Cell Transplantation, Vol. 16, pp. 57–65, 2007
Printed in the USA.

0963-6897/07 $90.00 + .00
E-ISSN 1555-3892
www.cognizantcommunication.com

Plasma as a Scaffold for Regeneration of Neural Precursor Cells After Transplantation Into Rats With Spinal Cord Injury

Mitsuko Takenaga,* Yuki Ohta,* Yukie Tokura,* Akemi Hamaguchi,* Noboru Suzuki,† Masaya Nakamura,‡ Hideyuki Okano,§ and Rie Igarashi*

*Institute of Medical Science, St. Marianna University School of Medicine, Kawasaki 216-8512, Japan
†Department of Immunology and Medicine, St. Marianna University School of Medicine, Kawasaki 216-8512, Japan
‡Department of Orthopaedic Surgery, Keio University School of Medicine, Tokyo 160-8582, Japan
§Department of Physiology, Keio University School of Medicine, Tokyo 160-8582, Japan

The present study investigated whether plasma could be useful as a scaffold for cell transplantation in rats with spinal cord injury (SCI). Transplantation of cells with plasma promoted the recovery of SCI-induced motor dysfunction. Immunohistochemical analysis revealed that the grafted cells had differentiated into the neural lineage. When dissociated neural precursor cells were cultured with plasma, extensive neurite outgrowth was observed along with increased expression of p35 and NF68. Neural markers were also expressed by the cultured cells. Culture with plasma reduced thymidine incorporation, but promoted cell growth and increased the RNA contents. These findings suggest that the cells underwent differentiation into neurons in the presence of plasma. In conclusion, plasma could be a promising scaffold for cell transplantation therapy.

Key words: Neural precursor cell; Plasma; Serum; BBB score; Spinal cord injury

INTRODUCTION

Neural cell transplantation is considered to be a promising therapy for neurodegenerative diseases as well as for trauma such as spinal cord injury (SCI) (20,25). Because embryonic stem (ES) cells differentiate preferentially into neural precursor cells under culture conditions that favor neurogenesis, transplantation of neural precursors derived from ES cells has been reported to promote recovery from SCI-induced motor dysfunction (18).

Scaffolds are considered important to maintain the viability of grafted cells and to promote neural maturation (10,12,24). Hurtado et al. (12) reported that a freeze-dried poly(D,L-lactic acid) macroporous scaffold combined with Schwann cells in a fibrin solution led to production and secretion of brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3). Prang et al. (21) showed that alginate-based hydrogel combined with adult neural progenitor cells promoted cell contact-mediated axonal regeneration. Scaffolds without any cells have also been reported to induce axonal regeneration (3,13,14,21,22).

Yamada et al. (26) found that platelet-rich plasma (PRP) could enhance the formation of new bone and that it is nontoxic, nonimmunoreactive, and accelerates wound healing. They demonstrated that a combination of PRP (as an autologous scaffold) and culture-expanded mesenchymal stem cells could increase osteogenesis compared with the scaffold alone or autogenous cancellous bone chips and marrow. Yang et al. (27) reported that plasma-treated, collagen-anchored polylactone facilitated cell transplantation and showed improved cellular affinity.

Plasma has various advantages as a scaffold. It contains growth factors that include neurotrophic factors (7,16), fibrinogen, and fibronectin, which are used for neural cell culture. Because autologous plasma can be used, the problem of immune reactions can also be overcome.

We have been investigating cell transplantation therapy after SCI (17,23). The present study was performed to determine whether plasma was potentially useful as a scaffold to promote the survival and differentiation of transplanted neural precursor cells in rats with SCI.

MATERIALS AND METHODS

Differentiation of Embryonic Stem (ES) Cells Into Neural Precursor Cells

Mouse ES cells (R-CMTI-1) were obtained from Dainippon Pharmaceutical Co. (Osaka, Japan) and used

Received May 2, 2006; final acceptance September 8, 2006.
Address correspondence to Mitsuko Takenaga, Ph.D., Institute of Medical Science, St. Marianna University School of Medicine, 2-16-1 Sugao, Miyamae-ku, Kawasaki 216-8512, Japan. Tel: +81-44-977-8111; Fax: +81-44-976-3747; E-mail: m2take@marianna-u.ac.jp

after 12–18 passages. Undifferentiated ES cells were propagated in the presence of leukemia inhibitory factor (LIF, Chemicon Technologies, USA), and then differentiation was induced as follows. Cells were cultured as embryonic bodies (EB) in the absence of LIF for 4 days, after which 1 μM all-*trans* retinoic acid (RA, Sigma Chemical Co., St. Louis, MO, USA) was added on the 4th and 6th days. Treatment of ES cells with RA has already been reported to induce neural differentiation (1,2), and about 80% of RA-treated cells become positive for neural cell adhesion molecule (NCAM) (8). On the 8th day, the cells were dissociated into a single-cell suspension (neural precursor cells) and then were used for the in vitro and in vivo studies. In some experiments, GFP-expressing ES cells were used (19).

Spinal Cord Injury (SCI) and Cell Transplantation

Adult female SD rats weighing 222.7 ± 17.8 g were used to create the SCI model. Under anesthesia, dorsal laminectomy was performed at the T9–10 level. Then a 10-g weight was dropped onto the spinal cord from a height of 25 mm. The Basso/Beattie/Bresnahan (BBB) score (4,5) was determined on the day before cell transplantation. On the 9th day after SCI, neural precursor cells (5×10^6 cells in 5 μl) were transplanted at the site of injury using a Hamilton syringe. Before and after cell transplantation, cyclosporine A (10 mg/kg/day) was injected subcutaneously for 4 days. Blood was collected from each rat on the day of cell transplantation. All animals were housed at a constant temperature (23 ± 1°C) and humidity (50–60%) with free access to a standard diet and water in an animal room with a 12-h light/dark cycle. The study protocol was approved by the Animal Experimentation Committee of St. Marianna University.

