Human Autologous Serum as a Substitute for Fetal Bovine Serum in *Human* Schwann Cell Culture

Parisa Goodarzi[1], Babak Arjmand[1,2], Seyed Hassan Emami-Razavi[1],

Masoud Soleimani[3], Abbas Khodadadi[4], Fereshteh Mohamadi-Jahani[1], and Hamid Reza Aghayan[1,2*]

[1] Clinical Grade Cell Manufacturing Facility, Brain and Spinal Cord Injury Research Center, Tehran University of Medical Sciences, Tehran, Iran

[2] Endocrinology and Metabolism Research Center, Endocrinology and Metabolism research Institute, Tehran University of Medical Sciences. Tehran, Iran

[3] Department of Hematology, Tarbiat Modares University, Tehran, Iran

[4] Iranian Tissue Bank, Research and Preparation Center, Tehran University of Medical Sciences, Tehran, Iran

Received: 23 Jan. 2013; Accepted: 19 May 2013

Abstract- Nowadays, cell -based and tissue engineered products have opened new horizons in treatment of incurable nervous system disorders. The number of studies on the role of Schwann cells (SC) in treating nervous disorders is higher than other cell types. Different protocols have been suggested for isolation and expansion of SC which most of them have used multiple growth factors, mitogens and fetal bovine sera (FBS) in culture medium. Because of potential hazards of animal-derived reagents, this study was designed to evaluate the effect of replacing FBS with human autologous serum (HAS) on SC's yield and culture parameters. Samples from 10 peripheral nerve biopsies were retrieved and processed under aseptic condition. The isolated cells cultured in FBS (1st group) or autologous serum (2nd group). After primary culture the cells were seeded at 10000 cell/cm^2 in a 12 wells cell culture plate for each group. At 100% confluency, the cell culture parameters (count, viability, purity and culture duration) of 2 groups were compared using paired t-test. The average donors' age was 35.80 (SD=13.35) and except for 1 sample the others cultured successfully. In first group, the averages of cell purity, viability and culture duration were 97% (SD=1.32), 97/33% (SD=1.22) and 11.77 (SD=2.58) days respectively. This parameters were 97.33% (SD=1.00), 97.55% (SD=1.33) and 10.33 days (SD=1.65) in second group. The difference of cell count, purity and viability were not significant between 2 groups (P>0.05). The cells of second group reached to 100% confluency in shorter period of time (P=0.03). The results of this study showed that autologous serum can be a good substitute for FBS in human SC culture. This can reduce the costs and improve the safety of cell product for clinical application.

© 2014 Tehran University of Medical Sciences. All rights reserved.

Acta Medica Iranica, 2014;52(4):241-245.

Keywords: Autologous serum; Cell culture; Cell transplantation; Fetal bovine serum; Schwann cell

Introduction

Nowadays, cell based therapies have introduced a novel perspective in treatment of chronic and hard-to-treat nervous system disorders. Among different cell types, Schwann cells have been used frequently for neuro-regeneration in various types of *in vitro* and pre-clinical studies. These studies elucidated potentials of these cells in treatment of cerebrospinal and peripheral nerve injuries (1-3). Based on previous *in vitro* and pre-clinical studies, a phase-1 clinical trial of autologous Schwann cell transplantation for chronic spinal cord injuries was performed by Brian and Spinal Cord Injury Research Center (BASIR- Tehran University of Medical Sciences). In this trial, autologous Schwann cells were retrieved from a small biopsy of patient's own Sural nerve and transplanted after *in vitro* expansion (4). In recent years cell-based research and therapies have grown dramatically in power and scope. Therefore, adhering to safety standards have become a matter of concern. Optimizing of laboratory environment, adhering to international standards, strict quality control and working in a GMP facility are several crucial issues to

Corresponding Author: HR. Aghayan
Clinical Grade Cell Manufacturing Facility,Brain and Spinal Cord Injury Research Center, Tehran University of Medical Sciences, Tehran, Iran
Tel: +98 21 61192856, Fax: +98 21 66938885, E-mail address: hr_aghayan@farabi.tums.ac.ir

improve safety and efficacy of cell products (5). Usually, due to limited amount of tissue biopsies which can be retrieved from patients, the isolated cells needed to be expanded *in vitro* before transplantation. Accordingly, Schwann cells should be expanded *in vitro* following retrieval from nerve biopsies to reach acceptable number and purity. Serum is one of the main components of culture solution which is routinely prepared from animal sources. Among different animal derived sera, FBS is used more frequent. It is expensive and has potential risk of viral, bacterial and prion transmission to the recipient. On the other hand, it can induce undesirable immune reactions against xenogeneic proteins (6).

