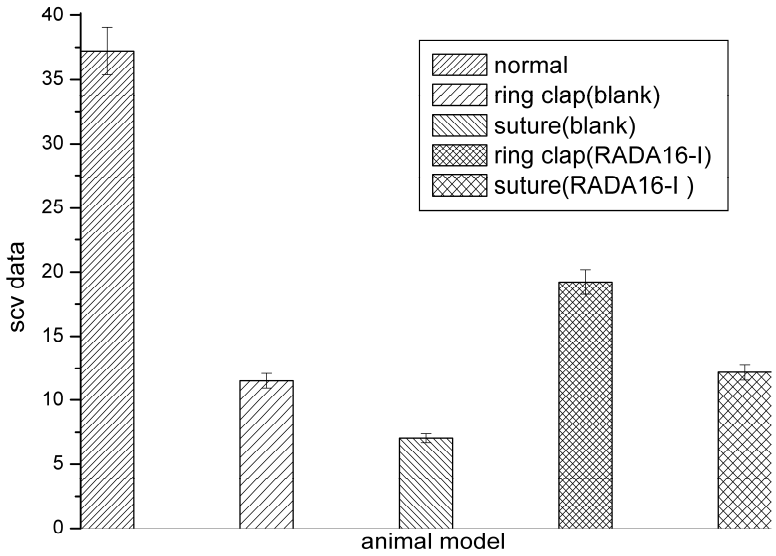

Fig. 1: The data of Sense Nerve Conduction Velocity parameters represented by histogram. The axis X represents animal models in different groups. Sense Nerve Conduction Velocity (SNCV) parameters (average) in different groups listed below: crush group treated with RADA16-I: 19.23 m/s; crush group treated with blank: 12.18 m/s; sever group treated with RADA16-I: 11.51 m/s; sever group treated with blank: 7.05 m/s; normal group: 37.28 m/s.

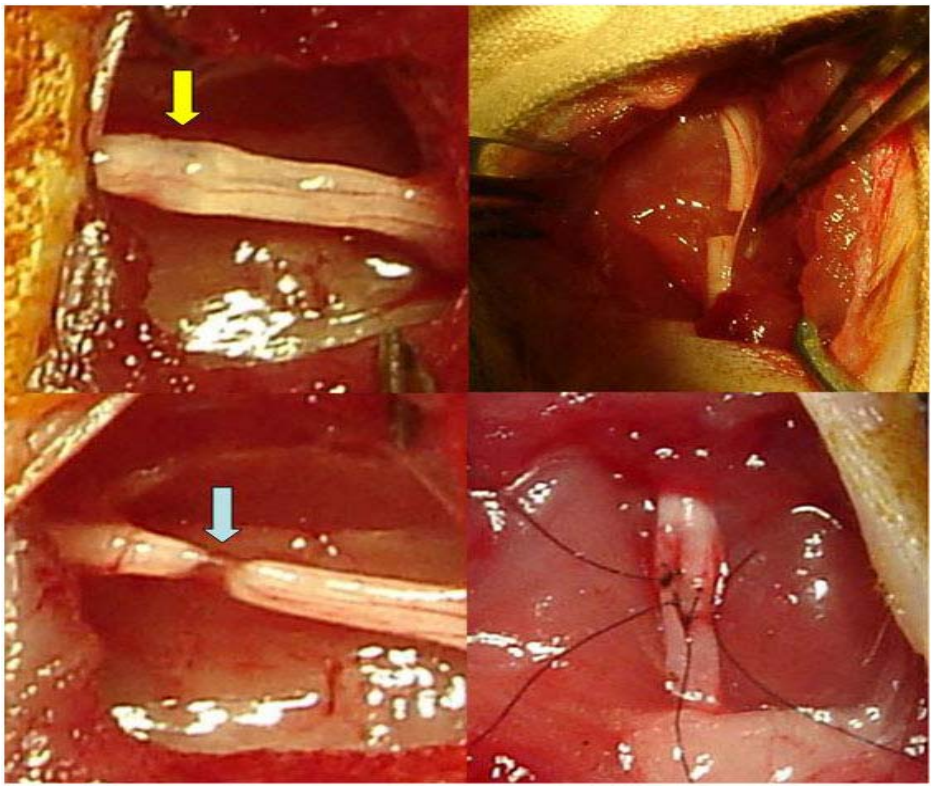

Fig. 2: Sciatic injury model. the wound on the left are crush group and the blue arrow refers to the crushed nerve segment and the upper shows the crushed segment injected with RADA16-Ⅰ(yellow arrow). The right wound shows the shear group and the wound was end to end sutured and the RADA16-Ⅰ was daubed on the broken end.