Immunohistochemical Analysis

Rats were anesthetized and perfused with 4% paraformaldehyde (pH 7.4) in PBS by intracardiac injection. Then specimens of the spinal cord (1.5 cm long) containing the site of injury were harvested. The following primary antibodies were used for immunohistochemistry: mouse anti-neurofilament 200 (NF200) and mouse anti-βIII tubulin for detection of neurons (Sigma Chemical Co.), mouse anti-glial fibrillary acidic protein (GFAP) for identification of astrocytes (Santa Cruz Biotechnology), and mouse anti-growth-associated protein 43 (GAP43, Zymed Laboratories Inc.). Incubation with the biotin-conjugated secondary antibody was followed by incubation with HRP-conjugated streptavidin, after which visualization was performed with 3,3′-diaminnobenzidine tetrahydrochloride and H_2O_2 in Tris-HCl-buffered saline (pH 7.5). A rhodamine-conjugated secondary antibody was used in some experiments. Fluorescent images were acquired by employing a conventional microscope equipped with a CCD camera (IX71/Cool SNAP-HQ, Olympus, Melville, NY, USA).

Enzyme-Linked Immunoassay

Spinal cord lysates were used for this assay. The primary antibodies were rabbit anti-BDNF (Chemicon Inc.), rabbit anti-glial cell-derived neurotrophic factor (GDNF, Santa Cruz Biotechnology Inc.), and mouse anti-ciliary neurotrophic factor (CNTF, R&D Systems Inc.). Then incubation was done with a biotin-conjugated secondary antibody, followed by incubation with HRP-conjugated streptavidin, after which 0.25% 2,2′-azino bis(3-ethylbenzthiazoline-6-sulfonic acid) (Sigma) was added to each well. The plates were incubated at room temperature, and color development was assessed from the absorbance at 415 nm. Results were expressed as the absorbance per milligram of protein (23).

Morphology

Neural precursor cells were seeded at 1×10^6/well in DMEM containing N2 supplement and fibronectin, and then were cultured at 37°C under a 5% CO_2/95% atmosphere. Subsequently, the cell morphology was observed by phase-contrast microscopy.

Cell Proliferation

Neural precursor cells were seeded at 1×10^5 or 2×10^5/well. After plasma or serum (10%) obtained from 6-week-old C57BL/6 male mice was added to the cells, incubation was done for 24 or 48 h. Then the uptake of [^{3}H]thymidine [methyl, 1′,2′-^{3}H thymidine (1.40 TBq/mmol)] was determined, and the MTT assay was also performed.

RT-PCR Analysis

Quantitative real-time RT-PCR was performed to assess changes in the expression of p35 and neurofilament 68 (NF68). Total RNA was isolated by using ISOGENTM (Nippon Gene, Tokyo, Japan). cDNA was synthesized by using a first-strand cDNA synthesis kit for RT-PCR (AMV)$^+$ (Roche Applied Science) and was labeled with Sybr Green 1 dye. PCR was done by using a Light Cycler System (Roche Molecular Biochemicals) and the following pairs of primers: p35 (PCR product of 923 bp), CGGCACGGTGCTGTCCCTGTCT (forward) and TCACCGATCCAGGCCTAGGAG (reverse); NF68 (327 bp), TGGAGAATGAGCTGAGAAGC (forward) and TTCGTAGCCTCAATGGTCTC (reverse); and G3PDH (452 bp), ACCACAGTCCATGCCATCAC (forward) and TCCACCACCCTGTTGCTGTA (reverse).

Statistical Analysis

Statistical analysis was performed by using the Mann-Whitney *U*-test and $p < 0.05$ was taken to indicate significance.

RESULTS

Neural Precursor Cell Transplantation Into SCI Rats

Rats with neural precursor cell transplantation showed improved movement of their hindlimbs and had significantly higher BBB scores than the SCI control group (Fig. 1), whereas the control group only showed minimal functional recovery. The BBB value was 1.5 ± 0.7 in the SCI control group versus 3.20 ± 0.7 in the cell transplantation group on the 14th day ($p < 0.05$). Rats that received cells plus plasma showed significantly better motor function (6.0 ± 1.10) than SCI controls ($p < 0.05$) and rats with cell grafts alone ($p < 0.05$). Transplantation of cells with serum also promoted functional recovery with a BBB score of 3.5 ± 1.08 on the 14th day, which was significantly higher compared with that of the SCI control group but lower than that of the cells plus plasma group ($p < 0.05$).

Immunohistochemical Findings

Immunohistochemical examination showed that the grafted cells had differentiated into neurons (NF200) and astrocytes (GFAP) (Fig. 2). GAP43 has been used as an index of cell growth status (6,15), and GAP43-positive cells were extensively observed in the spinal cord after grafting of cells plus plasma.

Determination of Neurotrophic Factor

When the levels of BDNF, GDNF, and CNTF in normal spinal cord tissue were set as 100%, SCI reduced these levels to 51.4 ± 5.6%, 59.0 ± 4.0%, and 90.1 ± 5.1%, respectively (Fig. 3). A significant reduction was observed for BDNF and GDNF, but not CNTF. Cell transplantation increased the levels of these neurotrophic factors to 67.1 ± 5.6% for BDNF and 70.2 ± 12.1% for CNTF, while transplantation of cells plus plasma augmented the levels to 90.1 ± 6.6% and 92.2 ± 7.1%, respectively.

