

Communication

Coculture of Schwann Cells and Neuronal Cells for Myelination in Rat

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ABSTRACT For *in vitro* myelination system, Schwann cells and neuronal cells of rat were cocultured. Schwann cells and neuronal cells, respectively, were obtained from dorsal root ganglion of rat embryos (E15). This method includes four steps: first step of suspension of the embryonic dorsal root ganglion cells, second step of addition of anti-mitotic cocktail, third step of purification of dorsal root cells, and fourth step of addition of Schwann cells to dorsal root ganglion cells. We made a highly purified population of myelination in a short period through this procedure and identified myelination basic protein using antibody of myelination basic protein.

Dorsal root ganglia (DRG) could generate a unique source of neurons which were different from non-neuronal cells.¹ Adult 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.

On the other hand, Schwann cell culture was isolated and purified from 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.

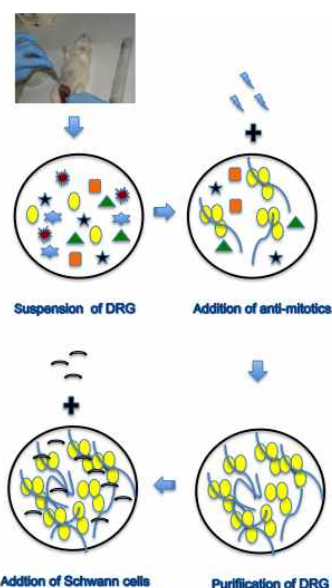


Fig. 1. Procedure for coculture of Schwann cells and neuronal cells for myelination from rat embryos.

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The method has also been used to prepare Schwann cells from rat embryos.⁵

Procedure of coculture of Schwann cells and neuronal cells for myelination from rat embryos was described in Fig 1.

This method consists of 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 cells⁴ and fourth step of addition of Schwann cells to dorsal root ganglion cells.¹ 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.

To observe 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 mouse anti-myelin basic protein (MBP) monoclonal antibody (dilution 1:1000, Abcam, Cambridge, MA). 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., Carpinteria, 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).

For formation of myelination, cultured DRG cells and neuronal cells, respectively, were prepared from rat embryo (E 16 day) (Fig. 2).

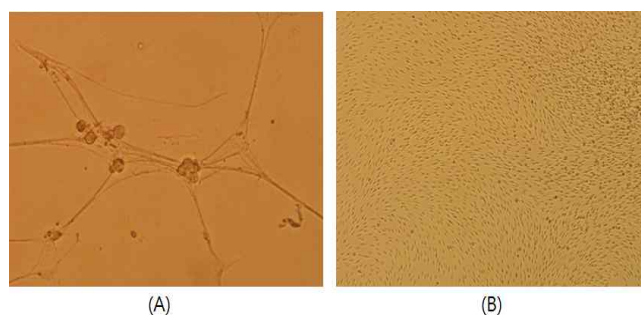


Fig. 2. Preparation of cultured DRG cells (A) and Schwann cells (B), respectively, from rat embryo (E 16 day).

To identify the myelin formed by coculture of DRG cells and neuronal cells, cultured cells were labeled with antibody of mouse anti-myelin basic protein (MBP) and were observed by fluorescent microscope. The green-fluorescent regions represent myelinated cells (Fig. 3).

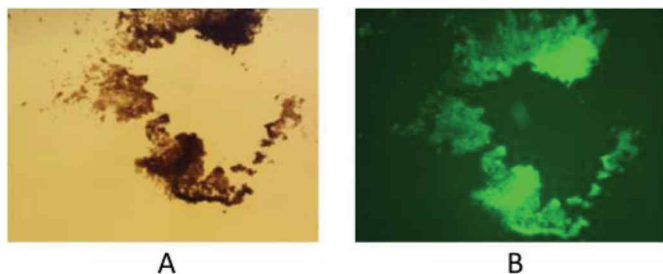


Fig. 3. Observation of myelination which is formed by coculture of DRG cells and neuronal cells (A: optical microscope, B: fluorescent microscope)

In this study, we used an efficiently modified method which was different from conventional three times of antimetabolic treatment in purification of DRG neuronal culture. This method includes treatment of a single high-dose (30 mM) of anti-mitotic cocktail. After this treatment, purification of DRG neuronal culture was taken 3 days instead of more 12 days in conventional method. Such a long procedure in performing experiment would increase risk of contamination with various infection of microorganism. Therefore, we could accomplish a purification of DRG culture using this highly efficient method. From coculture of this purified DRG neuronal cells and Schwann cells, we made a highly purified population of myelination in a short period and identified myelination proteins using antibody of myelination basic protein.

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KEYWORDS: coculture, Schwann cell, myelination, neuronal cell

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