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치의과학석사 학위논문

사람 말초신경에서 Cold jet 기법을
이용한 슈반세포의 분리 및 정제

Isolation and Purification of Schwann Cells
from Human Peripheral Nerves by Cold Jet
Technique

2017 년 8 월

서울대학교대학원

치의과학과 구강악안면외과학전공

나문 헨티

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지도교수 이 종 호

이 논문을 치의과학석사학위논문으로 제출함

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서울대학교 대학원

치의과학과 구강악안면외과학전공

나문 헨티

나문 헨티의 치의과학석사학위논문을 인준함

2017 년 6 월

위 원 장 김 성민 (인)

부 위 원 장 이 종호 (인)

위 원 장 정원 (인)

Abstract

Schwann cells (SCs) play an important role in peripheral nerve regeneration. However, established methods for SC preparation have limitations in that they are non-specific and time-consuming, as well as having a low yield and high cost. The purpose of this study was to assess the amount and purity of SCs isolated from human peripheral nerve tissue using the cold jet technique. The cold jet technique is an SC isolation and purification method that exploits the different responses of the SCs and fibroblasts to temperature shock. Specifically, the temperature shock causes specific detachment of SCs from fibroblasts. A total of 32 human peripheral nerve tissues (sensory and motor) were obtained from reconstruction procedures for oral cancer. After the length and weight of nerve segments were measured, the nerve fascicles were digested and SCs were isolated and purified by the cold jet technique. The purity of SCs was confirmed by morphology, reverse-transcription polymerase chain reaction, immunohistochemistry and flow cytometry. Phase-contrast photomicrographs, S100 mRNA expression rates and immunohistochemical staining show that the SC purification rate was greater with the cold jet technique. Flow cytometry showed the percentage (%) gated value increased from 56.10 to 95.95%. On average, $2.48 \pm 2.57 \times 10^6$ SC/g or $0.16 \pm 0.18 \times 10^6$ SC/cm were harvested efficiently from human peripheral nerve segments, where the SC yields of sensory and motor nerves did not show a significant

difference with the cold jet technique. Also, there was no difference in SC yields depending on gender. The cold jet technique was an efficient method for SC isolation and purification from the human peripheral nerves. The determined amount and the level of purity of the SC harvested by this method may be helpful for tissue engineering or cell therapy.

Keywords: Schwann cells, Human peripheral nerve, Cold jet technique, Purification and isolation, Flow cytometry

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I. Introduction

Schwann cells (SCs), the glial cells of the peripheral nervous system, are the main cell type within peripheral nerve stumps. SCs play a crucial role in functional recovery of injured peripheral nerves and are responsible for the formation and maintenance of the myelin sheath around axons in peripheral nerve fibers. After peripheral nerve injury, SCs proliferate and remove axon debris along with macrophages and also produce extracellular matrix molecules, integrin, and various neurotrophic factors to promote and guide axon growth in distal nerve stumps¹⁻³.

Methods have been developed to enrich SCs using antimetabolic treatment, a combination of antimetabolic treatment and antibody-mediated cytotoxicity employing complements, repeated explantations, differential adhesion, and immunoselection^{1, 4-6}. These techniques, however, have limitations. For example, antimetabolic agents are able to diminish contaminated fibroblasts but are harmful to SC function and can reduce SC yields because of their non-specific antimetabolic effects. Antibodies and complements are expensive for large scale preparation of SCs; thus, making it difficult to meet clinical goals that demand an economical approach. Repeated explantation and differential adhesion require comparatively complicated and time consuming procedures, which may lead to loss of SCs and possibly delay the

required therapy. Immunoselection is considered a good method for achieving high purity, but again requires expensive antibodies and special facilities¹.

In the current study, the cold jet technique was applied for human SC isolation as previously reported⁷. The cold jet technique has many advantages: no need for special equipment, low cost, and no utilization of antimetabolites that are clinically acceptable for a short period of SC growth. The use of artificial nerve grafts containing SCs is a promising method for peripheral nerve repair. Therefore, the production of a large number of viable SCs within a short period of time, which is essential for the application of tissue engineering techniques, is necessary for clinical application.

The purpose of this study was to assess and confirm the purity and number of SCs isolated from a specific amount (gram or centimeter) of human peripheral nerve tissue via the cold jet technique. Differences in SC yield were also evaluated according to nerve type, gender, and age.

II. Materials and Methods

1. Harvesting of human nerve segments

The use of human tissues for the experiments was approved by the Seoul National University Dental School Ethics Committee. Human peripheral nerve samples were obtained from oral cancer ablation and reconstruction procedures in patients at the Department of Oral and Maxillofacial Surgery, Seoul National University, Republic of Korea. A total 32 nerves (19 men and 13 women) were harvested from 28 patients (age range 31 to 77 years, average 63.2 years old). Sensory nerves included the cervical plexus of the neck (CP; n=4), the lingual nerve (LN; n=6), the forearm cutaneous nerve (FN; n=8), the greater auricular nerve (GAN; n=4), and the inferior alveolar nerve (IAN; n=2). The thoracodorsal nerve of the latissimus dorsi muscle served as a motor nerve (TDN; n=8).