Morphological Changes In Vitro

Cells cultured without additives showed slight neurite outgrowth (Fig. 4) and the number of neurites per cell was 1.25 ± 0.6. In contrast, neurite outgrowth in multiple directions (5.25 ± 1.2) was observed in cultures of plasma-treated cells and a gel was formed. The number of neurites was also increased by incubation with serum (5.32 ± 1.3), but the culture medium did not become a gel.

Cell Proliferation

In order to examine whether incubation with plasma affected the proliferation of neural precursor cells, the [^{3}H]thymidine incorporation assay was performed. As shown in Table 1, there was significant suppression of [^{3}H]thymidine uptake by both plasma and serum. In particular, cells cultured with plasma had the lowest uptake of [^{3}H]thymidine and the radioactivity was about one third of the control level after 24 h of incubation with 10% plasma. On the other hand, few apoptotic cells were seen and the MTT assay revealed that there was no adverse influence on cell viability. In fact, cell growth was promoted to some extent. The total RNA

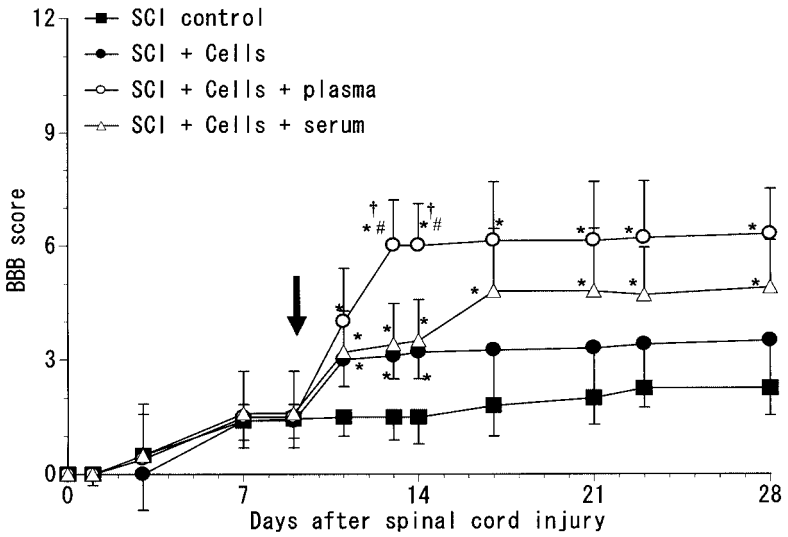

Figure 1. Functional recovery from SCI-induced motor dysfunction following neural precursor cell transplantation with plasma. On the 9th day after SCI (arrow), neural precursor cells (5×10^6) (5 μl) were transplanted at the center of the injured site. BBB scores were monitored before and after cell transplantation (mean ± SD, $n = 6$). *$p < 0.05$ versus the SCI control group, #$p < 0.05$ versus the cell graft group, and †$p < 0.05$ versus the cells plus serum group.