There are some safer FBS for clinical grade cell manufacturing which is more expensive and produced from qualified animals under specific condition with stricter quality control (GMP facility, gamma irradiation and comprehensive laboratory studies). In this manner animals are selected from specific geographic area such as: Australia or New Zealand. It has been recommended that when applicable, animal origin free materials should be used for clinical grade cell manufacturing (5). Serum-free media and human blood derivatives are two alternatives for animal derived serum. Serum-free media needs to be supplemented by different reagents such as transferrin, albumin, insulin, and selenium. Based on the cell type, different growth factors should be added to the culture medium. These materials considerably increase the costs and may decrease product safety (7-11). Previous studies, have demonstrated the feasibility of Schwann cell cultivation using serum-free media. Lopez *et al.* reported a successful Schwann cells expansion in serum-free media supplemented by insulin, transferrin, selenium, and growth factors (12).

Human blood-derived products such as serum are cost-effective and safer substitute for FBS in clinical cell transplantation trials. In our phase 1 Schwann cell transplantation trial for spinal cord injury, FBS and different reagents were used for cell expansion in initial cases (7). Then we performed a feasibility study for replacing FBS with HAS and depleting some reagents. The encouraging results of our pilot study resulted in designing this complimentary research. The objective of current study was to compare impacts of HAS with FBS on viability, purity, and culture duration of human Schwann cells. On the other hand, to decrease the costs and improve product safety we decided to eliminate the number and dosage of previously used reagents.

Materials and Methods

In this experimental study; 10 peripheral nerve biopsies were harvested under aseptic condition from brain death donors or amputated limbs after obtaining informed consent. The retrieved tissue placed in an ice-cold RPMI medium containing Penicillin-Streptomycin (1x concentration), then it was sent to clean room facility for further processing. Except for few reagents, which will be pointed, all media and reagents were purchased from PAA Company (Austria). Tissue processing and cell culture were performed under a Class100 vertical laminar flow safety cabinet (EHRET, Germany) that was located in a class 1000 clean room facility (affiliated to BASIR). Initially, nerve fascicles trimmed and cleaned off adjacent connective tissue, then cut into small pieces and were digested by 0.125% collagenase (cell preparation grade collagenase- MP Biomedicals, USA). During digestion, the sample was incubated at 37°C and agitated every 10 minutes up to complete digestion (2:30 to 3 hours). Then, collagenase activity was inhibited by adding DMEM with 10% of serum and the suspension was centrifuged at 1500 rpm for 10 minutes (Eppendorf 5810R- Germany). Supernatant was discarded and cell pellet re-suspended in culture media, then filtered through a 100µm filter and centrifuged at 1200 rpm for 10 minutes. Cells were re-suspended again in culture media which included; DMEM, transferrin and insulin (10µg/ml, MP Biomedicals, USA). Afterward, the cells were counted and checked for purity and viability by hemocytometer and trypan blue staining. Then, they seeded into 25 cm^2 flasks with a density of 10000 cell/cm^2. According to our previous experiences, fibroblast depletion was performed by serum free culture for 48 hours (fasting method). After that FBS and HAS with concentration of 10% were added to group 1 and 2 respectively. The culture medium was renewed every 3 days and cells were sub-cultured at 80% confluency. The primary yield of Schwann cells might be affected by donor's variable, so the cells from first sub-culture were used for comparing the serum effects. At 80% confluency cells were harvested with the following procedure. After decanting culture media, cells were washed with PBS and then detached from culture flask with TrypLE™ solution (Invitrogen, USA). The harvested cells were washed twice with PBS, and then cell counting and viability assessment were done. Afterwards, the cells from each group were seeded at a density of 5000 cell/cm2 into a 12 wells plate and incubated at 37°c, 5% CO2 and 98% humidity (Galaxy 170 R CO2 incubator-

New Brunswick Scientific, USA). The plates were examined daily and culture media changed every 3 days. The cells were harvested at 100% confluency, and then cell count, viability and purity was assessed. Purity was evaluated by S100 antibody and DAPI staining with immunocytochemistry technique (13). To prepare autologous serum, 25 to 30 ml of whole blood was withdrawn at donation time. The blood was transferred to a sterile 50-ml conical tube without anticoagulants and kept at room temperature for 2 hours. After clot formation, the serum was transferred to a 15-ml conical tube and centrifuged twice at 3,000 rpm for 10 minutes. Two samples (at 5th and 8th sub-culture) from second group were sent to Department of Medical Genetics (Sarem women's hospital, Tehran, Iran) for cytogenetics analysis. Data were entered in SPSS-19 and t-test was used to find any significant difference (P-value<0.05).

