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# 슈반세포와 뉴런세포의 수초화와 탈수초화

김현주<sup>1</sup> · 김지영<sup>1</sup> · 홍성갑<sup>2</sup>

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## Myelination and Demyelination of Schwann cells and Neuron cells

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### 요 약

취의 배아(배아 16일)의 척수신경절에서 슈반세포와 뉴런 세포가 실험실 상에서 분리되었고 정제되었다. 정제된 척수신경절 세포는 유사분열 억제제를 첨가하여 배양되었고 분리 정제된 슈반세포와 공동배양 배양시켰다. 이 결과로 마침내 수초화 과정이 구축되었다. 이 수초화는 *M. leprae*-specific phenolic glycolipid-1 (PGL-1) 성분으로 처리하였고 그 결과 neurofilament의 단클론 항체를 이용하여 수초화와 탈수초화를 비교 및 분석하였다.

### ABSTRACT

Schwann cells and neuron cells from dorsal root ganglion (DRG) of rat embryos (E16) were isolated and purified *in vitro* system. The purified DRG cells with anti-mitotic agents and purified Schwann cells, respectively, were cocultured and then consummated myelination processing. This myelination system was treated by *M. leprae*-specific phenolic glycolipid-1 (PGL-1) and then accomplished demyelination system. We compared with myelination and demyelination using neurofilament of monoclonal antibody.

### Keyword

demyelination, phenolic glycolipid-1, *M. leprae*, myelination, neurofilament

### I . INTRODUCTION

Thirty years ago, researchers developed a DRG neuron/Schwann cells co-culture system for myelin research *in vitro* [1]. The study of myelination has been facilitated by the availability to isolate and establish pure population of Schwann cells and Neuronal cells from primary Schwann cells. Mammalian DRG neuron cells can survive and regenerate in culture [2, 3, 4]. There are several researches on purified populations of these neurons. Coculture of purified DRG neurons and

Schwann cells 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 purified population of myelination by coculture of DRG neuronal cells and Schwann cells. The purified DRG cells with highly dose of anti-mitotic agent

and purified Schwann cells were cocultured and then accomplished myelination from coculture cells in a short time. Treatment of *M. leprae*-specific phenolic glycolipid-1 (PGL-1) into this coculture system was performed and then accomplished demyelination. Therefore, we identified demyelination processing using monoclonal antibody against neurofilament protein which is a component protein of myelinated cells.

## II. MATERIALS AND METHODS

### A. preparation of embryonic DRG

Embryos of mouse were decapitated with spring scissors and transferred 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°C 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. suspension of the embryonic dorsal root ganglion cells

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 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 resuspended in 1 ml of anti-Thy 1.2 antibody solution and incubated 30 min at 37 °C. After centrifugation, pellet was resuspended in 1 ml of rabbit complement solution and incubated 30 min at 37°C. Cells were plated onto a poly-L-lysine coated 100 mm culture dish in Schwann cell medium and incubated 30 min at 37°C. 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  $1 \times 10^6$  cells/100 mm plate.

### D. addition of anti-mitotic cocktail

Cultures were incubated at 37 °C, with 5% CO<sub>2</sub>. 24 h later, 150 mL of NGF stock solution (40 mM of 5-fluorodeoxyuridine and uridine, 1 mM Arabinofur-anosyl Cytidine (Ara C, Sigma-Aldich, Saint Louis, MO) in NG medium) was added into each well to make the final concentration of 5-fluorodeoxyuridine/uridine 20 mM. Cultures were incubated at 37 °C in a 5% CO<sub>2</sub> incubator. After 72 hours, 2/3 of the NGF stock solution was changed to NG medium and the cultures were re-fed every 2 days with NG medium. After three medium changes, the neurons were ready for the Schwann cell addition.

### E. addition of Schwann cells into DRG neuronal cell culture

Schwann cells were digested and washed once with DMEM (Invitrogen, Carlsbad, CA) containing 10% FBS, and then resuspended in C medium (MEM, 10% FBS, 2mM l-glutamine, 0.4% glucose, and 50 ng/mL 2.5 S NGF). 100 mL (approximately 200,000 cells) of Schwann cells was added to

each of the DRG cultures in C media. After 3 days, the co-cultures were supplemented with 50 mg/mL of ascorbic acid to initiate basal lamina formation and myelination. Myelination was allowed to proceed for up to 21 days. The process of myelination was observed and recorded under phase contrast microscope.