The harvested nerves were immediately transferred to Dulbecco's Modified Eagle's Medium (DMEM) with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin (Pen/Strep) (Gibco, Life Technologies Co., Grand Island, NY, USA) and were stored at 4°C.

2. Primary culture of human SCs

After removing perineural connective tissue and blood, the nerve segments were blotted with filter paper to remove excess water, and then their length and weight were measured. Following nerve dissection into 1-cm sections, the nerve fascicles were isolated from the nerve segments by stripping off the epineurium using microscissors and Dumont (no. 5) forceps under a microscope. Isolated nerve fascicles were pre-degenerated with DMEM containing 10% FBS (Gibco, Life Technologies Co., Grand Island, NY, USA), 2 μ M forskolin (FK; Calbiochem, Merck KGaA, Darmstadt, Germany), 10 ng/ml fibroblast growth factor (FGF; Invitrogen, Life Technologies Co., Carlsbad, CA, USA), 50 ng/ml glia growth factor (GGF; Reprokin Ltd., Valley Cottage, NY, USA) and 5 μ g/ml bovine pituitary extract (BPE; Invitrogen, Life Technologies Co., Carlsbad, CA, USA) for 2 weeks. The culture medium was changed two or three times per week.

Enzymatic dissociation of pre-degenerated nerve fascicles was carried out as described by Haastert et al⁸. Briefly, fascicles were minced using a sterile scalpel and were incubated in dissociation solution containing 10% FBS, 1% Pen/Strep, 0.125% collagenase (Type IV, Sigma-Aldrich Co., St. Louis, MO, USA), and 1.25 U/ml dispase (Roche Diagnostics GmbH, Penzberg, Germany) for 24 h at 37°C and 5% CO₂. Dissociation solution and tissue residues were transferred into 15-ml tubes, and the same volume of

Hank's Balanced Salt Solution was added for quenching enzymatic activity. Tissue residues were gently triturated using a glass pipette until a homogenous solution was observed. Samples were then centrifuged at 800 rpm for 5 minutes at room temperature. The supernatant was removed and the pellet was re-suspended in DMEM containing 10% FBS. Centrifugation was repeated once more, and primary peripheral nerve cells were plated at a density of 10^6 - 10^7 cells per well on a laminin-ornithine-coated six-well plate (35 mm²). DMEM supplemented with 10 ng/ml heregulin (HRG; R&D Systems Inc., Minneapolis, MN, USA), 2 μ M FK, 10 ng/ml FGF, 50 ng/ml GGF, and 5 μ g/ml BPE served as the growth medium, and 1% bovine serum albumin (BSA) was added to enhance the seeding efficiency during overnight culture. The next day, the medium was changed using growth medium without BSA, and primary nerve cells were cultured for 2-3 days at 37°C and 5% CO₂.

3. SC purification by the cold jet technique

For purification of SCs from primary cultures mixed with fibroblasts, the cold jet technique described by Jirsova et al.⁷ was applied. In order to collect SCs of high purity, colonies of SCs were marked on the bottom of the six-well plate, the medium was aspirated, and colonies of SCs were gently rinsed with a stream of ice-cold phosphate-buffered saline (PBS) and then immediately aspirated. Detachment of SCs growing on top of a bottom layer

of fibroblasts was conducted with ice-cold growth medium using a 1-ml blue pipette tip and monitored by phase-contrast microscopy. The suspension of floating cells, mainly SCs, was transferred into fresh laminin-ornithine-coated six-well plates and cultured at 37°C and 5% CO₂. The cold jet technique was repeatedly applied up to three times.

4. SC counting

SCs were marked as phase bright, bi-, tri- or multipolar with a small cytoplasm-to-nucleus ratio as seen under a phase-contrast microscope, while fibroblasts were identified by a much more flattened polymorphic shape with larger, rounded nuclei⁹. Total cell numbers and number of SCs were counted from six random fields (magnification 40X) with a phase-contrast microscope.

5. Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was extracted from serially purified SCs with Isol-RNA Lysis Reagent (5 PRIME Inc., Gaithersburg, MD, USA) according to the manufacturer's instructions from two different patients (greater auricular nerve). The yield of total RNA was determined by measuring the absorption at 260 nm. Reverse transcription of 1 µg of total RNA was performed in a final volume of 20 µl using 200 units of Superscript II Reverse Transcriptase, 0.5 µg of oligo dT₁₂₋₁₈ primer, 0.5 mM dNTP Mix, 10 mM DTT, and First

Strand Buffer (Invitrogen, Life Technologies Co., Carlsbad, CA, USA). The mixture of oligo dT₁₂₋₁₈ primer, dNTP Mix, total RNA, and DEPC-treated ddH₂O was heated at 70°C for 10 min. Then, the other components were added and the solution was incubated at 42°C for 1 h. Subsequent incubation at 70°C for 15 min was conducted to inactivate the reverse transcriptase.

