Mechanisms of Development 158 (2019) 103558

Contents lists available at ScienceDirect

Mechanisms of Development

journal homepage: www.elsevier.com/locate/mod

Use of *in vitro* electroporation and slice culture for gene function analysis in the mouse embryonic spinal cord

Shuanqing Li[a], Yunxiao Li[a], Han Li[a], Ciqing Yang[a,b,*], Juntang Lin[b,c,*]

[a] College of Life Science and Technology, Xinxiang Medical University, Xinxiang 453003, China
[b] Henan Key Laboratory of Medical Tissue Regeneration, Xinxiang Medical University, Xinxiang 453003, China
[c] College of Biomedical Engineering, Xinxiang Medical University, Xinxiang 453003, China

ARTICLE INFO

Keywords:
Mouse embryo
Spinal cord
Tissue culture
Electroporation

ABSTRACT

The spinal cord is an important part of the central nervous system (CNS). At present, the expression of the exogenous gene in the spinal cord of the embryonic mouse needs *in utero* spinal cord electroporation, but the success rate of this technique is very low. In this study, we have demonstrated the expression of an exogenous gene on one side of the spinal cord by combining two methods—*in vitro* electroporation of embryonic mouse spinal cord and organ spinal cord slices culture. We took 12-day embryonic mice, injected the green fluorescent protein (pCAGGS-GFP) plasmid into the spinal cord cavity *in vitro*, and then electroporated. The spinal cord was cut into 300-μm slices using a vibratory microtome. After cultured for 48 h, GFP-positive neurons were clearly observed on one side of the spinal cord, indicating that the exogenous gene was successfully transferred. The axon projection direction is basically unanimous from the inside to the lateral edge of the spinal cord. Compared to neurons *in vivo*, a single neuron in the culturing section has more complete neurites and is conducive to studying changes in the structure and behavior of individual neurons. Based on the above results, we have successfully established a convenient and efficient method for expressing the exogenous gene in the spinal cord of the mouse.

1. Introduction

Mammalian embryos develop in the maternal uterus. Therefore, it is difficult to study the nervous system abnormalities during embryonic development. The mouse is an animal model which is commonly used in biological studies. In previous research, we carried out *in utero* electroporation (IUE) in the cerebral cortex of mouse embryo, which resulted in the abnormal expression of the exogenous gene and analyzed the effect of target genes on the developing cerebral cortex (Liu et al., 2014). The emergence of *in vivo* electro-transformation technology has allowed the abnormal expression of the exogenous gene in different tissues and organs (Teh et al., 2003; Nakamura et al., 2004). The spinal cord is an important component of the central nervous system with functions, such as reflex, exercise, and conduction. (Shin et al., 2018). Damaging the spinal cord leads to serious consequences, such as muscle atrophy, respiratory paralysis, and even death (Danesin and Soula, 2017; O'Shea et al., 2017). Therefore, the establishment of an abnormal expression model of an exogenous gene has very important implications for studying the abnormal structure formation during embryonic spinal cord development. Yang et al. (2016) successfully used *in vivo* electroporation technology to transfect the exogenous gene into one side of the spinal cord of chicken embryos, which provided a method for the abnormal expression of an exogenous gene in the chicken embryonic spinal cord. Organotypic tissue culture is an experimental platform between cell culture and animal models. Gähwiler (1981) firstly performed organotypic tissue culture on the microporous membrane and achieved long-term tissue culture in *in vitro* conditions. It has been widely used in the study of the nervous system, especially in neurodevelopment (Raineteau et al., 2004). To study the effects of specific genes on the spinal cord development under *in vitro* conditions, we used E12 mouse embryos as an animal model, combined with *in vitro* electroporation and organotypic spinal cord culture technology, to explore the development of nerve fibers during the mouse spinal cord development. Our study provides a new technical method to investigate the abnormal expression of the exogenous gene in the spinal cord.

2. Materials and methods

Principles of laboratory animal care were followed, and all

* Corresponding authors at: College of Biomedical Engineering, Xinxiang Medical University, 453003 Xinxiang, China.
E-mail addresses: yangciqing@126.com (C. Yang), developmentlab@126.com (J. Lin).

https://doi.org/10.1016/j.mod.2019.103558
Received 10 April 2019; Received in revised form 4 June 2019; Accepted 14 June 2019
Available online 15 June 2019

procedures were conducted according to the guidelines established by the *National Institutes of Health*. All efforts were made to minimize the number of the used animals and their suffering. This study was approved by the Animal Experimentation Committee of Xinxiang Medical University, China.

2.1. Preparation of reagents and plasmid

The reagents included slice culture 25% horse serum media (37.5 mL of Neurobasal (Thermo Fisher, USA), 12.5 mL horse serum (HyClone, USA), 1 mL of B-27® Serum-Free Supplement (50 ×) (Thermo Fisher), 0.5 mL of 1% GlutaMAX (Thermo Fisher), 0.5 mL of 1% penicillin-streptomycin [Sigma]), and artificial cerebrospinal fluid (ACSF) (120 mmol/L NaCl, 1.3 mmol/L $MgCl_2$, 1.25 mmol/L NaH_2PO_4, 3.5 mmol/L KCl, 2.5 mmol/L $CaCl_2$, 25.6 mmol/L $NaHCO_3$, and 10 mmol/L glucose).

