Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

The ARRIVE guidelines 2.0: author complete answers

The ARRIVE Essential 10

These items are the basic minimum to include in a manuscript. Without this information, readers and reviewers cannot assess the reliability of the findings.

Study design 1

For each experiment, provide brief details of study design including:

a. The groups being compared, including control groups. If no control group has been used, the rationale should be stated.

Reply*: Positive control group is autograft, the comparison control group is pure PCL NGS group negative control group is blank implantation.*

b. The experimental unit (e.g. a single animal, litter, or cage of animals).

Reply*: The experimental unit is a single animal.*

Sample size 2

a. Specify the exact number of experimental units allocated to each

group, and the total number in each experiment. Also indicate the total number of animals used.

***Reply**: The total number of 40 male animals used. There were 10 animals in each group. Five animals of the same group live in a single animal cage.*

b. Explain how the sample size was decided. Provide details of any a priori sample size calculation, if done.

***Reply**: The Sample size was determined based on the number of experimental groups and different time points of analysis.*

Inclusion and exclusion criteria 3

a. Describe any criteria used for including and excluding animals (or experimental units) during the experiment, and data points during the analysis. Specify if these criteria were established a priori. If no criteria were set, state this explicitly.

***Reply**: Autograft transplantation was set as positive control group, because autogenous nerve transplantation is the gold standard for clinical treatment of peripheral nerve injury. Blank transplantation as negative control group. The study group was compared with the positive control group, the comparison group and the negative control group in terms of function, electrophysiology and histomorphology.*

b. For each experimental group, report any animals, experimental units or data points not included in the analysis and explain why. If there were no exclusions, state so.

Reply*: In order to perform data analysis on same number of data points, the sciatic nerve indexes of three animals in each group were randomly selected for statistical analysis at 2, 4, 8, 12 and 16 weeks after operation.*

c. For each analysis, report the exact value of n in each experimental group.

Reply: *For each analysis, the exact value of n in each experimental group is illustrated in the text of our manuscript and in the illustrations of supporting materials.*

Randomisation 4

a. State whether randomisation was used to allocate experimental units to control and treatment groups. If done, provide the method used to generate the randomisation sequence.

Reply*: The experiment was conducted in a random manner. Forty healthy rats of the same sex and weight in a certain range were randomly divided into four groups: A (autograft), B (blank), P1 (PU), P2 (PCL). First weigh the weight, and make it into 1, 2, 3 according to the weight*

from light to heavy In order to give each animal the same chance to be divided into four groups: a (autograft), B (blank), P1 (PU) and P2 (PCL), each random number can be divided into 4, the remaining 1 is assigned to group A, the remaining 2 is to group B, and the remaining 3 is to group P1; if all the animals are divided into group P2, the random number is divided into group P2 according to the remainder. The following table is as follows: 2,5,9,13,17,21,25,29,33,37,10,1,3,6,10,14,18,22,26,30,34,38,11,11,11,11,15,19,23,27,31,35,39,9 in group A and 11 in group B respectively; Group P2: 4,8,12,16,20,24,28,32,36,40,10 rats in total. In order to make the number of animals in four groups equal, one animal in group B should be transferred to group P1. Which animal in group B should be adjusted according to the following method. In order to make all 11 animals in group B have a chance to enter group P1, 11 can be divided into 00 and still get 0. Since there is no 0 in group B, a number of 34 is pushed back, and the remainder is 1 when 34 is divided by 11, then the No.1 animal in group B is transferred to group P1. The animal numbers are numbered by ear mark method.

b. Describe the strategy used to minimise potential confounders such as the order of treatments and measurements, or animal/cage location. If confounders were not controlled, state this explicitly.

***Reply**: According to the above random number, graft implantation, functional test and electrophysiological test were performed. Five animals of the same sex in the same nest and with similar body weight were used as the compatibility group. After the allocation, the number of animals in each group was equal. The weight of each group was similar, so as to reduce the experimental error.*

Blinding 5

Describe who was aware of the group allocation at the different stages of the experiment (during the allocation, the conduct of the experiment, the outcome assessment, and the data analysis).

***Reply**: The different stages of the experiment (allocation period, experiment Implementation) were carried out according to the above group numbers. Results the evaluation and data analysis results were entered according to the corresponding numbers of each group. According to the allocation of different stages of the experiment, there are corresponding records on the label outside the cage. Correspondingly, the result evaluation and data analysis are analyzed according to the random cage unit.*

Outcome measures 6

a. Clearly define all outcome measures assessed (e.g. cell death,

molecular markers, or behavioural changes).

***Reply**: Samples were excluded if anorexia, rapid weight loss, mental and behavioral burnout occurred after implantation.*

b. For hypothesis-testing studies, specify the primary outcome measure, i.e. the outcome measure that was used to determine the sample size.

***Reply**: For peripheral nerve regeneration experiment, according to the effect of 50% increase and 25% standard deviation compared with the positive control group.*

Statistical methods 7

a. Provide details of the statistical methods used for each analysis, including software used.