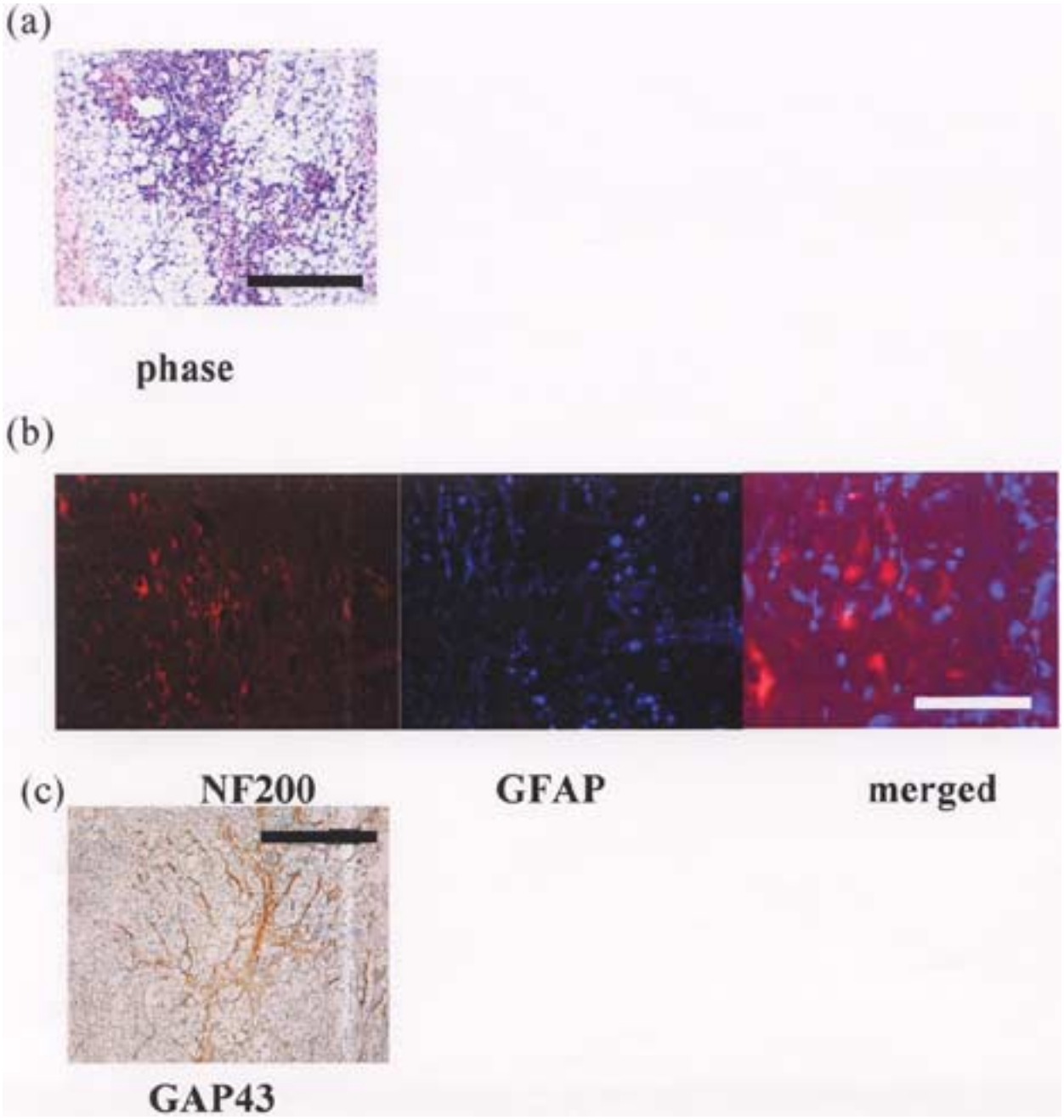

Figure 2. Immunohistochemical staining. The spinal cord from a rat grafted with cells plus plasma was removed at 7 days after cell transplantation for immunohistochemical staining. (a) H&E staining. Scale bar: 100 μm. (b) Cells expressing both NF200 and GFAP. Scale bar: 50 μm. (c) GAP43 expression is extensive. Scale bar: 100 μm.

content showed a slight, but significant increase as well (Table 2).

Neural Gene and Marker Expression In Vitro

Cells positive for βIII tubulin (neurons) and or GFAP (astrocytes) were observed when neural precursor cells that had differentiated from GFP-expressing ES were cultured with plasma (Fig. 5a). Expression of p35 was upregulated by 6.5-fold in the presence of plasma compared with that in cells treated without RA (Fig. 5b). Serum also promoted p35 gene expression by fivefold. Expression of NF68 was upregulated by over 20-fold

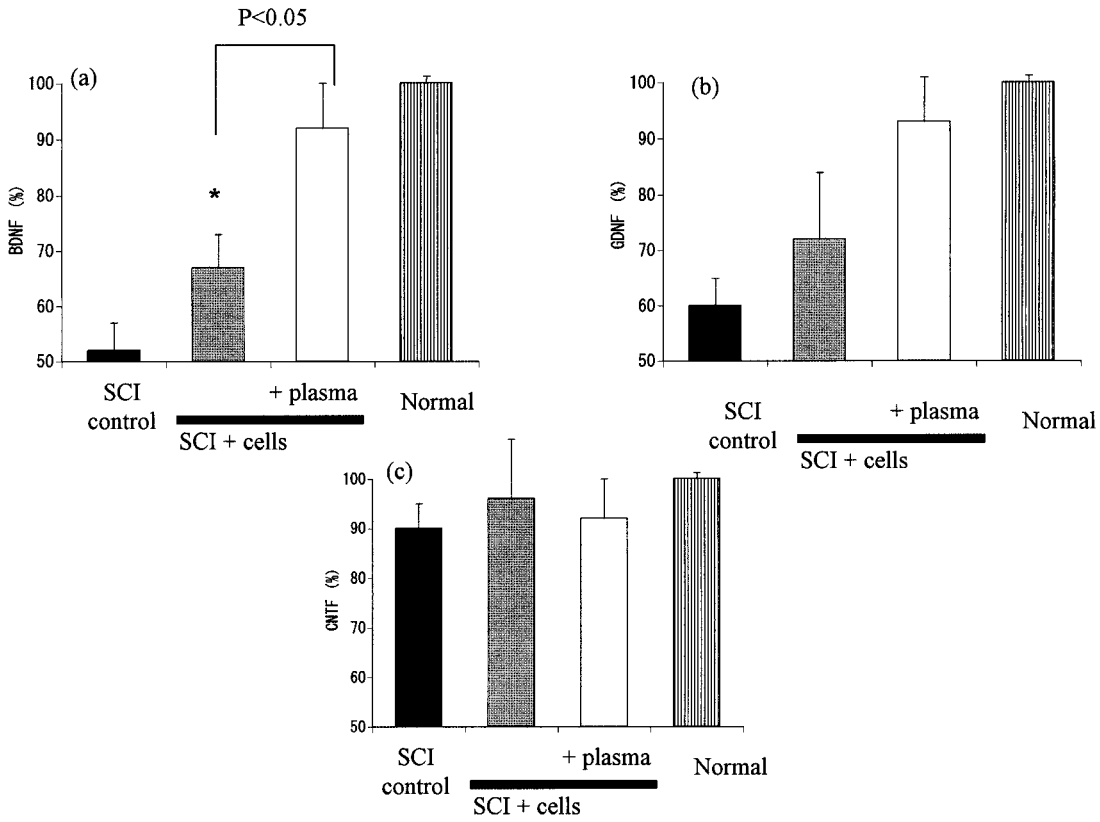

Figure 3. Neurotrophic factor. SCI reduced the levels of neurotrophic factors along with causing cell damage. Antibodies were used for quantification and spinal cords were removed at 7 days after cell transplantation. Values are expressed as a percentage of the control (mean ± SD, $n = 4$).

after addition of plasma to cultures and serum also increased its expression by 19-fold (Fig. 5c).