All plasmids used were extracted using a kit (Cwbio, China) and diluted in water.

2.2. The culturing of a 12-day pregnant mouse

At about 4:00 p.m. on the first day, a male mouse was caged with three female mice. The next morning at about 8:00 a.m., each female mouse was examined. If the female mouse was identified to have a white or creamy vaginal plug, it was pregnant for half a day. Subsequently, this pregnant mouse was fed until the 12th day when the experiment was started.

2.3. The electroporation of embryonic mouse's spinal cord

The pregnant mouse was put to death by cervical dislocation and its abdominal cavity was quickly cut open so that its uterus was exposed. Then, the embryonic mouse was removed from the uterus and placed in saline preheated to 37 °C (Fig. 1A). Under the stereo microscope (Haoyu Instrument, China), the spinal cord was peeled off along with the spinal canal and the skin on the back of the embryonic mouse was also removed (Fig. 1B). A transparent spinal cord cavity in the embryonic dorsal was visible (Fig. 1C). Next, we injected into the spinal cord cavity about 1 μL of 0.5 g/L pCAGGS-GFP plasmid with the help of a microinjected capillary glass needle such that the injected plasmid then formed a straight line along the spinal cord (Fig. 1D). At this time, the plate electrodes were placed on either side of the spinal cord (Fig. 1E), and then a suitable, square wave electric shock was delivered by an electroporator (CUY-21; Nepa Gene, Chiba, Japan). The electric shock parameters were voltage (35 V), current (100 mA), interval (60 ms), and electric pulse (6 times).

2.4. Spinal cord section and culture

The spinal cord was embedded in a 4% agar gel (Fig. 1F). After the agar gel was completely solidified, it was cut into small pieces and fixed on the vibratory microtome stage (VT1200S, Leica, Germany) (Fig. 1G). Pre-cooled Artificial Cerebrospinal Fluid (ACSF) was added to the solution tank. The small sections of solidified agar gel were cut with the parameters of 0.5 mm amplitude, 0.5 mm/s sectioning speed, and 300 μm section thickness (Fig. 1H). The spinal cord section was later transferred by a shovel to a microporous membrane (PICM03050, Millipore, USA) of 0.4 μm pore size (Fig. 1I). Finally, the microporous membrane was placed in a six-well plate to which 1 mL culture solution had been added and the plate was then incubated at 37 °C with 5% CO_2 in the atmosphere.

2.5. Observing the migration of a single neuron at the living cell workstation

After eight hours, we studied the culture under a fluorescence microscope to check whether the GFP was expressed in the spinal cord slice. The GFP positive spinal cord slices were then transferred and fixed into a glass culture dish with 1 mL of culture medium. The glass culture dish was then placed and observed at the living cell workstation where the culture condition was 37 °C with 5% CO_2 in the atmosphere. A picture was taken every 2 min for 24 h to keep a record of the migration of the nerve cells.

2.6. Staining and observation

After 48 h of culture, the culture medium was discarded, and the spinal cord slices were observed under a stereo fluorescence microscope (M205FA, Leica, Germany). The GFP positive spinal cord slices were placed in a 24 well plate, and 3 mL of 4% paraformaldehyde (PFA) was added to the wells, plates were incubated for 30 min at 4 °C, and then washed thrice (5 min for each wash) using 1 × PBS. Next, 0.1% Triton X-100 was used to permeabilize the membrane for 30 min. The slices were then incubated with the rabbit anti-microtubule-associated protein-2 (MAP2) (Abcam, UK), rabbit anti-glial fibrillary acidic protein (GFAP) (Abcam, UK), rabbit anti-neuron-specific nuclear protein (NeuN) (Abcam, UK), and mouse anti-proliferating cell nuclear antigen (PCNA) (Abcam, UK) at 4 °C overnight, respectively. The next day, the slices were washed thrice with 1 × PBS and incubated with goat anti-rabbit labeled with Cy3 secondary antibody (ZSGB-BIO, China) and goat anti-mouse labeled with Cy3 secondary antibody (ZSGB-BIO, China) at 4 °C for 6 h, respectively. After washing thrice with 1 × PBS, the slices were stained with anti-fluorescent quencher containing 4′,6-diamidino-2-phenylindole (DAPI) for 10 min, covered with a cover glass, observed, and photographed under a confocal microscope (Nikon Eclipse 80i, Japan).

2.7. Image analysis and processing

Photographs were taken under the confocal microscope and processed using Photoshop CS3 software.

