

## PDGF-bb가 BDNF

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### ***In Vitro* Neural Cell Differentiation Derived from Human Embryonic Stem Cells: Effects of PDGF-bb and BDNF on the Generation of Functional Neurons**

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**Objective:** This study was to investigate the generation of the functional neuron derived from human embryonic stem (hES, MB03) cells on *in vitro* neural cell differentiation system.

**Methods:** For neural progenitor cell formation derived from hES cells, we produced embryoid bodies (EB: for 5 days, without mitogen) from hES cells and then neurospheres (for 7~10 days, 20 ng/ml of bFGF added N2 medium) from EB. And then finally for the differentiation into mature neuron, neural progenitor cells were cultured in i) N2 medium only (without bFGF), ii) N2 supplemented with 20 ng/ml platelet derived growth factor-bb (PDGF-bb) or iii) N2 supplemented with 5 ng/ml brain derived neurotrophic factor (BDNF) for 2 weeks. Identification of neural cell differentiation was carried out by immunocytochemistry using  $\beta_{III}$ -tubulin (1:250), MAP-2 (1:100) and GFAP (1:500). Also, generation of functional neuron was identified using anti-glutamate (Sigma, 1:1000), anti-GABA (Sigma, 1:1000), anti-serotonin (Sigma, 1:1000) and anti-tyrosine hydroxylase (Sigma, 1:1000).

**Results:** *In vitro* neural cell differentiation, neurotrophic factors (PDGF and BDNF) treated cell groups were high expressed MAP-2 and GFAP than non-treated cell group. The highest expression pattern of MAP-2 and  $\beta_{III}$ -tubulin was indicated in BDNF treated group. Also, in the presence of PDGF-bb or BDNF, most of the neural cells derived from hES cells were differentiated into glutamate and GABA neuron *in vitro*. Furthermore, we confirmed that there were a few serotonin and tyrosine hydroxylase positive neuron in the same culture environment.

**Conclusion:** This results suggested that the generation of functional neuron derived from hES cells was increased by addition of neurotrophic factors such as PDGF-bb or BDNF in b-FGF induced neural

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(01-PJ10-PG8-01EC01-0010)

This study was supported by a grant (01-PJ10-PG8-01EC01-0010) of the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea.

cell differentiation system and especially glutamate and GABA neurons were mainly produced in the system.

**Key Words:** Human embryonic stem cell, Neural progenitor cell, BDNF, PDGF-bb, Functional neuron

(embryonic stem cell)  
 (blastocyst)  
 (inner cell mass)  
 (pluripotent cell)  
 가 . 2001 Thomson Reubinoff  
 200 가  
 1981 Evans Martin  
 ,<sup>12</sup> 1998 Thomson 가 .<sup>6,7</sup>  
 .<sup>3</sup> 2000 Reubinoff 5 ,  
 Shuldiner 가 .<sup>4</sup> ,<sup>8</sup>  
 (mouse embryonic fibroblast, STO)  
 feeder-free  
 MB (Maria Biotech) 02 MB03  
 가  
 .<sup>5</sup> MB01, 02, 03 NIH  
 ,  
 feeder-free  
 MB03  
 (Neurodegenerative disorders) PDGF-  
 bb BDNF  
 , (Parkinson's disease) 가가 .  
 (Alzheimer's disease), (Huntington's disease),  
 (Multiple system strophy),  
 (Amyotrophic lateral sclerosis; ALS) (Spinal cord injury) 1.  
 MB03 STO cell (ATCC, CRL 1503) feeder 10 ( 2 ,40 population doublings)가 , Matrigel (Collaborative Biomedical Productive)  
 (cell replacement therapy)