PCR amplification was performed with PCR-PreMix (AccuPower PCR PreMix, Bioneer Inc., Daejeon, Korea) in a My Cycler Thermal Cycler (Bio-Rad Laboratories Inc., Hercules, CA, USA) with a specific S100 primer set: sense 5'-TGGTTGCCCTCATTGATGTC- 3', antisense 5'-TCAAAGAACTCATGGCAGGC-3' (Cosmo Genetech Co., Ltd, Seoul, Korea). The reactions were started at 94°C for 5 min and amplified for 32 cycles of 45 sec at 94°C, 45 sec at 61°C and 1 min at 72°C. A final extension was continued for 10 min at 72°C to complete polymerization. GAPDH was used as an internal control to confirm equal loading of the samples: sense 5'-ACACCCACTCCTCCACCTTT-3', antisense 5'-TGCTGTAGCCAAATTCGTTG-3' (Cosmo Genetech, Co., Ltd, Seoul, Korea). The PCR products were separated on a 2% agarose gel, imaged using a ChemiDoc XRS molecular imaging system (Bio-Rad Laboratories Inc., Hercules, CA, USA), and analyzed with Multi Gauge software (version 3.0, Fuji Photo Film, Co., Ltd. Tokyo, Japan).

6. Immunohistochemistry

Purified cells were cultured on laminin-ornithine-coated cover slips and fixed with 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2) for 30 min at room temperature and then rinsed with PBS three times for 5 min each. Cells were then permeabilized with 0.2% Triton X-100 and 1% BSA in PBS for 10 min, blocked with 5% goat serum in PBS for 1 h, and then incubated with mouse anti-S100 (1:1000 dilution, Chemicon International Inc., Temecula, CA, USA), mouse anti-p75 (nerve growth factor receptor) (1:200 dilution, Chemicon International Inc., Temecula, CA, USA), or rabbit anti-glial fibrillary acidic protein (GFAP) (1:100 dilution, Chemicon International Inc., Temecula, CA, USA) overnight at 4°C. Cells were then rinsed in PBS three times, and the bound primary antibodies were visualized by incubating with Cy3-conjugated goat anti-mouse or anti-rabbit IgG (1:100 dilution, Jackson Immuno Research Lab, West Grove, PA, USA) or Alexa488-conjugated goat anti-mouse for 1 h at room temperature. For counterstaining, cells were incubated with DAPI solution (0.2 µg/ml, Sigma-Aldrich Co., St. Louis, MO, USA) for 10 min. Stained cells on the cover slips were washed in PBS, mounted carefully onto glass slides with Gel/Mount (Biomedex, Foster City, CA, USA), and then observed under a confocal laser scanning microscope (LSM510 META, Carl Zeiss, Jena, Germany). The photographic images were captured at 40X objective magnification. Negative

controls were processed in a parallel manner omitting primary antibodies.

7. Fluorescence activated cell scanning (FACS) analysis

Cells obtained by the cold jet technique were analyzed by a FACS Calibur flow cytometer (Becton, Dickinson and Company, Franklin Lakes, NJ, USA) directly after the staining procedure. The percentage of SCs purified by the cold jet procedure was determined by measuring the fraction of S100-positive cells in the fluorescence intensity dot plot compared to the total amount of intact cells. A histogram was subsequently prepared. The percentage of putative SCs among total cells was obtained from three independent trials, although only representative data are shown. For each assay, 10,000 cells were analyzed using 488-nm excitation for FITC. The data were analyzed with a software program (Cytomics RXP, Beckman-Coulter Co., Pasadena, CA, USA).

8. Comparison of donor nerve types and statistical analysis

Total SCs were counted after the second cold jet treatment and expressed as SC number per mg and per mm of nerve tissue. Parameters based on the type of donor nerve from patients were analyzed by one-way analysis of variance (ANOVA), and preplanned comparisons with the controls were

performed by the *post hoc* Fisher's Protected Least Significant Difference (PLSD) test using StatView software (Version 5.0.1, SAS Institute, Cary, NC, USA). Significance was set at $P < 0.05$. All values are presented as mean \pm standard deviation (SD).

III. Results

1. SC purification by the cold jet technique

The purity of SCs isolated from human nerve segments was confirmed by morphology, immunocytochemistry, RT-PCR, and flow cytometry. Phase-contrast photomicrographs showed that the SC purification rate gradually increased by the cold jet technique (Fig. 1). After 24 h of primary culture, two types of cells—fibroblasts and SCs—were mixed on the culture dish (Fig. 1A). However, after the first and third cold jet treatments, SCs showing the typical bipolar or tripolar morphology were highly enriched, and only a few fibroblasts remained (Figs. 1B, 1C).