3. Results

3.1. In vitro electroporation for mouse embryonic spinal cord

Since it is technically difficult to perform *in vivo* electroporation in mouse embryonic spinal cord, we have used the 12-day old mouse embryos from the uterus (Fig. 1A). The spinal cord was peeled *in vitro* (Fig. 1B), the target gene plasmid was injected into the spinal cord cavity (Fig. 1C) using a glass capillary needle, and then electroporated (Fig. 1E). The spinal cord was then sectioned and cultured for 48 h. Our results showed that the reporter gene GFP was successfully expressed on one side of the spinal cord (Fig. 1J, K) indicating that the exogenous gene vector was introduced into the spinal cord epithelial cells by *in vitro* electroporation, and can be expressed during the culture process. Removing embryos from the uterus and stripping the spinal cord are performed for easy observation and operation under the microscope. After electroporation, the spinal cord was immediately sectioned and cultured to ensure the cells were alive.

3.2. In vitro cultivation for mouse spinal cord slices

In this experiment, the spinal cord tissue was embedded in agar with appropriate temperature (Fig. 1F) and further sectioned by the vibratory microtome (Fig. 1H). Since the tissue structure is relatively small after sectioning, the tissue sections were transferred to the hanging cell culture dish membrane with the agar. Then, we transferred the culture dish to a six-well plate with 1 mL of culture medium. Under this condition, the culture medium just contacted the bottom of the slices, ensuring that the cells in the tissue slice can obtain nutrients, and the upper surface of the spinal cord can be fully exposed to the gas environment to ensure gas exchange. In the culture process, the

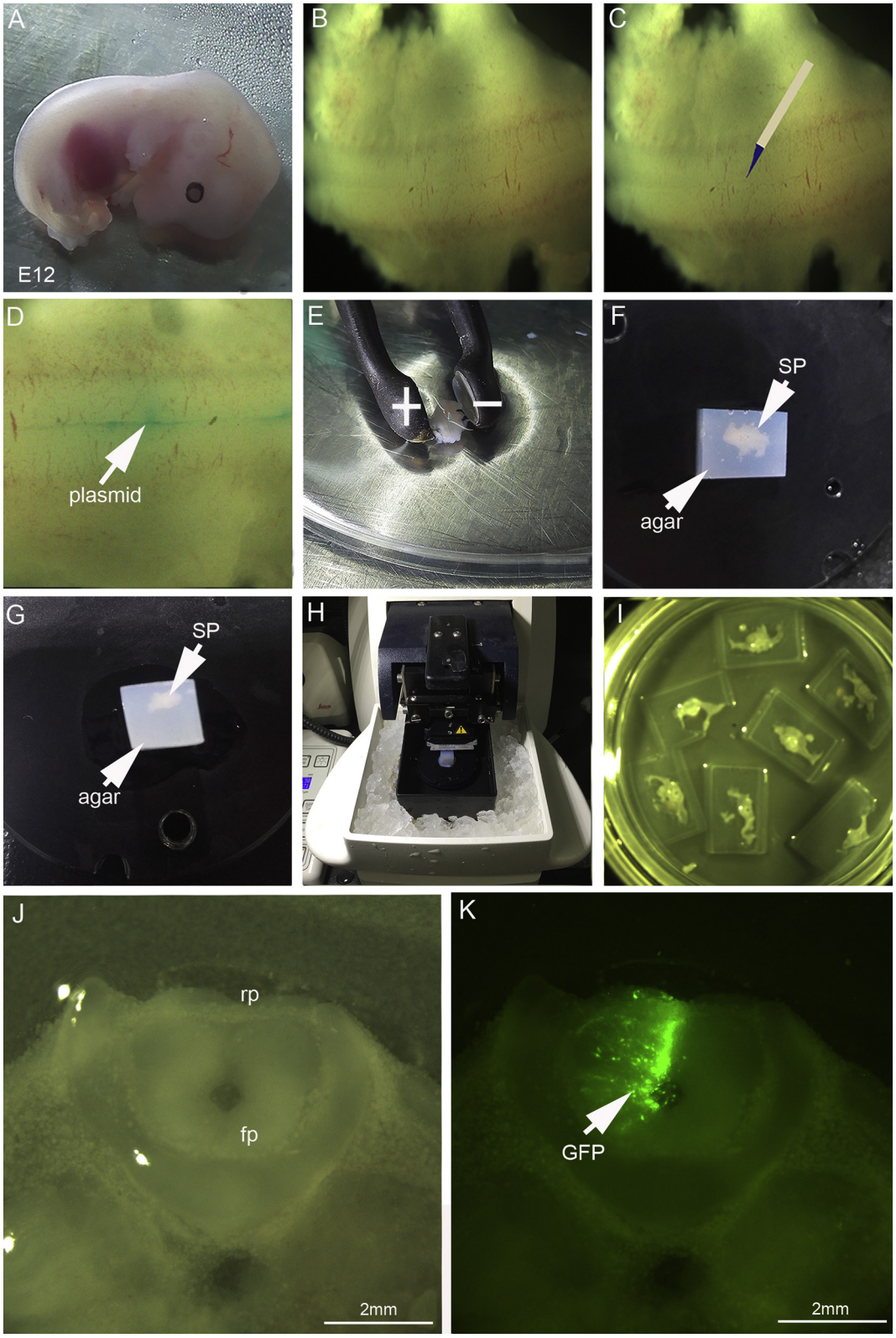

Fig. 1. *In vitro* electroporation and slice culture of mouse embryonic spinal cord.
A–K shows a complete process from embryos collecting to slice observation. A, removing the embryonic mice from the uterus of pregnant mice; B, separation of the spinal cord with the spinal canal; C and D, injection the pCAGGS-GFP plasmid into the spinal cavity; E, spinal cord electroporation; F, embedding the spinal cord after the electroporation; G, fixing the agar block on the carrier table; H, the spinal cord section; I, transferring 6–8 slices of spinal cord into the micropore membrane for culture; J, after 48 h culture, the spinal cord was observed under fluorescence stereomicroscope; K, expression of positive GFP cells on one side of the spinal cord. Abbreviation: fp, floor plate; nc, notochord; rp, roof plate; SP, spinal cord. Scale bar = 2 mm in J, K.