STO cell conditioned medium 20  
 MB03 alkaline phosphatase (Sigma)가  
 SSEA-4 (stage-specific mouse embryonic antigen-4, DSHB)가 SSEA-3, SSEA-1  
 TRA-1-60 (tumor rejection antigen-1-60, gift of Dr. P.W. Andrews) TRA-1-81 (gift of Dr. P.W. Andrews) 가  
 RT-PCR Oct-4 (octamer-binding transcription factor-4)<sup>8</sup> telomerase activity (Intergen) 가 G-banding karyotyping (46, XX)  
 2. 가  
 , embryoid body , (neural progenitor cell) , (functional neuron, mature neuron) 4가

### 1) Embryoid body (EB)

EB 3  
 가 가  
 MB03 0.1% collagenase 2 , bacteriological dish (Falcon, # 1007) 4~5 ×10<sup>6</sup> 10% FBS (Hyclone), 1 mM L-glutamine 1% non-essential amino acid가 DMEM/F12 (Dulbecco's modified Eagle's medium/F12, Gibco) 5 EB (Figure 1A).

### 2) (neural progenitor cell)

EB 0.1% gelatin (Falcon, cat #3002) 20 ng/ml basic-FGF (KOMA)가 가 N2 7~10 . N2 DMEM/F12 N2 supplement, insulin (Sigma, 5 mg/L),

putrescine (Sigma, 100 μM), sodium selenite (Sigma, 30 nM), apo-transferrin (Sigma, 100 μg/ml), progesterone (Sigma, 20 nM) 가 EB가

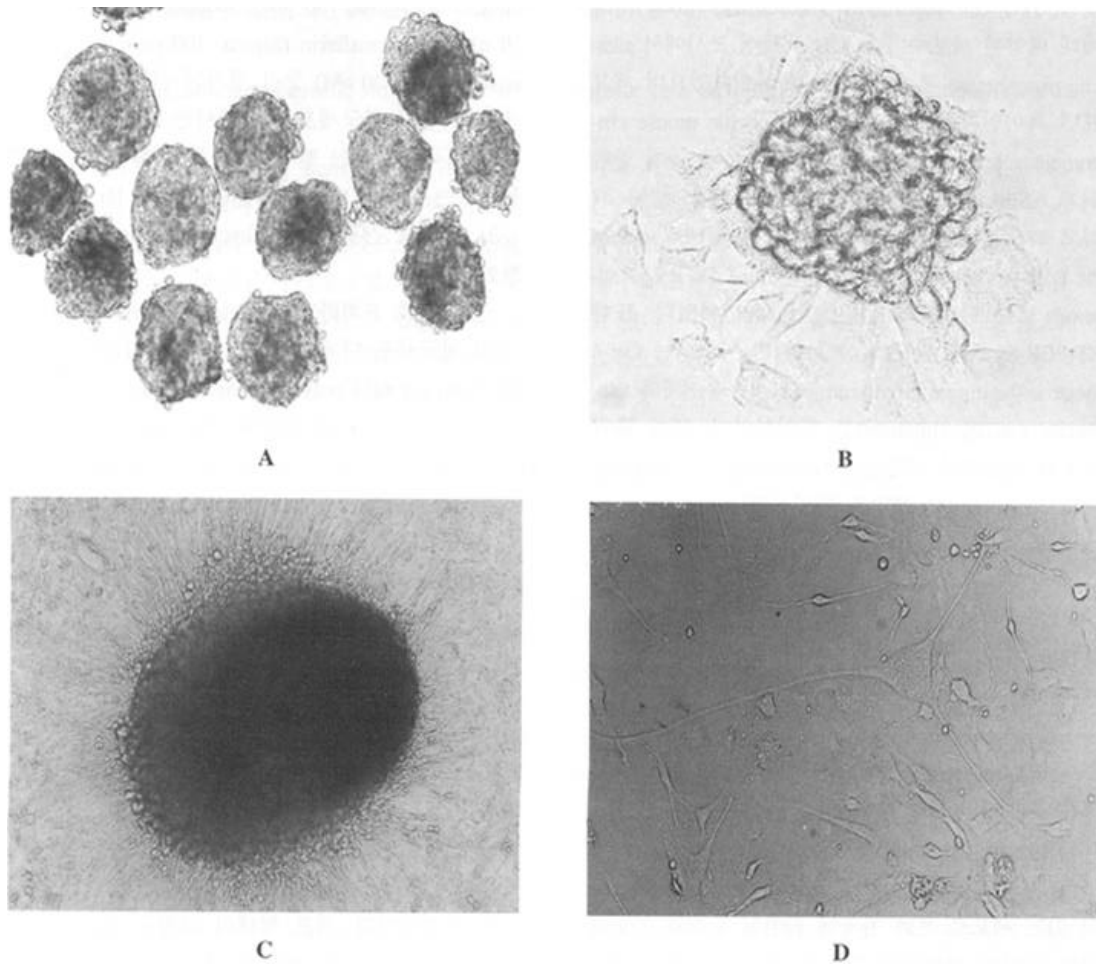
(Figure 1B, C).