The purity of SCs was characterized at RNA and protein levels. Expression levels of S100 mRNA were investigated in SCs obtained after repeated cold jet treatments. Figure 2 shows that S100 mRNA expression rates of SC obtained from greater auricular nerve (GAN) of two different patients gradually increased with the cold jet method; 132.19% and 160.02% after the second and third cold jet for the first sample, and 154.60% and 198.79% after the first and second cold jet for the second sample, compared to primary cells (100%), respectively. This result suggests that the purity of SC was improved by the cold jet technique. The purity of cells was also confirmed by immunohistochemistry with Schwann cell markers such as

S100, p75, and GFAP antibodies¹⁰⁻¹³ (Figs. 3 and 4). Specifically, expression of S100 and p75 proteins was greater following cold jet treatments, and cell morphologies maintained the bipolar or tripolar shape of SCs. Flow cytometry of SCs using FACS¹⁴ was performed with FITC-labeled S100 antibody to assess the purity of SCs obtained from repeated cold jet treatments. S100-positive staining cells were quantified (green histogram, Fig. 5). The results showed that the fluorescence histogram of the cells shifted to the right compared to the primary cells, and the percentage of gated cells in the population increased from 56.10 to 95.95 (Table 1). This comparison indicates that the purity of SC cells was highly increased by repeated cold jet treatments.

2. SC yield based on the type of donor nerve

A total of $2.48 \pm 2.36 \times 10^6$ SCs per gram or $0.16 \pm 0.18 \times 10^6$ per centimeter of nerve tissue were obtained. Tables 2, 3, 4 show the difference in numbers of SCs isolated according to nerve type (sensory or motor), gender, and age. Calculation per gram of nerve tissue (Table 2) demonstrated that the number of SC isolates from sensory nerves tended to be higher than the one from motor nerves ($2.86 \pm 2.57 \times 10^6$ vs. $1.36 \pm 1.05 \times 10^6$, $P=0.122$). Isolation of SCs per centimeter from sensory nerves (Table 2) also obtained more SCs compared to isolation from motor nerves ($0.18 \pm 0.20 \times 10^6$ vs.

0.11±0.09x10⁶, *P*=0.33). Gender-dependent analysis (Table 3) of SCs isolates per gram and centimeter did not show significance. Age-dependent analysis (Table 4) of SC isolates showed that sensory and total SCs isolations per gram from patients over 65 years old were markedly greater in comparison with ones under 65 years (4.18±3.16 x10⁶ vs. 1.92±1.59 x10⁶ in sensory; 3.39±2.84 x10⁶ vs. 1.68±1.53 x10⁶ in total nerves). Sensory and total SCs isolation per centimeter from patients over 65 years old was also significantly higher when compared with those under 65 years old (0.29±0.27 x10⁶ vs. 0.11±0.09 x10⁶ in sensory; 0.24±0.24 x10⁶ vs. 0.10±0.09 x10⁶ in total nerves *P*<0.05).

Concerning nerve phenotype-related purification of SCs, the total yield of SCs from the motor thoracodorsal nerve of the brachial plexus (TDN) was 1.36±1.05 x 10⁶ SC/g (Fig. 6A) and 0.11±0.09x10⁶ SC/cm (Fig. 6B). The SC yield per gram from the sensory lingual nerve (LN) was significantly higher than that of the TDN, cervical nerve (CP): 2.91±3.15 x 10⁶; LN: 4.70±3.66 x 10⁶; forearm nerve (FN): 2.38±1.52 x 10⁶; greater auricular nerve (GAN): 1.07±0.59 x 10⁶; and inferior alveolar nerve (IAN): 2.75±1.89 x 10⁶). The SC number from the LN was also significantly higher per centimeter than the ones from the other nerve types. (LN: 0.38±0.31 x10⁶; FN: 0.11±0.06 x10⁶; GAN: 0.05±0.02 x 10⁶; and IAN: 0.22±0.14 x10⁶).

IV. Discussion

SCs play a pivotal role in peripheral nerve regeneration, and highly purified SCs are essential for constructing tissue-engineered artificial nerve grafts for peripheral nerve regeneration^{15, 16}. However, a common problem in obtaining highly purified SCs is fibroblast contamination of SC cultures. This problem is the reason that existing results have a negative effect on engineered nerve repair by disturbing SC-mediated axonal regeneration¹. Over the past 30 years, many efforts have been made to harvest highly enriched SCs^{1, 17-21}. The current methods, however, require special equipment or have complicated procedures resulting in relatively low cell yields. In contrast, clinical application of SC therapy requires high yields of SCs obtained by safe, rapid, and easy purification methods.

The protocol used in present study was similar to procedures involving regular cell passage; therefore, it is relatively easy to perform. Additionally, it does not require special facilities or agents, except for ice cold culture medium. More importantly, this simple procedure was very efficient and posed no harm to cells; therefore, it is possible to apply this procedure in the clinic. In present study, it took about 27-40 days to obtain the purified SCs after third trial using cold jet technique. The time required from the primary seeding up to the first cold jet treatment was 10.5 days on average, from the

first to second cold jet treatment was 13 days on average, and from second to third was 15 days on average. It took a relatively long time because the cell culture was started from a small cell number and the cold jet method was performed when the cell confluency was 100%. The purity of SCs isolated with the cold jet technique was assessed by evaluating protein and RNA levels using SC-specific RT-PCR, flow cytometry analysis, and immunohistochemical staining. As shown in Figure 2, S100 mRNA expression rates of SC obtained from GAN of two different patients was slightly greater with the cold jet method. Flow cytometry data showed that the percentage of gated cells in the population increased from 56.10 to 95.95% after the third cold jet treatment. Collectively, these results suggest that the purity of SCs was much greater using repeated cold jet treatments, although the collection rate of SCs was lower.