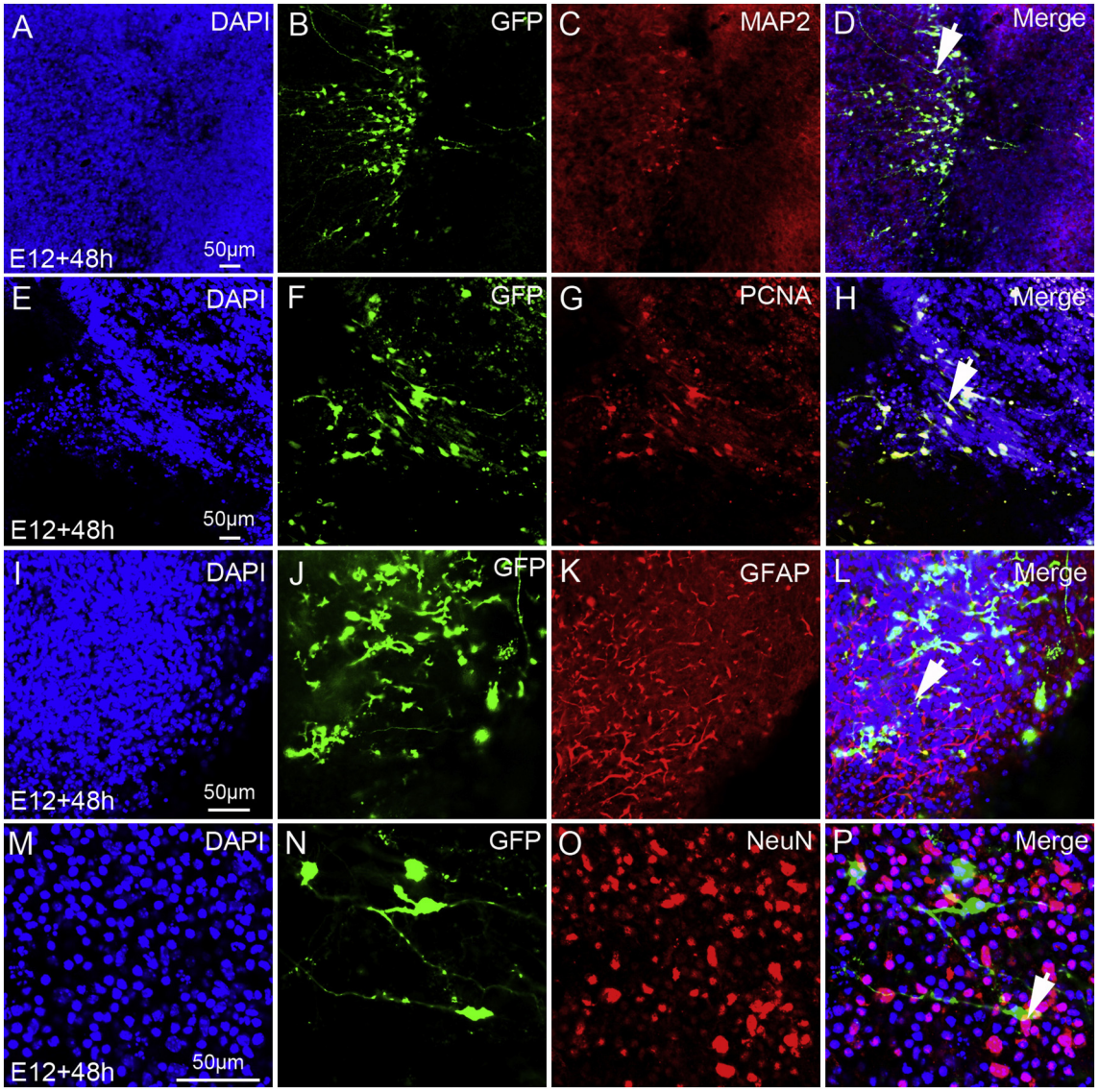

Fig. 2. The expression of MAP2, PCNA, GFAP and NeuN in cultured mouse embryonic spinal cord slice.
A–P were imaged using a confocal microscope. A–D the expression of MAP2 in pCAGGS-green fluorescent protein (GFP) positive slices at E12 culture to 48 h; 4′,6-diamidino-2-phenylindole (DAPI) nuclear staining (A, blue), GFP expression (B, green), MAP2 expression (C, red), and the merged image (D) are shown. E–H the expression of PCNA in pCAGGS-GFP positive slices at E12 cultured for 48 h; DAPI nuclear staining (E, blue), GFP expression (F, green), PCNA expression (G, red), and the merged image (H) are shown. I–L the expression of GFAP in pCAGGS-GFP positive slices at E12 culture to 48 h; DAPI nuclear staining (I, blue), GFP expression (J, green), GFAP expression (K, red) and the merged image (L) are shown. M-P the expression of NeuN in pCAGGS-GFP positive slices at E12 culture to 48 h; DAPI nuclear staining (M, blue), GFP expression (N, green), NeuN expression (O, red), and the merged image (P) are shown. Scale bars, 50 μm in (A, E, I, M) for (A-P) respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

hanging cell culture dish membrane is 0.4 μm, which allows nutrients to pass through. Fig. 2A–D is the expression of MAP2 in pCAGGS-GFP positive slices in E12 culture at 48 h. MAP2 is a neuronal dendritic marker which reflects the state of neuronal growth. The positive expression of MAP2 can be observed in the culturing slice (Fig. 2C). In Fig. 2D, the cells indicated by the white arrow appear co-labeled with GFP and MAP2. Moreover, according to the MAP2 staining