### 3) (functional neuron)

EB  
 ( ) 0.1% Trypsin-EDTA 100 μg/ml poly-D-lysine (Sigma) , i) N2 ii) N2 20 ng/ml PDGF-bb (platelet derived growth factor-bb; Biosource) iii) N2 5 ng/ml BDNF (brain derived neurotrophic factor; Chemicon) 7~14 가

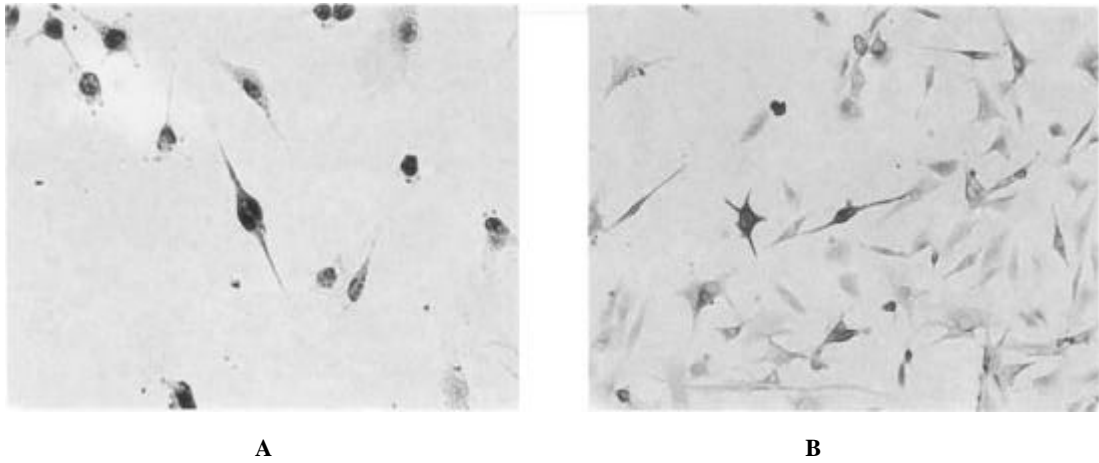
### 3.

가  
 4% paraformaldehyde (Sigma) 10 , 0.02% Triton X-100 (Sigma) 10 . 5% normal goat serum (Vector) 1 1 . 1 anti-human nestin (rabbit IgG, Chemicon, 1:100) , anti-MAP2 (mouse IgG, Sigma, 1:1000) anti-β tubulin isotype III (mouse IgG, Sigma, 1:250)가 anti-NF160 (mouse IgG, Sigma, 1:40) mature anti-NF200 (mouse IgG, Sigma, 1:40) . (astrocyte)



**Figure 1.** Neural cell differentiation from embryoid body (EB) derived human embryonic stem MB03. **(A)** Floating human EBs.  $\times 150$ . **(B)** Attached EB on 0.1% gelatin.  $\times 300$ . **(C)** Neural progenitor cell formation from EB induced by bFGF.  $\times 40$ . **(D)** Neural cell differentiation from neural progenitor cells.  $\times 100$ .

