마우스 배아에서 슈반세포-뉴런 네트워크의 분리와 슈반세포의 분리

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Isolation of Schwann Cell and Separation of Schwann Cell-Neuron Network from Mouse Embryo

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요 약

슈반세포의 수초화에 대한 연구는 일차 슈반세포의 분리와 배양의 성공에 의해 가능해지고 있다. 본 연구에서는 슈반세포의 근원으로서 마우스 배아의 척수신경절이 사용되었다. 이 방법에는 세 가지 단계가 있다. 첫 단계는 배아의 척수신경절의 파쇄이고 두 번째 단계는 섬유아세포로부터 슈반세포-뉴런 연합체의 기계적인 분리에 의한 슈반세포의 전구세포의 확장이며 세 번째 단계는 뉴런으로부터 슈반세포의 분리와 분리된 슈반세포의 확장이다. 우리는 본 과정을 통해 짧은 시간이내에 슈반세포-뉴런 연합체와 슈반세포를 고순도로 분리하였다.

ABSTRACT

The study of Schwann cell myelination has been facilitated by the availability to isolate and establish pure population of primary Schwann cells. Dorsal root ganglia (DRG) of mouse embryo as source of Schwann cells were used in this study. This method includes three steps: first step of dissociation of the embryonic DRG, second step of expansion of Schwann cell precursors, followed by mechanical separation of the Schwann cell-neuronal network from the underlying fibroblasts, and third step of purification of Schwann cells from the associated neurons and subsequent expansion of the purified Schwann cells. We made a highly purified population of Schwann cells and Schwann cell-neuron networks in a short period using this procedure.

Keyword
dorsal root ganglia, Schwann cell, Schwann cell-neuron network, myelination,

Ⅰ. INTRODUCTION

Dorsal root ganglia provide distinguishable source of neurons from nonneuronal cells [1]. Adult mammalian DRG neuron cells can survive and regenerate in culture [2,3]. There are several researches on purified populations of these neurons [5,6,7]. Cultures of purified DRG neurons can be used in myelin formation.

The most widely used method for preparing primary Schwann cell culture uses DRG as the primary source of Schwann cells. The procedure is very simple and produces a highly purified population of Schwann cells in a short time. The method has also been used to prepare Schwann cells from mouse embryos.

In this study, we performed a procedure for preparing Schwann cells from embryos of rat DRG using modified method including protocols for

Schwann cell purification, expansion, and storage.

II. MATERIALS AND METHODS

A. dissection and dissociation of embryonic DRG

Under a dissecting microscope, mose embryos were decapitated with spring scissors and transfered the head to a pre-labeled 1.5 mL Eppendorf tube prepared. The tips of spring scissors vertically were inserted into the rostral end, just anterior to the vertebral column and begin making 3-4

sequential cuts from rostral to caudal (from head to tail) direction. The ganglia from the dorsal root starting from one end of the spinal cord were pinched off. All the ganglia (approximately 37 -42 ganglia/spinal cord/embryo) were removed and repeated with the step for all the spinal cords. The 1.5 mL of 0.25% trypsin were added to each dish and placed the dishes into a 37℃ air incubator for 15-20 min. It were added with 10 mL of L-15/10% FBS into each tube and centrifuged the ganglia at 50 *g* for 5 min. The pellet was added and resuspended with 10 mL of L-15/10% FBS. After centrifugation for 5 min as above, it was added with 1 mL of DRG plating medium to each tube. Using narrow-bored glass Pasteur pipettes by flaming the tips, the pellet was triturated 10-15 times and added with additional 1 mL of DRG plating medium to the cell suspension. This dissociated cells in an uncoated 35 mm dish were incubated.

B. Expansion of Schwann cell precursors and separation of the Schwann cell-neuronal network from fibroblasts

After 18 hr, plating of the dissociated

DRG was replaced the medium with 2 mL of N2/NGF. Under the serum-free condition, fibroblast growth is suppressed while axon-associated Schwann cell precursors continue to proliferate. There is no need to change the medium. After 6-7 days, Schwann cell-neuron network forming axon bundles was transferred into culture medium.

C. purification and expansion of Schwann cells.

After collecting Schwann cell-neuron networks, the cells were pelleted by centrifugation at 200 *g* for 5 min.