results, the transfected neurons have an ideal dendritic length. Fig. 2E–H are the results of PCNA expression in the spinal cord slice. PCNA is an important marker for the initiation of cell proliferation and is a good marker of the state of cell proliferation. GFP-positive neurons and PCNA-labeled cells overlap, indicating that our transfected cells have a proliferative capacity (Fig. 2H). Fig. 2I–L indicates the distribution of GFAP in the spinal cord slice. GFAP is a marker for astrocyte activation and is mainly distributed in the central nervous system. Further, we found GFP and GFAP co-labeled nerve cells (indicated by the white arrow), which indicates that the transfected cells contain some astrocytes (Fig. 2K). To observe the distribution of mature neurons in the spinal cord, we performed NeuN staining (Fig. 2M–P) which is a specific marker for mature neurons. The results showed that in some cases, GFP-positive cells and NeuN-labeled cells were co-expressed (Fig. 2P).

3.3. Exogenous gene expression in cultured mouse spinal cord slices in vitro

After the spinal cord was cultured for 48 h, the GFP expression of the spinal cord tissue was observed under the confocal microscope (Fig. 3A–I). We could clearly observe several GFP-positive cells with a clear axon protrusion in the transfected spinal cord (Fig. 3B). The axon projection direction was unidirectional from the inside of the spinal cord to the lateral edge (Fig. 3C). However, the GFP-positive neurons were not concentrated in the spinal cord epithelium and some of the cells migrated to the gray matter area of the spinal cord (arrows in