anti-GFAP (rabbit IgG, DAKO, 1:200)	.		FITC	520 nm, TRITC
	.	anti-gluta-	630 nm	
mate (mouse IgG, Sigma, 1:1000), anti-tyrosine hydro-	.		sample	
xylase (mouse IgG, Sigma, 1:100), anti-serotonin (rabbit	.		ABC (Avidin-biotin peroxidase complex, DAB staining,	
IgG, Sigma, 1:1000), anti-GABA (rabbit IgG, Sigma,	.		Vector)	
1:1000)	.	2	ABC	1
(tetramethyl rhodamine isothiocyanate) conjugated goat	.		, 2	biotin labelled goat anti-mouse or
anti-mouse or rabbit IgG (Jackson Immunoresearch, 1:	.		rabbit IgG	1
200)	.		streptavidin peroxidase	30
FITC (fluorescein isothiocyanate) conjugated	.		2	
goat anti-mouse or rabbit IgG (Jackson Immunorese-	.		H <sub>2</sub> O <sub>2</sub>	DAB (3,3'-diaminoben-
arch, 1:100)	.	1	가	가
sample	.	mounting (Biomeda)	5~10	가



**Figure 2.** Nestin/ $\beta$ -tubulin stained neural progenitor cells with DAB staining. **(A)** A number of neural progenitor cells were stained with anti-nestin.  $\times 200$ . **(B)** A few neural cells stained with  $\beta$ -tubulin were presented in neural progenitor cell stage.  $\times 100$ .

2.

10~14

가

1.

(hES, MB03) EB가

, 0.1% gelatin bFGF 7~10

가

ABC

(negative control)

2

2

1

EB가

EB 10 ng/ml bFGF

(intermediate filament) nestin ,

가 50%

(Figure 2A).

$\beta$ -tubulin isotype III가

(Figure 2B).

2

nestin 10%

(Figure 3A),

MAP2 (microtubule associated protein 2)  $\beta$ -tubulin isotype III가

60%

(Figure 3E, F).

Neurofilament

(NF) 200

NF160

(Figure 3C, D).