DISCUSSION

When rats with SCI were monitored using the BBB score, it was shown that cell transplantation promoted functional recovery and this was associated with expression of the axonal growth marker GAP43. It should be noted that transplantation of cells with plasma was more effective than the other methods tested.

Plasma and serum both contain many factors involved in the regulation of cell growth and survival. Chiaretti et al. (7) showed that neurotrophic factors (BDNF, GDNF, and NGF) exist in human plasma, although the levels are lower than in cerebrospinal fluid. Kim et al. (16) reported that the BDNF level in human plasma was 1352.6 pg/ml. Fibronectin, which is used for neural culture, is also found in plasma. In order to survive at the site of transplantation, grafted cells need various cytokines and growth factors. Thus, certain factors might have the potential to promote the cell survival, growth, and differentiation at the graft site. In the present study, plasma might have at least acted as a provider of various factors in the early stage after cell transplantation, in addition to being a scaffold.

Neurotrophic factors, such as BDNF and GDNF, showed an increase in the spinal cord after cell transplantation. The levels of these factors were higher in the cells plus plasma group, indicating that grafted cells had differentiated into neurons after transplantation and then started to secrete these factors. An increase of neurotrophic factors would promote regeneration further. The actions of plasma as a scaffold and provider of neurotrophic factors would have already finished at an earlier stage.

p35 is a neuron-specific activator of cyclin-dependent kinase-5 (cdk5), to which it binds and then triggers neurite sprouting and neurite differentiation in vivo (9). Neurofilaments are the predominant structure in large myelinated axons, where they are considered to function by maintaining the axonal caliber (11). When the cells were cultured with plasma, neurite outgrowth was observed along with increased expression of neural genes and neural markers. Although [^{3}H]thymidine incorpora-

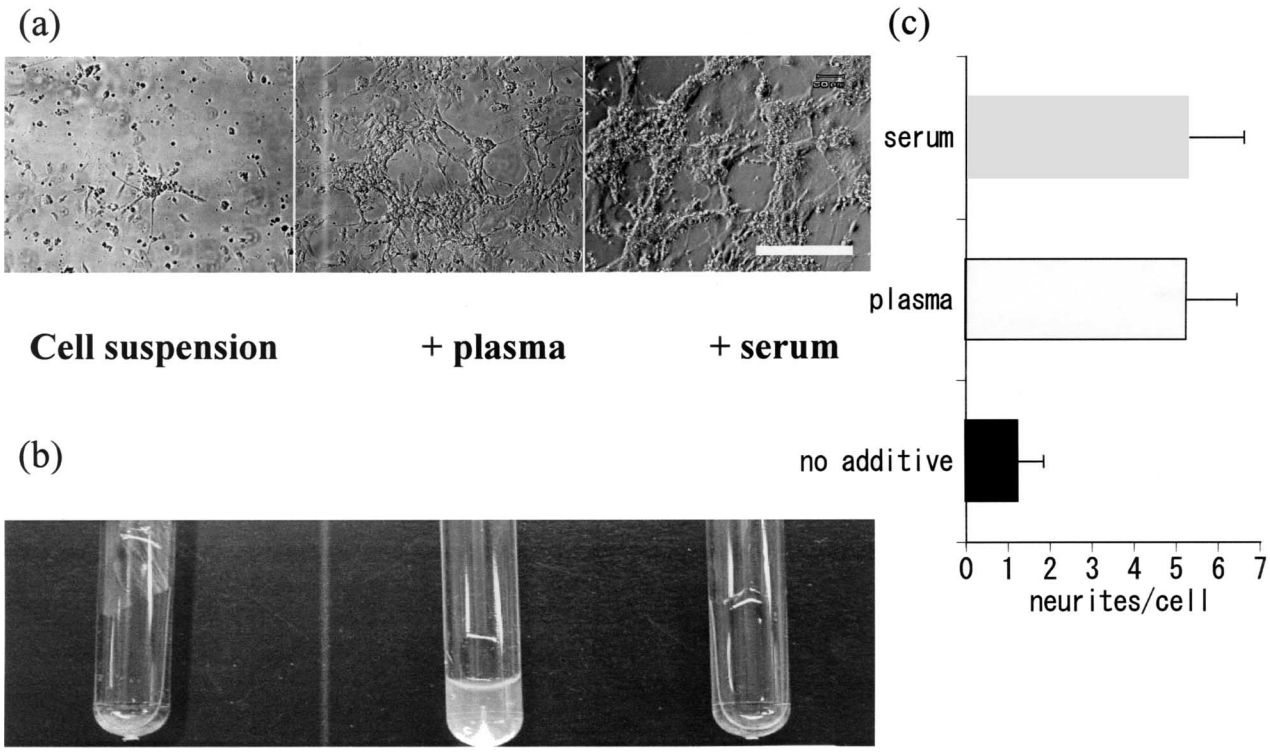

Figure 4. Neurite outgrowth and gel formation. (a) Plasma and serum promoted extensively neurite outgrowth. Neural precursor cells were incubated in the presence of plasma or serum (10%) for 24 h and then photographed. Scale bar: 100 µm. (b) A cell suspension with plasma (10%) forms a gel, while a suspension with serum (10%) does not. (c) Neural precursor cells were incubated with plasma or serum for 24 h and the number of neurites more than 25 µm long was counted from photographs taken under a phase-contrast microscope as shown in (a). Five separate experiments were carried out per group, and the number of neurites per cell was expressed as the mean ± SD.