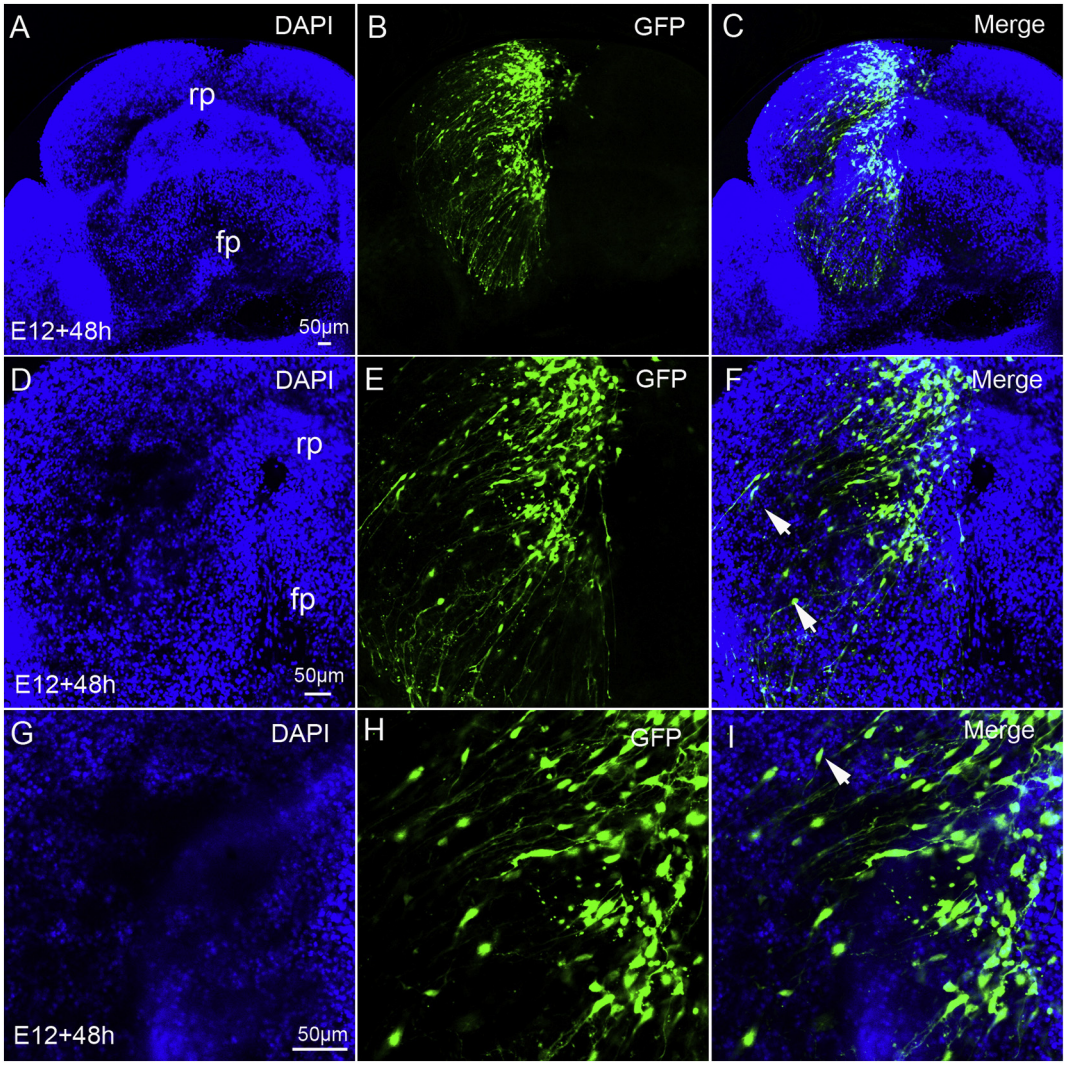

Fig. 3. GFP expression after electroporation of mouse embryonic spinal cord.
A–I are the results of spinal cord culture for 48 h after electroporation; Among them, A, D, G are staining results for DAPI; B, E, H show GFP expression; E is a partial enlargement of B, H is a partial magnification of E, F is a partial enlargement of C, and I is a partial magnification of F. Abbreviation: fp, floor plate; ne, neuroepithelium; rp, roof plate; sp., spinal cord. Scale bar = 50 μm in A–I.

Fig. 3C). This result can be further clarified after enlargement (Fig. 3D–F). When the transfected neuroepithelial cells differentiated to neurons, they migrated to a specific site under the guidance of axons (Fig. 3F). Further enlargement of the map could reveal a clearer structure of a single neuron (Fig. 3G–I).

3.4. Observing single cell migration using a living cell work station

In order to observe the migration of single neuron on the spinal cord, we transferred the spinal cord cultured for eight hours to a living cell workstation for culture. A picture was taken every two minutes for 24 h (Fig. 4A–H). During this process, the position of the neuron indicated by the red arrow did not move significantly. At the same time, the neuron indicated by the white arrow moved from the edge of the spinal cord (Fig. 4A) and then slowly migrated to the side of the spinal cord cavity (Fig. 4H). The neuron migration distance is 294 μm. We also found that the migration rate at the beginning of migration was slow, but as it migrated to a nearby cell, the migration speed was accelerated. The contact of two cells may lead to the exchange of information. In this process, we observed the complete migration pathway of individual neuronal cell and the change of its migration rate. This provides a good method for further observation of the effects of certain exogenous target genes on spinal cord development.

4. Discussion

The spinal cord is an important part of the central nervous system and its normal development is important for the organism. Once an abnormal expression of certain spinal cord-related genes occurs, its structure and function will be affected, thereby causing damage to the organism. Eventually, it may induce irreversible serious consequences, such as amyotrophic lateral sclerosis, progressive spinal atrophy, and so on (Nicholls and Saunders, 1996). Mouse embryo is a commonly used animal model for studying the function of the central nervous system. In this experiment, we found that in more than 13 days old embryos, the injected plasmid diffused throughout the spinal cord and it could not be accurately injected into the spinal cavity. Thus, we used E12 embryonic mice as a model where, after the plasmid is injected into the spinal cavity, it spread in a straight line along the spinal cavity. The application of IUE allows the abnormal expression of exogenous genes in the mouse embryonic cortex (Xu et al., 2017; Shinmyo and Kawasaki, 2017). Our group has used IUE technology to successfully express GFP-positive cells in the mouse embryonic cerebral cortex (Liu et al., 2014). However, because of the difficulty in operating on a mouse spinal cord,