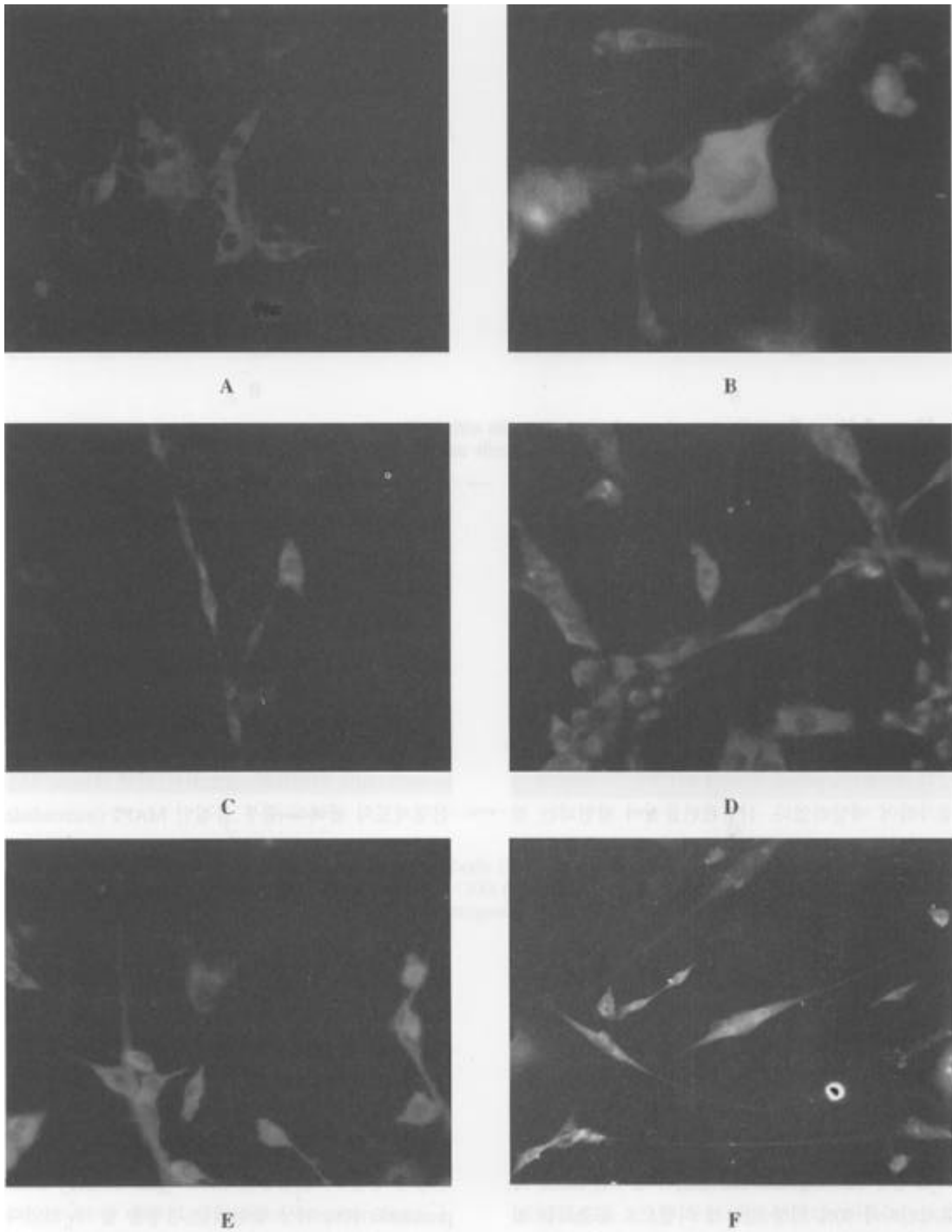
(astrocyte)

GFAP (glial fibrillary acidic protein) 10%

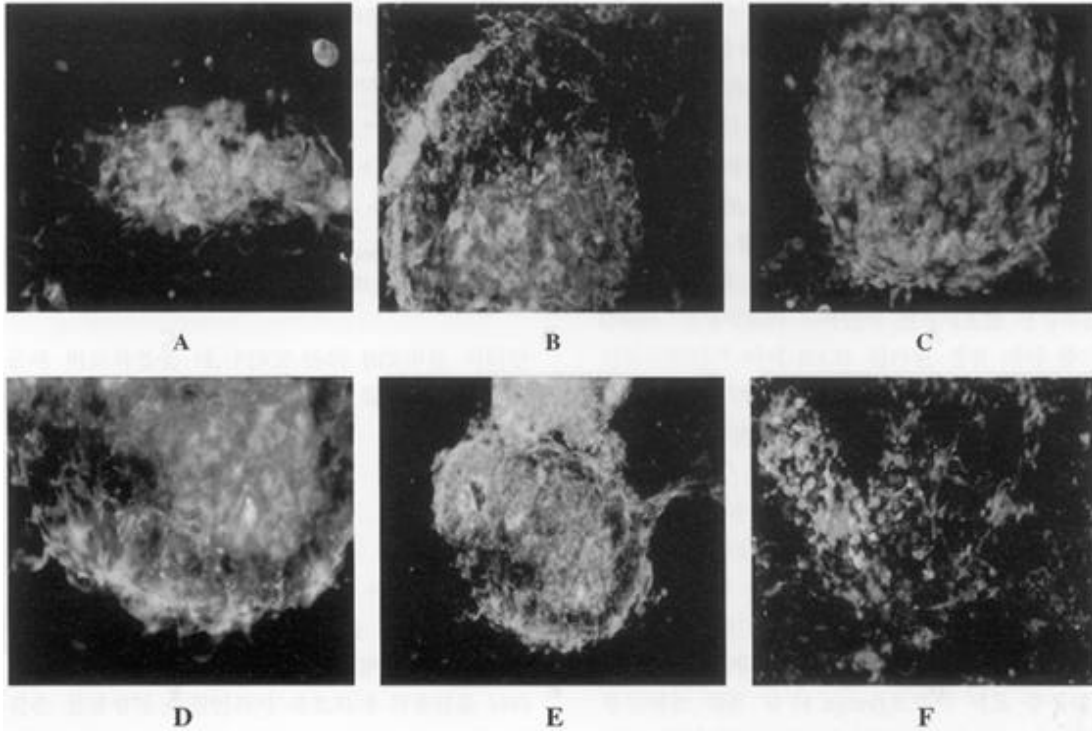
(Figure 3B).