Table 1. Effect of Plasma and Serum on the Thymidine Uptake Assay and MTT Assay

No. of Seeded Cells/ Treatment	Thymidine Uptake Assay (dpm)		MTT Assay (ABS)	
	24-h Incubation	48-h Incubation	24-h Incubation	48-h Incubation
1×10^5				
No additive	622.6 ± 46.4	237.8 ± 120.6	0.5872 ± 0.0162	0.5036 ± 0.0074
Plasma	176.3 ± 35.87*†	102.3 ± 58.06*‡	0.6343 ± 0.0162*	0.7177 ± 0.0527*
Serum	236.8 ± 37.0*	223.7 ± 60.6	0.6063 ± 0.0417	0.7166 ± 0.054*
2×10^5				
No additive	1134.3 ± 158.1	608.2 ± 319.1	0.9480 ± 0.0276	0.9671 ± 0.0231
Plasma	302.8 ± 104.8*	803.8 ± 199.3‡	1.0404 ± 0.1101§	0.9962 ± 0.0884
Serum	307.3 ± 89.4*	1290.7 ± 179.03*	0.8916 ± 0.1003§	0.9683 ± 0.1713

Cells were cultured in the presence or absence of serum or plasma (10%). Values are mean ± SD ($n = 3$).
*$p < 0.01$ versus RA alone—pulsed control cells (no additive).
†$p < 0.05$ versus serum-treated cells.
‡$p < 0.01$ versus serum-treated cells.
§$p < 0.05$ versus RA alone—pulsed control cells (no additive).

Table 2. Effect of Plasma and Serum on the Total RNA Content

Incubation Time/Treatment	RNA Content (mg/ml)
24 h	
No additive	0.332 ± 0.005
Plasma	0.382 ± 0.009*
Serum	0.412 ± 0.0045†
48 h	
No additive	0.381 ± 0.007
Plasma	0.575 ± 0.021*
Serum	0.656 ± 0.05*

Values are mean ± SD ($n = 3$). Cells (2×10^6/ml/well) were cultured in the presence or absence of serum or plasma. Total RNA was extracted and suspended in the same volume of DPEC-DW.
*$p < 0.05$ versus control cells (no additive).
†$p < 0.01$ versus control cells (no additive).

tion was significantly reduced by culture with plasma, it promoted cell growth and an increase of total RNA. Taken together, these results suggest that plasma increased cell growth slightly, but also promoted differentiation.

Unlike serum, plasma contains fibrinogen and forms a fibrin gel on exposure to air. Because culture was done under aerobic conditions, plasma formed a gel as shown in Figure 4. Although little air would be injected during cell transplantation with plasma under our experimental conditions, there is a possibility that fibrinogen would form a scaffold for the cells even if a gel did not develop. The superiority of plasma as a scaffold needs to be confirmed by more detailed comparison with serum, but plasma seems to be a good candidate scaffold for neural cell transplantation.

Scaffolds without cells have been reported to induce axonal regeneration, including substances such as algi-