***Reply**: Data are presented as the mean ± standard deviation (s.d.). Groups were compared by the one-way analysis of variance (ANOVA) using GraphPad Prism Software (GraphPad, La Jolla, CA, USA). A difference of $p < 0.05$ was considered statistically significant.*

b. Describe any methods used to assess whether the data met the assumptions of the statistical approach, and what was done if the assumptions were not met.

***Reply**: If the position of implant material changes significantly after*

surgery, the sample is excluded.

Experimental animals 8

a. Provide species-appropriate details of the animals used, including species, strain and substrain, sex, age or developmental stage, and, if relevant, weight.

***Reply**: Sprague-Dawley rat is rattus norregicus strain, SPF grade, male, 6~8 weeks age, 180~200 g weight.*

b. Provide further relevant information on the provenance of animals, health/immune status, genetic modification status, genotype, and any previous procedures.

***Reply**: Number of animal use permit: SYXK (Yue): 2018-0186, the test results are healthy.*

Experimental procedures 9

For each experimental group, including controls, describe the procedures in enough detail to allow others to replicate them, including:

a. What was done, how it was done and what was used.

***Reply**: At week 0, the graft was performed and the blank negative control group was set up. At the 2nd, 4th, 8th, 12th and 16th week, one animal was randomly selected from each group. After euthanasia, the*

regenerated nerve samples were taken for macroscopic HE staining and analysis. At the 4th, 8th, 16th and 16th week, 3 animals in each group were randomly selected for electrophysiological test after general anesthesia. At the 14th week, 3 animals in each group were randomly selected, and the regenerated nerve samples were taken after euthanasia. At the 14th week, one animal in each group was randomly selected. After euthanasia, the regenerated nerve samples were taken for ammonia silver staining and myelin sheath density analysis. Red ink, white paper, carton track, electrophysiological equipment, HE staining solution, ammonia silver staining solution, epoxy resin, paraffin, slicing knife, embedding box were used. Laser confocal microscope, transmission electron microscope.

b. When and how often.

Reply*: The SFI index analysis is tested at the 2nd, 4th, 8th, 12th and 16th weeks postoperatively. On the basis of SFI index, electrophysiological analysis was conducted at 14th weeks, and HE staining analysis was conducted every 4 weeks. Immunofluorescence, ammonia silver staining and TEM were performed at the 14th week.*

c. Where (including detail of any acclimatisation periods).

Reply*: The transplantation, SFI index and neuroelectrophysiology were*

performed in the aseptic operation room of SPF animal room. After transplantation, all animals were placed in the rat strain feeding room designated by SPF animal room. The staff of animal center regularly fed, changed bedding and observed. The sampling place is to move the animals from the special exit to the designated animal sampling room outside the SPF shielding environment for sample collection.

d. Why (provide rationale for procedures).

***Reply**: It is the requirement of experimental setup to abide the animal ethics and welfare Commission (AEWC).*

Results 10

For each experiment conducted, including independent replications, report:

a. Summary/descriptive statistics for each experimental group, with a measure of variability where applicable (e.g. mean and SD, or median and range).

***Reply**: The sample size of each round included at least three animals in each case. Data are presented as the mean ± standard deviation (s.d.). Groups were compared by the one-way analysis of variance (ANOVA) using GraphPad Prism Software (GraphPad, La Jolla, CA, USA).*

*Statistical significance of $P < 0.05$ was accepted and reported as follows: * $P < 0.05$, * * $P < 0.01$, * $P < 0.001$, * * * $P < 0.0001$. More information can be found in a specific legend.*

b. If applicable, the effect size with a confidence interval.

***Reply**: For the sciatic nerve regeneration experiment, the sample size of n = 3 was selected according to the effect of 50% increase and 25% standard deviation compared with the control group. The acceptable error rate is 5%.*

The Recommended Set

These items complement the Essential 10 and add important context to the study. Reporting the items in both sets represents best practice.

Abstract 11

Provide an accurate summary of the research objectives, animal species, strain and sex, key methods, principal findings, and study conclusions.

***Reply**: The aim is to develop a tissue-engineered polymer nerve guiding scaffold which can achieve the same results as autograft transplantation through animal experiments. The animal species is Sprague Dawley (SD), which belongs to rat family, rat genus and male. The key method is to prepare block copolymer scaffolds with*

different surface properties in vitro. Our study found that the block copolymer PU NGS can simulate the mechanical properties of rat sciatic nerve and provide a better micro-environment for host immune cells and Schwann cells in nerve defects. The results of sciatic nerve index, nerve electrophysiology, histochemistry and immunofluorescence confirmed that the copolymer NGS showed a similar result to that observed in autograft transplantation.

Background 12

a. Include sufficient scientific background to understand the rationale and context for the study, and explain the experimental approach.