BDNF PDGF-bb

double staining



**Figure 3.** *In vitro* differentiated neural cells after 2 weeks from neural progenitor cells derived human embryonic stem cells. **(A)** Neural progenitors with anti-nestin.  $\times 200$ . **(B)** Astrocyte with anti-GFAP.  $\times 400$ . **(C)** Immature neural cell with anti-NF160.  $\times 200$ . **(D)** Mature neural cell with anti-NF200.  $\times 200$ . **(E)** Neural cell with anti-MAP2.  $\times 200$ . **(F)** Neural cell with anti- $\beta$  tubulin.  $\times 200$ .

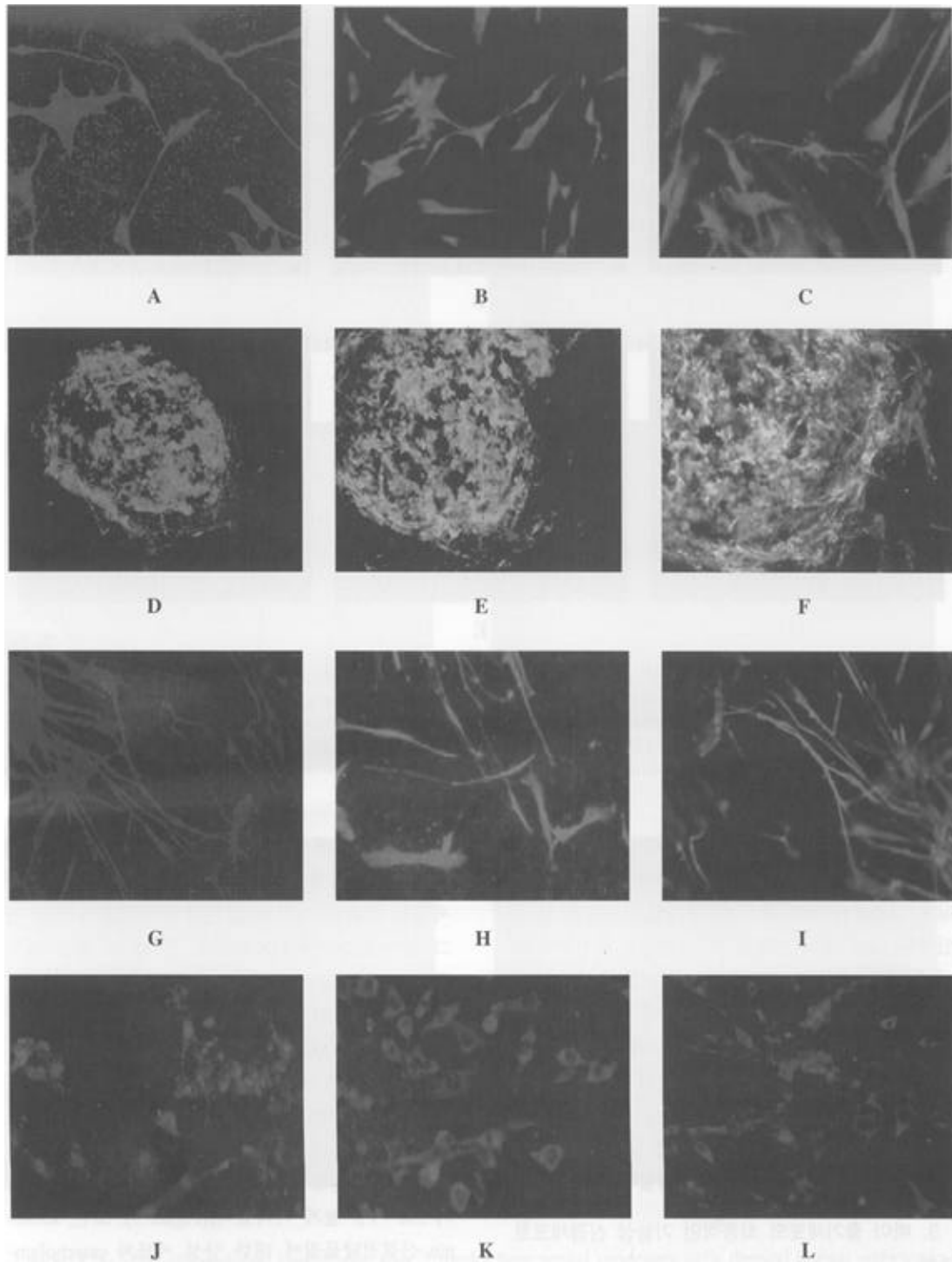


**Figure 4.** Double staining of MAP2 (green, monoclonal antibody) and GFAP (red, polyclonal antibody) examined in mature neuron stage according to the treatment groups. MAP2 and GFAP staining represented as indicators the neuron and glial cells, respectively. Green color is the more, neuron formation is the more. Each group was treated with no addition of growth factor (A and D), PDGF-bb (B and E) and BDNF (C and F) in the neural cell differentiation stage for 14 days. (A, E and F)  $\times 100$ . (B, C and D)  $\times 300$ .

BDNF PDGF-bb  
 가  
 가

BDNF PDGF-bb  
 glutamate GABA 가  
 , BDNF 20%  
 PDGF-bb  
 MAP2 가  
 glutamate GABA 가  
 GFAP BDNF  
 PDGF-bb 가 PDGF-bb  
 PDGF-bb choline glutamate GABA (?-amino  