A variety of different prototypes in the cells isolated from individual nerve segments was also observed. During the cell culture period, a relationship between cell phenotype and SC yield was observed. More numbers of SCs were harvested from sensory nerves than from motor nerves. Possible governing mechanisms behind this phenomenon may include differences in biochemical markers, SC phenotype, neurotrophic support, and/or nerve architecture^{22, 23}. Chemical and cellular differences between sensory and motor nerves may also play a relevant role²⁴. SC number per

gram and per centimeter of LN was higher than the ones of the other nerves. The reason might be that LN itself has relatively more SCs than compared with other nerves and has fewer peri-neural tissue, or low proportion of epi- or inter-fascicular epineurium.

It was suggested that there are slight differences in female and male peripheral tissues²⁵, so gender-based analysis was performed. Nonetheless, no differences were observed herein in SC yield between male and female patients (Table 3). However, SC yield depended on age and showed significant differences in both SC numbers per gram and centimeter. Although initially we were going to divide samples into young and old ages with the cut-off at 50 years old, there was only one nerve sample from a patient under 50 years old, thus we adjusted the cut-off to 65 years old; there were 15 nerve samples from patients over 65 years old and 17 samples from patients under 65 years old. Interestingly, the sensory and the total SCs isolations per gram and per centimeter of patients over 65 years old were significantly higher in comparison with ones from patients under 65 years old. In the further SC yield studies that investigate the effect of age, it will be necessary to collect more nerve samples from younger patients, specifically those under 50 years old.

V. Conclusion

In conclusion, $2.48 \pm 2.36 \times 10^6$ SC/g and $0.16 \pm 0.18 \times 10^6$ SC/cm were efficiently harvested from human peripheral nerves, an important finding that might be useful in future studies on tissue-engineered nerve conduits constructed with SCs for nerve regeneration. In addition, the yield of SCs isolated from the sensory lingual nerve using the cold jet technique was significantly higher than that of motor nerves, and there was no difference in the SC yield depending on gender. These findings can be used to improve the current nerve regeneration techniques by accurate calculation of nerve length or weight necessary for isolation of SCs. Thus, present investigation has implications for immediate clinical management of nerve injuries.

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VII. Figures and Figure Legends

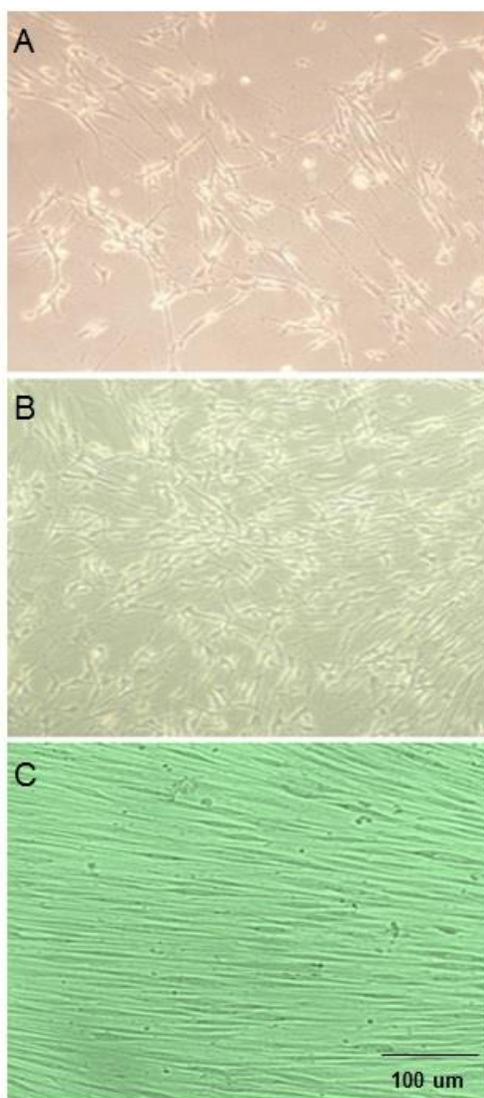


Figure 1

Phase-contrast photograph of Schwann cell (SC) purification and enrichment using the cold jet technique. (A) After 24 h of primary culture, most suspended cells adhered onto the plate and developed one of two distinct

shapes that represented two types of cells: SCs and fibroblasts. (B) SC culture obtained after the first cold jet treatment. Both SCs and fibroblasts proliferated, but many SCs were seen on the plate together with few fibroblasts. (C) Schwann cell culture obtained after the third cold jet treatment. Purified SCs showed the typical bipolar or tripolar morphology of SCs. Original magnification for photomicrographs: 100X. Scale bar: 100 μ m.