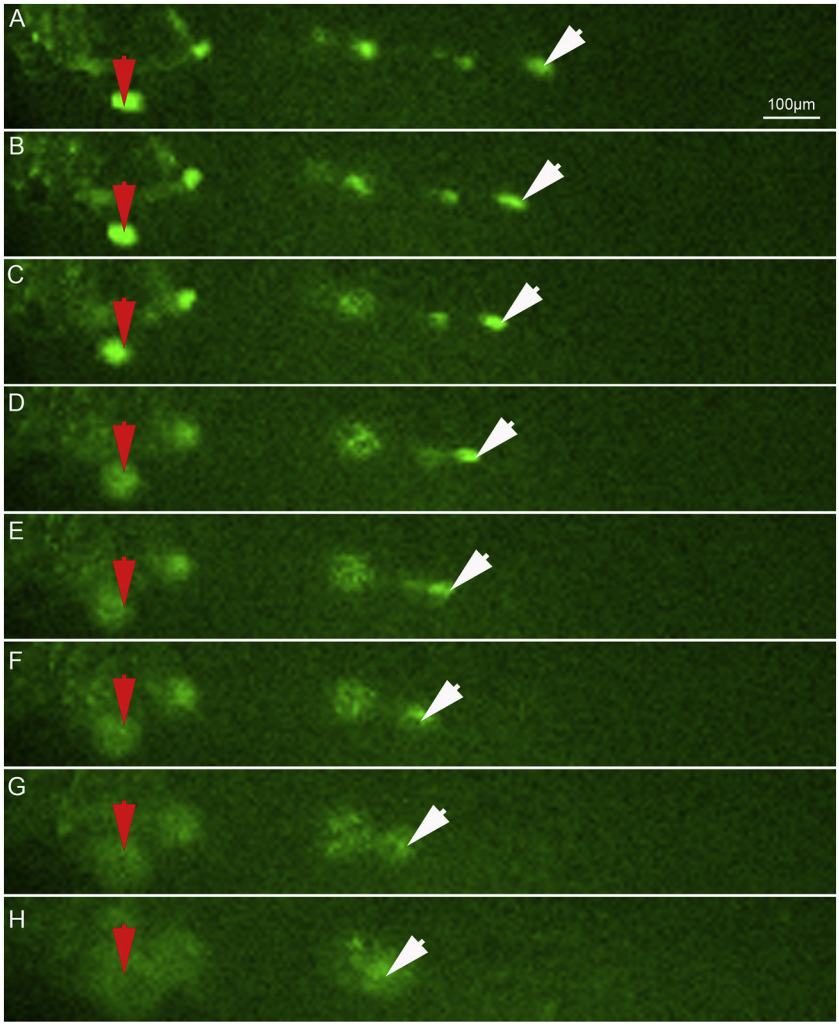

Fig. 4. Living cell workstation to observe neuronal migration.
A–H is a picture of the neuron migration process taken by the living cell workstation. The neurons pointed by white arrows are the same neurons at different times. The neurons indicated by the red arrows do not move significantly during the entire process. Scale bar = 100 μm in A–H. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

it is almost impossible to accurately inject exogenous genes into the spinal cord. Thus, we hypothesized that the exogenous gene can be injected at a specific in the spinal cord using *in vitro* electroporation to study the effect of abnormal expression of the transfected gene on neural development.

Organotypic tissue culture is an experimental platform that combines both cell culture and animal model. Gähwiler (1981) set up this method for the first time in 1981 to successfully perform tissue culture. For organotypic tissue culture, a microporous membrane was used as a carrier, tissue slices were put on the membrane, and the culture medium was added to culture the tissue slices under the membrane. This technique has been applied for cultivation of the central nervous system tissues such as hippocampus, medulla, cerebellum, spinal cord, *etc.* (Doisy et al., 2015; Vostrikov et al., 2005; Yoshida et al., 2012; Pandamooz et al., 2016). Recently, a new, simple, and reproducible method has been successfully established for culturing mouse spinal cord (Liu et al., 2017) in which a large number of active spinal cord slices can be obtained. In this study, we combined these two experimental techniques for the first time and successfully established a method for the cultivation of organotypic spinal cord slices based on the *in vitro* electroporation of the mouse embryonic spinal cord. With this method, we successfully achieved a positive expression of pCAGGS-GFP in the mouse embryonic spinal cord. We could clearly observe the morphology and structure of the neurons and the process of differentiation and migration, which overcomes the technical challenge of the embryonic spinal cord electroporation in the uterus. Compared with the IUE of the spinal cord, our method reduces the operation difficulty and improves the success rate of the experiment. This study provides a simpler method for abnormal expression of exogenous genes in the mouse spinal cord.

Frozen section or isolated neuron culture can cause great damage to neurons, which impacts the observation of the structure of individual neurons (Yang et al., 2017; Chen et al., 2018). However, spinal cord tissue culture can solve this problem. Due to the thickness of spinal cord slices, it can maintain the integrity of neurons which is significant for studying the structure and behavior of individual neurons. To better observe the migration of individual neurons in real time, we used living cell workstations. In this experiment, we can observe the migration of a single neuron, and as the two nerve cells approach, the rate of migration was accelerated. To further understand the spinal cord morphology during development, we can abnormally express candidate exogenous genes and observe the abnormal phenomenon and the target genes function. However, this new method has some disadvantages. Compared with frozen sections of spinal cord obtained during *in vivo* embryonic development, spinal cord slices lack stringent developmental regulation and the spatiotemporal regulation of the neurons is

disturbed, which leads to a certain gap between live developments.

Acknowledgments

Funding

This work was supported by grants from National Science Foundation of China (81771226), the Henan Province Foundation (162300410214, 19IRTSTHN003), the Xinxiang City Foundation (CXRC16003, ZD17008), the support project for the Disciplinary group of Psychology and Neuroscience, Xinxiang Medical College Research Innovation Support Program (YJSCX201801Z), the PhD Research Startup Foundation (505090) of Xinxiang Medical University.