Reply*: Damage to the peripheral nervous system is a major source of disability, usually resulting in neuralgia or impaired muscle movement and / or normal sensation. For rodents with gaps less than 10 mm, almost normal functional recovery can be achieved; for larger gaps, there are still insurmountable challenges. The current clinical gold standard for bridging long, non-healing nerve spaces, autograft, has several drawbacks. Despite our best efforts, the design of alternative "nerve bridges" for peripheral nerve repair is still in the distant future. Therefore, there is an urgent need to design new methods to match or exceed the performance of trans critical nerve gap autograft.*

b. Explain how the animal species and model used address the scientific objectives and, where appropriate, the relevance to human biology.

***Reply**: This approach is a shift from the current clinical and laboratory approaches to nerve repair, which may stimulate alternative paradigms of stimulating peripheral nerve repair.*

Objectives 13

Clearly describe the research question, research objectives and, where appropriate, specific hypotheses being tested.

***Reply**: An immunomodulatory method was used to stimulate nerve repair in nerve guided scaffolds to explore the regenerative effect of reparative monocyte recruitment.*

Ethical statement 14

Provide the name of the ethical review committee or equivalent that has approved the use of animals in this study, and any relevant licence or protocol numbers (if applicable). If ethical approval was not sought or granted, provide a justification.

***Reply**: All animal research protocols were approved by the animal ethics and welfare Commission (AEWC) before the experiment. The approval No. is GFAC-AWEC-005.*

Housing and husbandry 15

Provide details of housing and husbandry conditions, including any environmental enrichment.

Repl*y: The housing is SPF level barrier environment. SD rats have the advantage of group living. Multiple rearing in the same cage. Sufficient fresh and dry feed is added regularly in the hopper of cage box, and feed is added two days a week. If needed, it can be added at any time according to the situation. We changed the aseptic and dry bedding material before each feed addition.*

Animal care and monitoring 16

a. Describe any interventions or steps taken in the experimental protocols to reduce pain, suffering and distress.

Repl*y: During the operation, the SD rats were anesthetized with isoflurane (2%) and maintained a constant temperature of 36.5 degrees. After operation, carbofen (PHR1452, Sigma) 4 mg/kg was injected subcutaneously 10 minutes after operation for analgesia.*

b. Report any expected or unexpected adverse events.

Repl*y: If there are unexpected adverse events, the samples will be excluded. Then the experiment would be repeated.*

c. Describe the humane endpoints established for the study, the signs that were monitored and the frequency of monitoring. If the study did not have humane endpoints, state this.

Repl*y: If the experiment may cause adverse consequences such as eating difficulty, nerve disorder, movement difficulty, severe infection, severe pain and other adverse consequences, we administered anesthesia and analgesia and other methods for nursing upon observation twice per day and weighting twice per week. If the symptoms are not relieved after nursing, a humane end point of animal euthanasia is carried out. The way to euthanasia is to inhale excess carbon dioxide.*

Interpretation/scientific implications 17

a. Interpret the results, taking into account the study objectives and hypotheses, current theory and other relevant studies in the literature.

Reply: *The phenomenon of amphiphilic PU NGS regulating host macrophages to promote regeneration at the site of nerve injury is consistent with the reports of other research groups (Proc Natl Acad Sci. USA, 2017, 114 (26), E5077; BMC Neuroscience., 2017, 18 (1), 53; Biomater. Sci., 2018, 6, 2987; J. Mater. Chem. B, 2019, 7, 940).*

b. Comment on the study limitations including potential sources of bias, limitations of the animal model, and imprecision associated with the results.

Reply: *The total number of experimental animals may be a limitation even if the experiment was conducted in a random way. The sample size of each subset includes at least 3 animals in each case, and the acceptable error rate is 5%, which may lead to inaccuracies related to the results.*

Generalisability/ translation18

Comment on whether, and how, the findings of this study are likely to generalise to other species or experimental conditions, including any relevance to human biology (where appropriate).

Reply*: The correlation between the reported rat peripheral nerve regeneration and analogous human healing-associated peripheral nerve regeneration will be important for immune engineering nerve regeneration methods.*

Protocol registration 19

Provide a statement indicating whether a protocol (including the research question, key design features, and analysis plan) was prepared before the study, and if and where this protocol was registered.

***Reply**: In vivo experiment protocol was performed in Guangzhou Yongnuo Medical Laboratory Animal Center on 40 Sprague-Dawley (SD) male rats.*

Data access 20

Provide a statement describing if and where study data are available.

***Reply**: Study data is available in the online version of the paper.*

Declaration of interests 21

a. Declare any potential conflicts of interest, including financial and non-financial. If none exist, this should be stated.

***Reply**: There are no conflicts to declare.*

b. List all funding sources (including grant identifier) and the role of the funder(s) in the design, analysis and reporting of the study

***Reply**: National Natural Science Foundation of China (No.82070695 and No.81900619), Guangdong Provincial Key Laboratory of Research in Structural Birth Defect Disease (No: 2019B030301004) and Postdoctoral Science Foundation of China (No.2018M630936 and No.2018M643252), SIAT Innovation Program for Excellent Young Researchers (No. Y8G032), Chinese Academy of Sciences President's International Fellowship Initiative (No.2019PM0006). These projects*

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