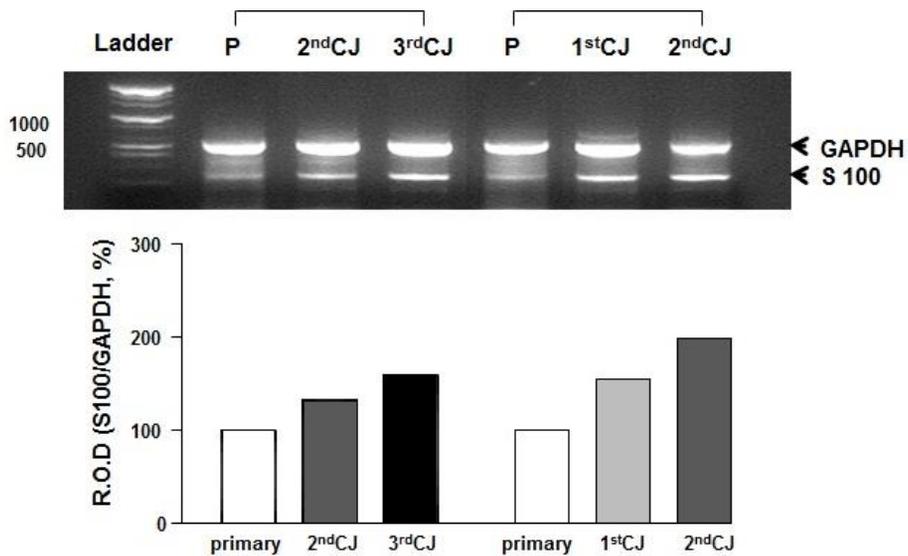


Figure 2

S100 mRNA expression after repeated cold jet treatments. RT-PCR analysis was performed with total RNA isolated from SCs obtained from two different

nerve. Signals were normalized against the corresponding GAPDH signal and quantified by Multi Gauge software (Version 3.0). P; primary culture cells, CJ; cold jet technique.

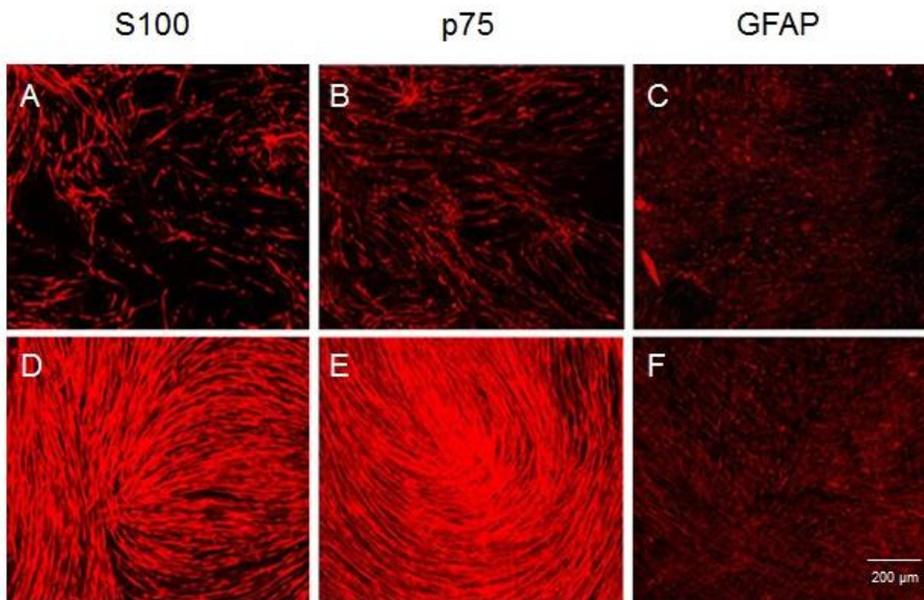


Figure 3

SC characterization by immunohistochemistry with S100 (A, D), p75 (B, E), and GFAP (C, F) antibodies. Cells were immunostained with each primary antibody (then with Cy3-conjugated secondary antibodies for visualization) following the first (A, B, C) and second (D, E, F) cold jet treatments on laminin-ornithine-coated six-well plates. Original magnification for photomicrographs: 100X. Scale bar: 200 μm.