Declaration of competing interest

The authors declare that they have no known conflicts of interest associated with this publication.

Author contributions

Conceived and designed the experiments: Juntang Lin. Performed the experiments: Shuanqing Li, Yunxiao Li, Han Li, Ciqing Yang. Analyzed the data: Shuanqing Li. Wrote the manuscript: Shuanqing Li.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.mod.2019.103558.

References

Chen, X., Zhang, S., Huang, J., Dong, W., Xiao, H., Shao, H., Cheng, J., Wu, H., Qi, Y., 2018. Hyper-SUMOylation of K^+ channels in sudden unexplained death in epilepsy: isolation and primary culture of dissociated hippocampal neurons from newborn mice for subcellular localization. Methods Mol. Biol. 1684, 63–71.

Danesin, C., Soula, C., 2017. Moving the Shh source over time: what impact on neural cell diversification in the developing spinal cord? J. Dev. Biol. 5 (2), 1–17.

Doisy, E.T., Wenzel, H.J., Mu, Y., Nguyen, D.V., Schwartzkroin, P.A., 2015. Nodule excitability in an animal model of periventricular nodular heterotopia: c-fos activation in organotypic hippocampal slices. Epilepsia 56 (4), 626–635.

Gähwiler, B.H., 1981. Organotypic monolayer cultures of nervous tissue. J. Neurosci. Methods 4 (4), 329–342.

Liu, Y., Fu, S., Niu, R., Yang, C., Lin, J., 2014. Transcriptional activity assessment of three different promoters for mouse in utero electroporation system. Plasmid 74, 52–58.

Liu, J.J., Huang, Y.J., Xiang, L., Zhao, F., Huang, S.L., 2017. A novel method of organotypic spinal cord slices culture in rats. Neuroreport 28 (16), 1097–1102.

Nakamura, H., Katahira, T., Sato, T., Watanabe, Y., Funahashi, J., 2004. Gain- and loss-of-function in chick embryos by electroporation. Mech. Dev. 121 (9), 1137–1143.

Nicholls, J., Saunders, N., 1996. Regeneration of immature mammalian spinal cord after injury. Trends Neurosci. 19 (6), 229–234.

O'Shea, T.M., Burda, J.E., Sofroniew, M.V., 2017. Cell biology of spinal cord injury and repair. J. Clin. Invest. 127 (9), 3259–3270.

Pandamooz, S., Salehi, M.S., Nabiuni, M., Dargahi, L., Pourghasem, M., 2016. Evaluation of epidermal neural crest stem cells in organotypic spinal cord slice culture platform. FoliaBiol (Praha) 62 (6), 263–267.

Raineteau, O., Rietschin, L., Gradwohl, G., Guillemot, F., Gähwiler, B.H., 2004. Neurogenesis in hippocampal slice cultures. Mol. Cell. Neurosci. 26 (2), 241–250.

Shin, J.E., Jung, K., Kim, M., Hwang, K., Lee, H., Kim, I.S., Lee, B.H., Lee, I.S., Park, K.I., 2018. Brain and spinal cord injury repair by implantation of human neural progenitor cells seeded onto polymer scaffolds. Exp. Mol. Med. 50 (4), 39.

Shinmyo, Y., Kawasaki, H., 2017. CRISPR/Cas 9- mediated gene knockout in the mouse brain using in utero electroporation. Curr. Protoc. Neurosci. 79, 3.32.1–3.32.11.

Teh, C., Chong, S.W., Korzh, V., 2003. DNA delivery into anterior neural tube of zebrafish embryos by electroporation. Biotechnigues 35 (5), 950–954.

Vostrikov, V.M., Kolomeets, N.S., Aleksandrova, O.P., Viktorov, I.V., Uranova, N.A., Sukhikh, G.T., 2005. Organotypic cultures of free-floating slices of human embryo medulla oblongata. Neurosci. Behav. Physiol. 35 (1), 9–15.

Xu, D., Zhu, Y., Xu, Z., 2017. Efficient genetic manipulation in the developing brain of tree shrew using in utero electroporation and virus infection. J. Genet. Genomics 44 (10), 507–509.

Yang, C., Li, X., Wang, C., Fu, S., Li, H., Guo, Z., Zhao, S., Lin, J., 2016. N-cadherin regulates beta-catenin signal and its misexpression perturbs commissural axon projection in the developing chicken spinal cord. J. Mol. Histol. 47 (6), 541–554.

Yang, C., Li, X., Li, Q., Zhang, B., Li, H., Lin, J., 2017. Combined use of in ovo electroporation and cultured neurons for gene function analysis of embryogenesis in the chicken optic tectum. Neuroreport 28 (17), 1180–1185.

Yoshida, Y., Suzuki, Y., Al-Jahdari, W.S., Hamada, N., Funayama, T., Shirai, K., Katoh, H., Sakashita, T., Kobayashi, Y., Nakano, T., 2012. Evaluation of the relative biological effectiveness of carbon ion beams in the cerebellum using the rat organotypic slice culture system. J. Radiat. Res. 53 (1), 87–92.