 butric acid)  
 5% (Figure 5). , seroto-  
 nin catecholam-  
 ines  
 TH (tyrosine hydroxylase)  
 가

3.



**Figure 5.** Generation of functional neurons from neural progenitor cells treated with BDNF or PDGF; no addition of growth factor (control; **A, D, G** and **J**), PDGF treated group (**B, E, H** and **K**) and BDNF treated group (**C, F, I** and **L**). Indirect immunostaining of GABA (**A-C**), Glutamate (**D-F**), Serotonin (**G-I**) and dopamine (**J-L**) secreting neural cells. (**A-L**,  $\times 200$ ).



**Table 1.** Effect of neurotrophic factors on the generation of functional neuron

	GABA	Glutamate	Serotonin	TH
BDNF	+++*	+++	++	+
PDGF	++	++	++	+
W/O	+	+	+	+

\*(+): Tendency of generation to specific neural cell derived from human embryonic stem cells

, PDGF-bb BDNF (Figure 5 Table 1).

B49  
 GDNF가  
 RA 가 4+4 protocol  
 GABA RA가 가  
 bFGF (4 ng/ml) (20 ng/ml)  
 PDGF 1995  
 PDGF-aa  
 PDGF-bb  
 BDNF  
 TrkB (synapses)  
 bFGF  
 MB03  
 EB  
 PDGF-bb BDNF 가  
 GDNF (glial cell line-derived neurotrophic factor) 1993  
 PDGF-bb BDNF

Choline  
 glutamate GABA  
 Zhang Reubinoff  
 7,8  
 (corpus striatum)  
 GABA glutamate  
 vivo  
 in  
 bFGF  
 가  
 가

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