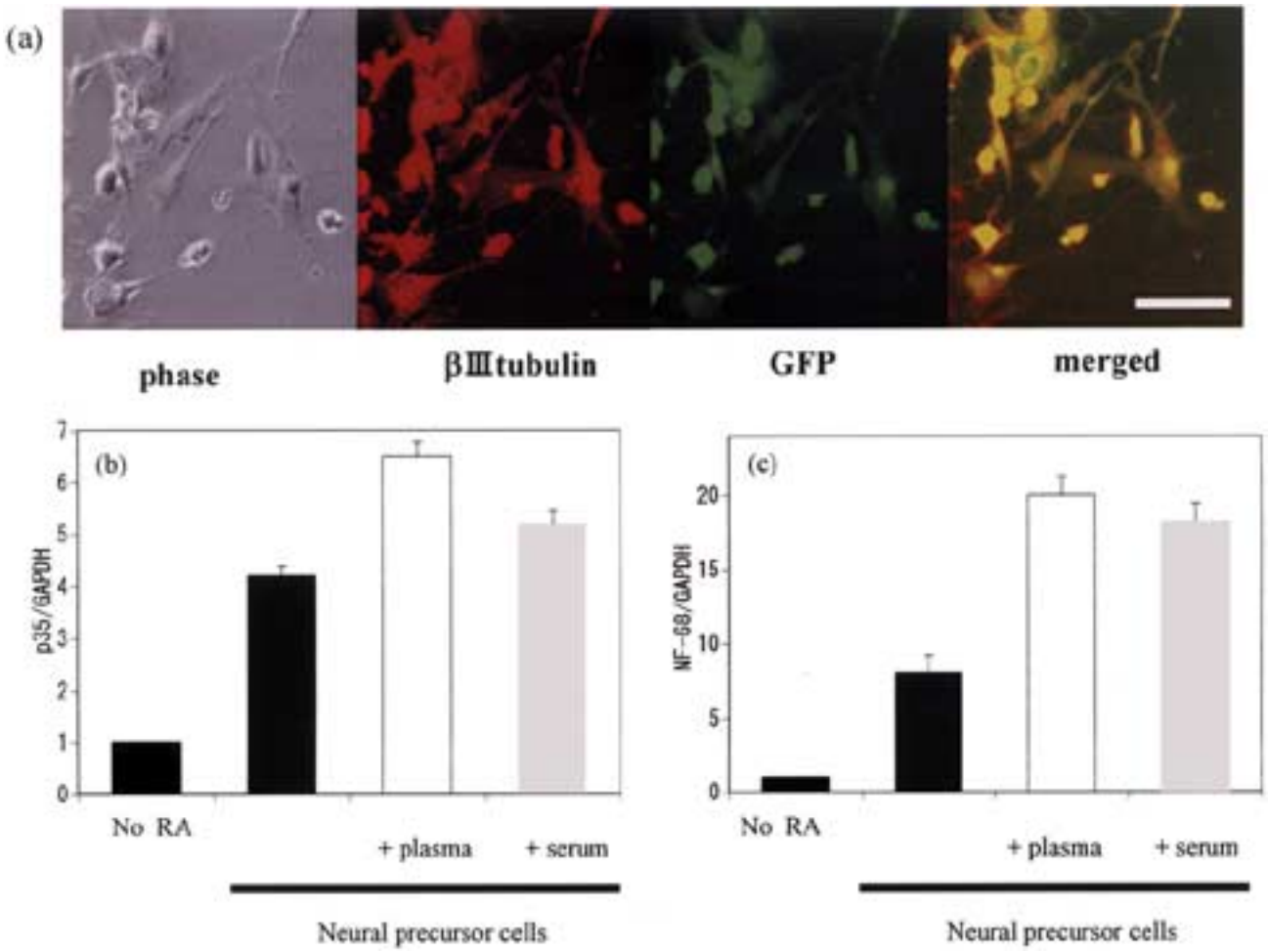

Figure 5. Promotion of neural marker and gene expression. Neural precursor cells derived from GFP-expressing ES were incubated with plasma or serum (10%) for 24 h. (a) Immunohistochemical staining. Scale bar: 50 μm. (b) Measurement of p35 and (c) 68kDa neurofilament (NF68) gene expression by RT-PCR. Values are shown as the mean ± SD ($n = 3$).

nate-based anisotropic hydrogel (21), freeze-dried agarose (22), and freeze-dried alginate cross-linked sponge (14). Bakshi et al. (3) studied a nonbiodegradable hydrogel, poly(2-hydroxyethylmethacrylate) (PHEMA), and they demonstrated that PHEMA combined with BDNF achieved sustained drug delivery to promote axonal growth and functional recovery after SCI. Jain et al. (13) used agarose gel as both a scaffold and a carrier of BDNF-releasing microtubules, and demonstrated that BDNF encouraged neurite outgrowth into the scaffold. Therefore, it might be important to combine cell therapy with the controlled delivery of neurotrophic factors by using a suitable scaffold.

Plasma is not only a scaffold but also provides the controlled release of various growth factors. In the future, the combination of plasma and other neurotrophic factors might lead to successful in vivo neural regeneration.

ACKNOWLEDGMENTS: This study was supported by grants from the Japanese Ministry of Education, Culture, Sports, Science, and Technology for Development of Stem Cell Therapy for Spinal Cord Injury and a Neural Stem Cell Bank.