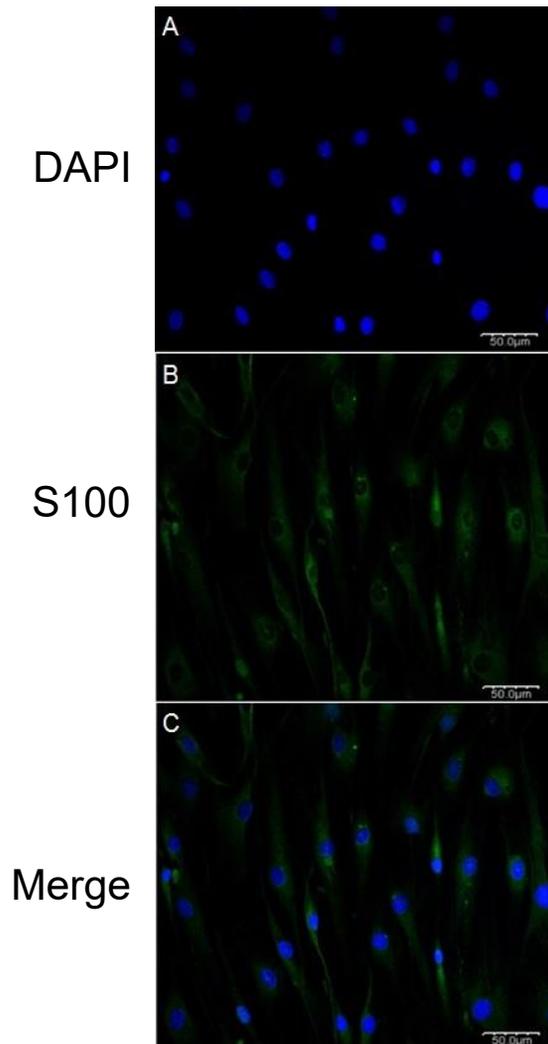


Figure 4

Immunohistochemistry for S100 protein in cells after the third cold jet treatment. Cells were stained with DAPI (A) and S100 antibody (then with Alexa488-conjugated secondary antibody for visualization) (B) and merged (C). Scale bar: 50 μm. Original magnification for photomicrographs: 400X.

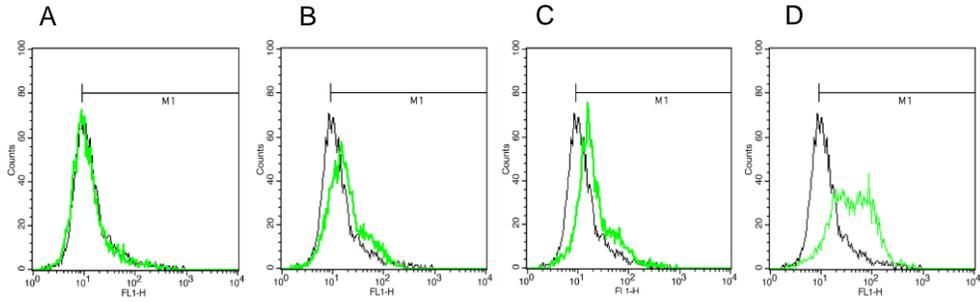


Figure 5

Flow cytometry of SCs serially purified via the cold jet technique. Flow cytometry was used to determine the purity of SCs using S100 expression as a Schwann cell marker (A; primary culture, B; first cold jet treatment, C; second cold jet treatment, D; third cold jet treatment). The histogram shows the negative control of primary culture cells (black line) and S100-specific fluorescent SCs serially purified by the cold jet technique (green line). For each assay, 10,000 cells were analyzed using 488 nm excitation for FITC.

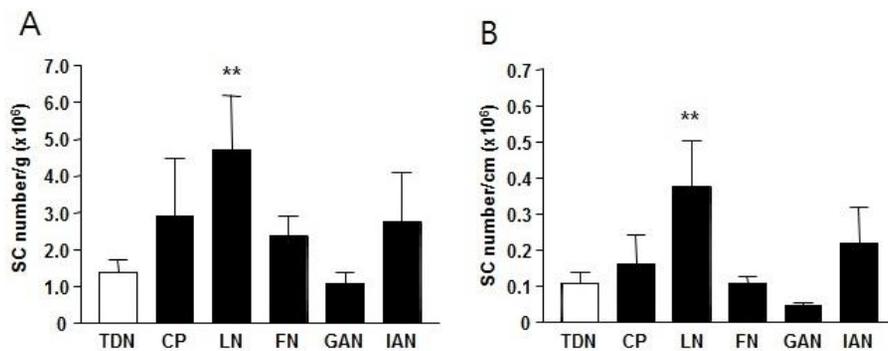


Figure 6

SC numbers per mg or mm according to nerve phenotype. SC numbers per g (A) and cm (B) were counted from cells obtained after the final cold jet treatment of several different types of nerves including the thoracodorsal nerve (TDN) of the brachial plexus (motor nerve type) and cervical (CP), lingual (LN), forearm (FN), greater auricular (GAN), and inferior alveolar nerves (IAN) (sensory nerve type). Data are expressed as the mean \pm SEM. Significance was set at $**P<0.01$.

VIII. Tables

Table 1. Flow cytometry of S100 positive SCs obtained after sequential cold jet technique.

	Primary cells	1 st cold Jet	2 nd cold Jet	3 rd cold Jet
% Gated (M1)	56.10	81.57	90.44	95.95
Mean (M1)	21.32	25.58	28.07	55.99

Table 2. Nerve types of Schwann cell proliferation following Cold jet technique.