The pellet was resuspend in trypsin-collagenase solution and incubated at 37!C for 30 min. The reaction was stopped by adding Schwann cell medium and the cells were pelleted as above. The pellet was resupended in 1 ml of anti-Thy 1.2 antibody solution and incubated 30 min at 37℃. After centrifugation, pellet was resupended in 1 ml of rabbit complement solution and incubated 30 min at 37℃. Cells were plated onto a poly-L-lysinecoated 100 mm culture dish in Schwann cell medium and incubated 30 min at 3 7℃. Next day, bipolar, spindle-shaped Schwann cells were changed the medium to N2-Schwann cell growth medium. Neuregulin and Forskolin contained in the growth medium start to stimulate Schwann cell proliferation while growth of any contaminating fibroblasts is suppressed under the serum-free condition. Neurons should die off within 2 days in the absence of NGF in the medium. After 6-7 days, the cells were replated onto new poly-L-lysine-coated plates at a density of 1×10^6 cells/100 mm plate.

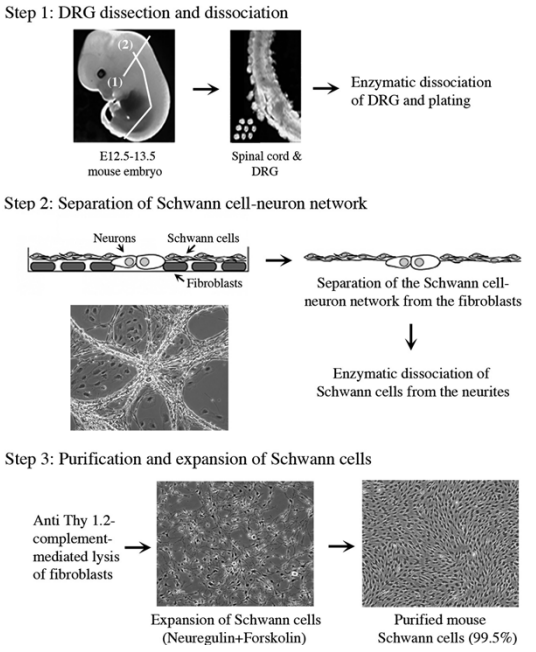

Fig. 1. Procedure for isolating Schwann cells from a mouse embryo.

III. RESULTS AND CONCLUSIONS

Procedure of used method described in Fig 1. and this includes three steps [4]: first step of dissociation of the embryonic DRG (Fig.2), second step of expansion of Schwann cell precursors,

followed by mechanical separation of the Schwann cell-neuronal network from the underlying fibroblasts (Fig. 3), and third step of purification of Schwann cells from the associated neurons and subsequent expansion of the purified Schwann cells (Fig. 4).

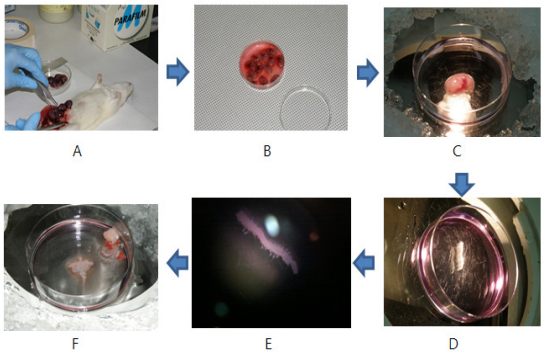

Fig. 2. Disssection and dissociation of DRG from mouse embryo (E 16 day).

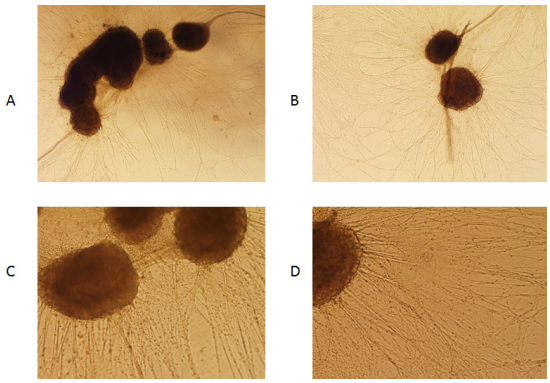

Fig. 3. Separation of the Schwann cell-neuronal network from fibroblasts.

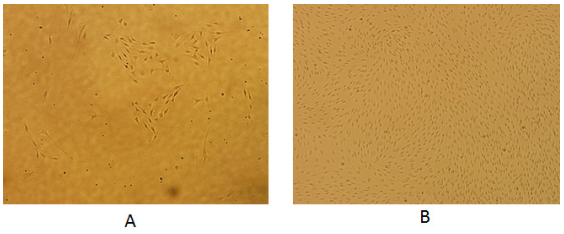

Fig. 4. Purification of Schwann cells from the associated neurons and subsequent expansion of the purified Schwann cells.

We made a highly purified population of Schwann cells and Schwann cell-neuron networks in a short period using this modified method.

This Schwann cells will use for research of myelination with rat neurons.

ACKNOWLEDGMENTS

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