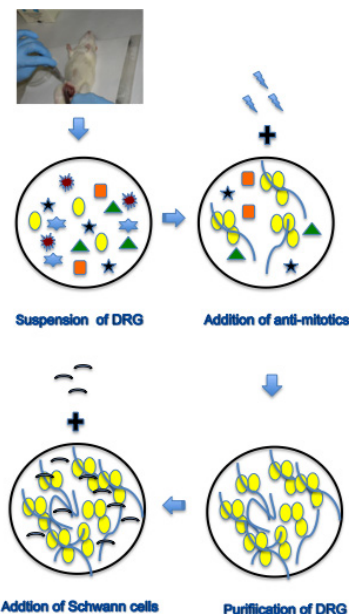
#### F. Immunocytochemistry

To observed the formation of the myelin, the DRG neuron/SC co-cultures (14 days after initiation myelin formation) were then labeled with primary antibodies, which included monoclonal antibody (mAb) against myelin basic protein (MBP). After three washes with PBS, the coverslips were further incubated with Alexa Fluor 488 mouse anti-rabbit IgG and Alexa Fluor 594 goat anti-mouse IgG (Invitrogen, Carlsbad, CA) for 60 min at room temperature. After a final wash in PBS, the slides were mounted with mounting fluid (DAKO Ltd., Carpenteria, CA) and visualized under a fluorescence microscope (Olympus, Tokyo, Japan). The images were digitally recorded and processed with Image-Pro Plus (Media Cybernetics, Atlanta, GA).

### III. RESULTS AND CONCLUSIONS

Procedure of coculture with Schwann cells and neuronal cells for myelination from DRG of rat embryos contains following four steps: first step of suspension of the embryonic dorsal root ganglion cells [4], second step of addition of anti-mitotic cocktail [1], third step of purification of dorsal root cells [4], and fourth step of addition of Schwann cells to dorsal root ganglion cells [1].

Construction of myelination was performed through coculture with cultured DRG cells and cultured neuronal cells, respectively, of rat embryos (E 16 day) (Fig. 2).



<Procedure of coculture with Schwann cells and neuronal cells for myelination>

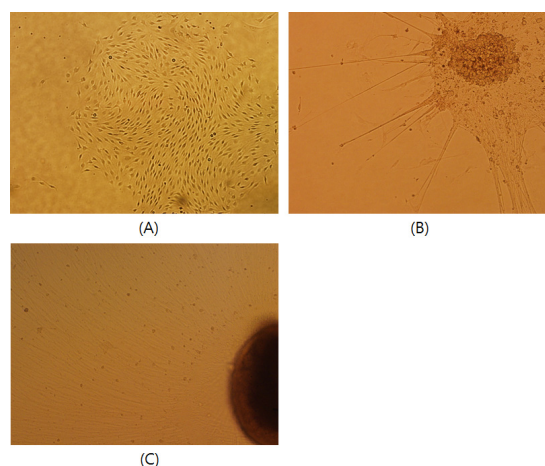


Fig. 1. Isolation and Purification of cultured Schwann cells and DRG neuronal cells, respectively, from rat embryo (E 16 day). (A: Schwann cells; B: DRG neuronal cells; C: myelinated cells)

To detect and identify the myelination formed by coculture of DRG cells and neuronal cells, cultured cells were labeled with monoclonal antibody of neurofilament and were analyzed by fluorescent microscope. The green-fluorescent domains represent neurofilament proteins of myelinated cells (Fig. 3 B).

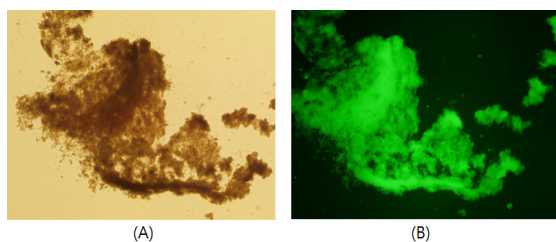


Fig. 2. Detection and Identification of myelination that is formed with coculture of DRG neuronal cells and Schwann cells using monoclonal antibody of neurofilament protein (A:optical microscope, B: fluorescent microscope).

In this study, we accomplished a purified population of myelination through this procedure and identified myelination using monoclonal antibody against neurofilament protein. After treatment of *M. leprae*-specific phenolic glycolipid-1 (PGL-1) into this myelinated coculture system were also identified demyelination of this myelinated coculture *in vitro* system because of absence of monoclonal antibody against neurofilament protein.

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