SC isolation (x10 ⁶)	Nerve type		
	Sensory	Motor	Total
SC number* (NO/g)	2.86 ± 2.57	1.36 ± 1.05	2.48 ± 2.36
SC number † (NO/cm)	0.18 ± 0.20	0.11 ± 0.09	0.16 ± 0.18

* $P=0.122$ Sensory vs. Motor

† $P=0.33$

Table 3. Gender-dependent analysis of Schwann cell proliferation following Cold jet technique.

SC isolation (x10 ⁶)	Gender					
	Male			Female		
	Sensory	Motor	Total	Sensory	Motor	Total
SC number (NO/g)	2.33 ± 1.95	1.80 ± 1.09	2.19 ± 1.75	3.60 ± 3.22	0.62 ± 0.38	2.92 ± 3.08
SC number (NO/cm)	0.15 ± 0.18	0.15 ± 0.10	0.15 ± 0.16	0.23 ± 0.24	0.04 ± 0.02	0.19 ± 0.22

Table 4. Age-dependent analysis of Schwann cell proliferation following Cold jet technique.

SC isolation (x10 ⁶)	Age					
	≤65			>65		
	Sensory	Motor	Total	Sensory	Motor	Total
SC number (NO/g)	1.92 ± 1.59	0.57 ± 0.29	1.68 ± 1.53	4.18* ± 3.16	1.83 ± 1.06	3.39* ± 2.84
SC number (NO/cm)	0.11 ± 0.09	0.05 ± 0.03	0.10 ± 0.09	0.29* ± 0.27	0.14 ± 0.10	0.24* ± 0.24

*P<0.05 vs. ≤ 65 Years

IX. Abstract in Korean

사람 말초신경에서 Cold jet 기법을 이용한 슈반세포의 분리 및 정제

치의과학과 구강악안면외과학전공

(지도교수 이 종 호)

나문 헨티

1. 목 적

슈반세포(Schwann cell; SCs)는 신경교세포로서 말초 신경재생 및 기능회복에 중요한 역할을 하는 것으로 보고되어있다. 슈반세포를 분리하기 위해서는 세포용해제와 항유사분열제 등을 이용하는 기술이 보편적으로 이용되어 왔다. 그러나 이러한 기술적 방법들은 약물 처리로 인해 임상 적용에 어려울 뿐 아니라 경우에 따라 고가의 항체와 특수시설을 필요로 하여 고비용을 필요로 하는 문제점도 가지고 있다. Cold jet 기법은 특별한 장비를 필요치 않고, 비용이 저렴하며, 빠른 임상적 용이 가능하다는 장점이 있다.

이 연구는 Cold jet 기법을 사용하여 인체 유래 말초 신경 조직으로부터 분리된 슈반세포의 양과 순도를 관찰하고 신경학적 유형, 성별, 연령에 따라 슈반세포 수율의 차이를 평가하고자 하였다.

2. 방법

슈반세포는 28명의 환자 (31-77세, 평균 63.2세)에서 총 32개의 말초신경 (남성 19명, 여성 13명)으로부터 분리하였다. 말초신경 중 감각신경은 경부 신경총 (CP; n=4), 설신경 (LN; n=6), 대퇴피부신경 (FN; n=8), 대이개신경 (GAN; n=4), 하치조신경 (IAN; n=2)이었고, 운동신경은 광배근의 흥배신경 (TDN; n=8)이었다. 1cm 길이로 신경을 자른 뒤, 해부현미경하에서 상피를 박리하여 신경다발을 노출시킨 뒤 배양하였다. 슈반세포 정제를 위해 Cold jet 기법을 이용하였고, 최대 3회 반복하여 실시하였다. 위상차현미경 (x40)을 통해 슈반세포 계수측정을 실시하였다. 슈반세포의 순도는 형태학적 관찰, 역전사 중합효소 연쇄반응, 면역조직화학 및 유동 세포 계측법으로 확인하였다.

3. 결과

위상차 현미경 사진, S100 mRNA 발현을 및 면역 조직화학 염색법에 의한 결과, Cold jet 기법에 의해 점차적으로 슈반세포의 정제율이 증가하는 것으로 관찰되었다. 유동세포 계측법 (%)에서 역치값이 56.10%에서 95.95%로 증가한 것으로 관찰되었다. 평균적으로 $2.48 \pm 2.57 \times 10^6$ SC/g 또는 $0.16 \pm 0.18 \times 10^6$ SC/cm가 인체 유래 말초 신경부위로부터 효율적으로 증가하였다. 콜드 제트 기술에 의한 감각 및 운동 신경의 슈반세포 수율은 통계적으로 유의한 차이를 나타내지 않았다. 또한 성별에 따른 슈반세포 수율에는 차이가 없었다.

4. 결 론

Cold jet 기법은 인체 유래 말초신경에서 슈반세포 분리 및 정제를 위한 효율적인 방법으로 사료되었다. 이 방법으로 얻은 슈반세포의 정량 및 순도는 조직공학 또는 세포치료에 대한 향후 계획에 도움이 될 수 있을 것으로 사료되었다.

주요어: 슈반세포, 사람 말초신경, 콜드제트기법, 분리 및 정제,
유동세포계측법

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