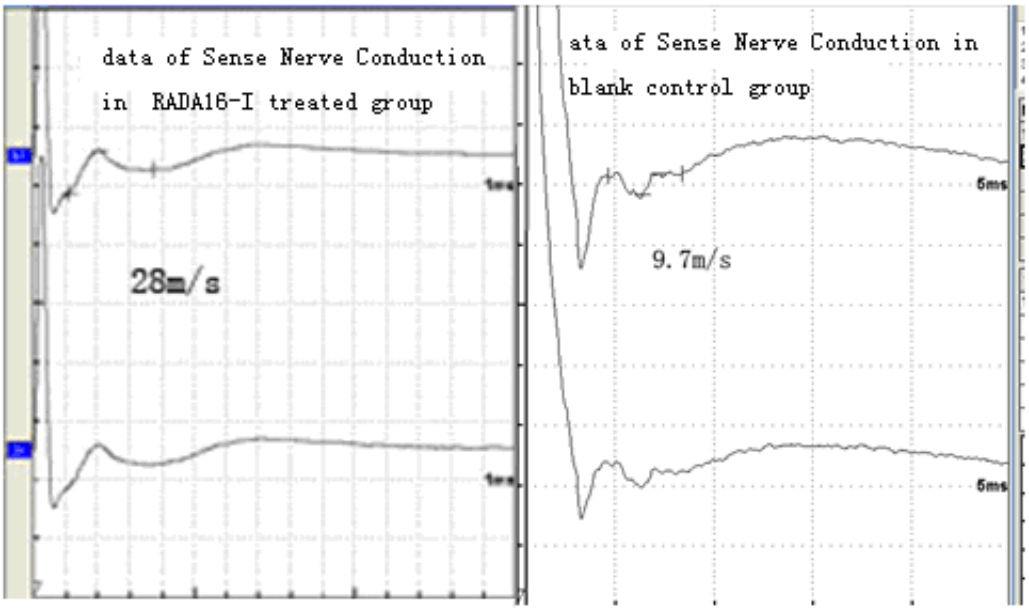

Fig. 3: Graphs of the Sense Nerve Conduction Velocity (SNCV) parameters. graph on the left shows the data of RADA16-Ⅰtreatment and right graph shows the blank treatment.

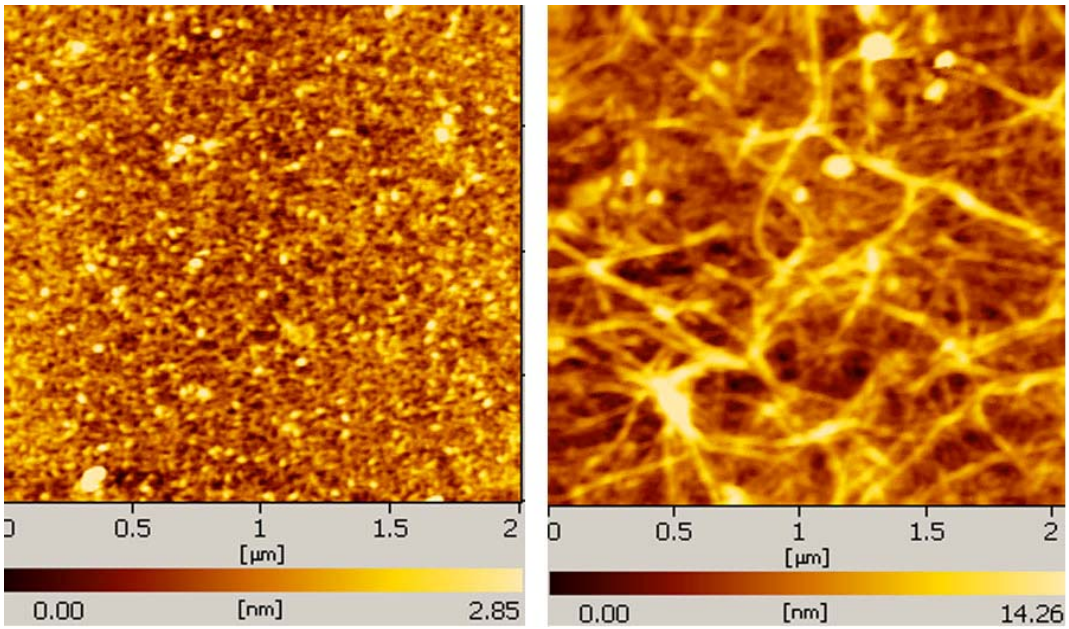

Fig. 4: The typical AFM height images. Right: 10mg/ml RADA16-Ⅰpeptide(1%(w/v) PuraMatrix™ diluting with Milli-Q water), Left: 1%Chitosan acetic acid (1%) solution. Right: The representative morphology is the some globular pieces should be assembled into nanofiber-like aggregation, the height and the weight of the globular pieces are 5.9nm～6.9nm, and 78.9nm～105.9nm, respectively, the diameter data should be corrected; All errors are the 95% confidence level.

Atomic force microscopy (AFM)

Employed with AFM (Figure. 4), researchers investigated the morphological properties of 10mg/ml RADA16-Ⅰ peptide (right), and a commercially 10mg/ml chitosan fluid dressing (left), shown in Figure. 4, the most difference 10 mg/ml RADA16-Ⅰpeptide with other topical dressings is its nanofiber 3D scaffold matrix, in which the nanofibers the height and the weight of the globular pieces are 5.9nm～6.9nm, and 78.9nm～105.9nm, moreover, and offered with 17～43% surface porosity(%), which is very benefit for the healing requirements of the injured tissue and cell.

DISCUSSION

The regeneration of nerve is a striking example of plasticity within lorenervous system. Research on regeneration and functional recovery after peripheral nerve injury, both clinical and basic, has come a long way. Research has hitherto focused on the methods of repair and of bridging nerve gap. One of the key questions to be answered is which technique offers the peripheral nerve the best environment to promote regeneration and functional recovery. Biological glues including collagen, Chitosan, poly (DL) lactic acid (PDLA) et al. have been explored for the use of promoting nerve regeneration but their antigenicity more or less limited their use13(Stipcevic T 2006). People are still searching for the new type of proper material.

Concluded from above research, Sense Nerve Conduction Velocity (SNCV) parameter in RADA16-Ⅰtreated sciatic nerve has been enhanced obviously and thus reflect the healing of the demyelination. The healing mechanism needs further investigation.

CONCLUSION

These experiments demonstrate that self-assembling peptides can create nanofiber microenvironments in the injured sciatic nerve and that these microenvironments promote nerve regeneration. Because these nanofibers may be modified in a variety of ways, this approach may enable injectable tissue regeneration strategies.

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