REFERENCES

1. Bain, G.; Kitchens, D.; Yao, M.; Huettner, J. E.; Gottlieb, D. I. Embryonic stem cells express neuronal properties in vitro. Dev. Biol. 168:342–357; 1995.
2. Bain, G.; Ray, W. J.; Yao, M.; Gottlieb, D. I. Retinoic acid promotes neural and represses mesodermal gene expression in mouse embryonic stem cells in culture. Biochem. Biophys. Res. Commun. 223:691–694; 1996.
3. Bakshi, A.; Fisher, O.; Dagci, T.; Himes, B. T.; Fischer, I.; Lowman, A. Mechanically engineered hydrogel scaffolds for axonal growth and angiogenesis after transplantation in spinal cord injury. J. Neurosurg. Spine 1:322–329; 2004.
4. Basso, D. M.; Beattie, M. S.; Bresnahan, J. C. A sensitive and reliable locomotor rating scale for open field testing in rats. J. Neurotrauma 12:1–21; 1995.
5. Basso, D. M.; Beattie, M. S.; Bresnahan, J. C.; Anderson, D. K.; Faden, A. I.; Gruner, J. A.; Holford, T. R.; Hsu, C. Y.; Noble, L. J.; Nockels, R.; Perot, P. L.; Salzman, S. K.; Young, W. MASCIS evaluation of open field locomotor scores: Effects of experience and teamwork on reliability. Multicenter animal spinal cord injury study. J. Neurotrauma 13:343–359; 1996.
6. Cafferty, W. B. J.; Gardiner, N. J.; Das, P.; Qiu, J.; McMahon, S. B.; Thompson, S. W. N. Conditioning injury-induced spinal axon regeneration fails in interleukin-6 knock-out mice. J. Neurosci. 24:4432–4443; 2004.
7. Chiaretti, A.; Piastra, M.; Polidori, G.; DiRocco, C.; Caresta, E.; Antonelli, A.; Amendola, T.; Aloe, L. Correlation between neurotrophic factor expression and outcome of children with severe traumatic brain injury. Intensive Care Med. 29:1329–1338; 2003.
8. Chiba, S.; Sekino, H.; Suzuki, N. Induction of neuroepithelial stem cells from mouse embryonic stem cells treated with retinoic acid. Inflammation and regeneration (in Japanese). 22:543–549; 2002.
9. Desbarats, J.; Birge, R. B.; Mimouni-Rongy, M.; Weinstein, D. E.; Palerme, J. S.; Newell, M. K. Fas engagement induces neurite growth through ERK activation and p35 upregulation. Nat. Cell Biol. 5:118–125; 2003.
10. Friedman, J. A.; Windebank, A. J.; Moore, M. J.; Spinner, R. J.; Currier, B. L.; Yaszemski, M. J. Biodegradable polymer grafts for surgical repair of the injured spinal cord. Neurosurgery 51:742–751; 2002.
11. Hoffman, P. N.; Cleveland, D. W.; Griffin, J. W.; Landes, P. W.; Cowan, N. J.; Price, D. L. Neurofilament gene expression: A major determinant of axonal caliber. Proc. Natl. Acad. Sci. USA 84:3472–3476; 1987.
12. Hurtado, A.; Moon, L. D.; Maquet, V.; Blitd, B.; Jerome, R.; Oudega, M. Poly (D,L-lactic acid) macroporous guidance scaffolds seeded with Schwann cells genetically modified to secrete a bi-functional neurotrophin implanted in the completely transected adult rat thoracic spinal cord. Biomaterials 27:430–442; 2006.
13. Jain, A.; Kim, Y. T.; McKeon, R. J.; Bellamkonda, R. V. In situ gelling hydrogels for conformal repair of spinal cord defects, and local delivery of BDNF after spinal cord injury. Biomaterials 27:497–504; 2006.
14. Kataoka, K.; Suzuki, Y.; Kitada, M.; Hashimoto, T.; Chou, H.; Bai, H.; Ohta, M.; Wu, S.; Suzuki, K.; Ide, C. Alginate enhances elongation of early regenerating axons in spinal cord of young rats. Tissue Eng. 10:493–504; 2004.
15. Kim, D. H.; Jahng, T-A. Continuous brain-derived neurotrophic factor (BDNF) infusion after methylprednisolone treatment in severe spinal cord injury. J. Korean Med. Sci. 19:113–122; 2004.
16. Kim, D. J.; Roh, S.; Kim, Y.; Yoon, S. J.; Lee, H. K.; Han, C. S.; Kim, Y. K. High concentrations of plasma brain-derived neurotrophic factor in methamphetamine users. Neurosci. Lett. 388:112–115; 2005.
17. Kitagawa, A.; Nakayama, T.; Takenaga, M.; Matsumoto, K.; Tokura, Y.; Ohta, Y.; Ichinohe, M.; Yamaguchi, Y.; Suzuki, N.; Okano, H.; Igarashi, R. Lecithinized brain-derived neurotrophic factor (PC-BDNF) promoted the differentiation of embryonic stem cells both in vitro and in vivo. Biochem. Biophys. Res. Commun. 328:1051–1057; 2005.
18. McDonald, J. W.; Howard, M. J. Repairing the damaged spinal cord: a summary of our early success with embryonic stem cell transplantation and remyelination. Prog. Brain Res. 137:299–309; 2002.
19. Okada, Y.; Shimazaki, T.; Sobue, G.; Okano, H. Retinoic-acid-concentration-dependent acquisition of neural cell identity during in vitro differentiation of mouse embryonic stem cells. Dev. Biol. 275:124–142; 2004.
20. Park, K. I.; Teng, Y. D.; Snyder, E. Y. The injured brain interacts reciprocally with neural stem cells supported by scaffolds to reconstitute lost tissue. Nat. Biotechnol. 20: 1111–1117; 2002.
21. Prang, P.; Muller, R.; Eljaouhari, A.; Heckmann, K.; Kunz, W.; Weber, T.; Faber, C.; Vroemen, M.; Bogdahn, U.; Weidner, N. The promotion of oriented axonal regrowth in the injured spinal cord by alginate-based anisotropic capillary hydrogels. Biomaterials 27:3560–3569; 2006.
22. Stokols, S.; Tuszynski, M. H. Freeze-dried agarose scaffolds with uniaxial channels stimulate and guide linear axonal growth following spinal cord injury. Biomaterials 27: 443–451; 2006.
23. Takenaga, M.; Ohta, Y.; Tokura, Y.; Hamaguchi, A.; Nakamura, M.; Okano, H.; Igarashi, R. Lecithinized superoxide dismutase (PC-SOD) improved spinal cord injury-induced motor dysfunction through suppression of

oxidative stress and enhancement of neurotrophic factor production. J. Control Rel. 110:283–289; 2006.
24. Teng, Y. D.; Lavik, E. B.; Qu, X.; Park, K. I.; Ourednik, J.; Zurakowski, D.; Langer, R.; Snyder, E. Y. Functional recovery following traumatic spinal cord injury mediated by a unique polymer scaffold seeded with neural stem cells. Proc. Natl. Acad. Sci. USA 99:3024–3029; 2002.
25. Vroemen, M.; Aigner, L.; Winkler, J.; Weidner, N. Adult neural progenitor cell grafts survive after acute spinal cord injury and integrate along axonal pathways. Eur. J. Neurosci. 18:743–751; 2003.
26. Yamada, Y.; Ueda, M.; Naiki, T.; Takahashi, M.; Hata, K.; Nagasaka, T. Autogenous injectable bone for regeneration with mesenchymal stem cells and platelet-rich plasma: Tissue-engineered bone regeneration. Tissue Eng. 10:955–964; 2004.
27. Yang, J.; Wan, Y.; Yang, J.; Bei, J.; Wang, S. Plasma-treated, collagen-anchored polylactone: Its cell affinity evaluation under shear or shear-free conditions. J. Biomed. Mater. Res. 67:1139–1147; 2003.