Result

Ten nerve biopsies (10-14 cm lengths) were retrieved from 8 male and 2 female donors. The average donors' age was 35.80 (SD=13.35) and except for 1 sample the others cultured successfully. Figure 1 demonstrates Schwann cells' morphology and the results of S100 staining in each group. Cytogenetic reports at 5th sub-culture revealed an apparently normal karyotype and the samples of 8th sub-culture did not yield any metaphase chromosome (Figure 2).

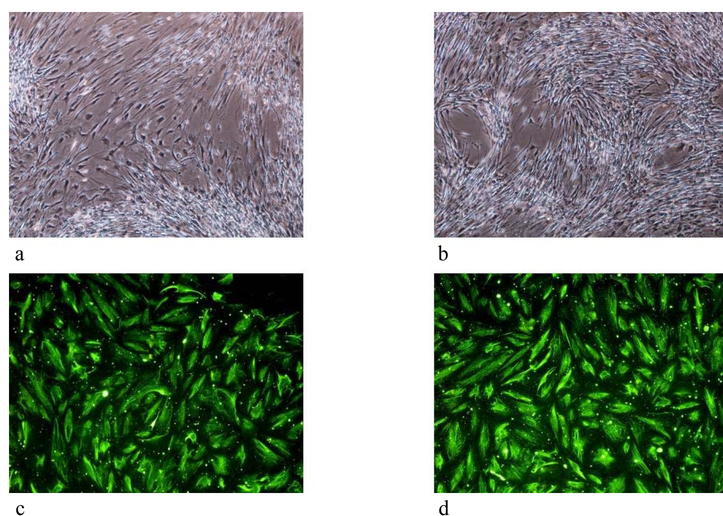

Figure 1. Schwann cells Morphology at first sub-culture under inverted microscope (40X) in first (a) and second group (b). Anti S100 staining (100X) performed at second sub-culture to determine Schwann cell purity in first (c) and second group (d).

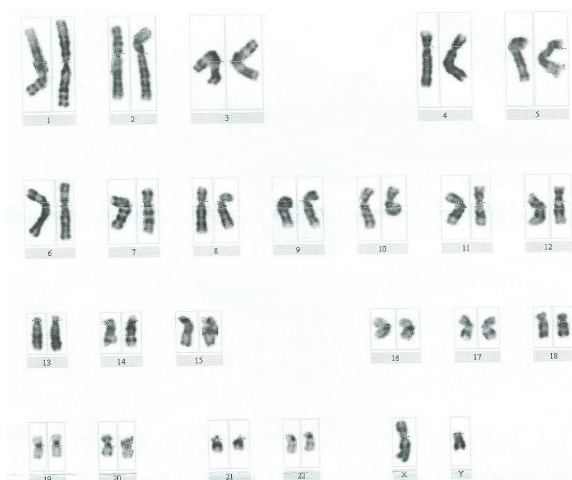

Figure 2. Cytogenetic report of Schwann cell at 5th passage from 16 years old male donor, revealed 46-XY normal karyotype

In first group, the averages of cell purity, viability and culture duration (at 100% confluency) were 97% (SD=1.32), 97.33% (SD=1.22) and 11.77 (SD=2.58) days respectively. This parameters were 97.33% (SD= 1.00), 97.55% (SD=1.33) and 10.33 days (SD=1.65) in second group. Table 1 shows donors' demographic information and culture variables of each group. The difference of cell count, purity and viability were not significant between 2 groups (P>0.05). The cells of second group reached to 100% confluency in shorter period of time (P=0.03). Average cell count was 71955.55 cells/cm^2 (SD=21295.13) in first group and 75388.88 cells/cm^2 (SD=19658.42) in second group (P> 0.05).

Table 1. Donors' demographic and cell culture variables in study groups

Donors' No.	Age / Sex	First group (FBS)				Second group (HAS)			
		Cells/cm^2	Viability (%)	Purity (%)	Culture duration (days)	Cells/cm^2	Viability (%)	Purity (%)	Culture duration (days)
1	26 /M	53600	8	96	12	54900	99	97	10
2	33 /F	67100	98	97	16	65400	98	98	14
3	53 /M	51700	95	96	14	52400	95	96	11
4	16 /M	83800	98	95	10	85900	98	96	8
5	29 /M	118600	99	96	8	117100	98	98	9
6	32 /M	52700	97	98	9	81500	97	97	10
7	40 /M	78600	97	98	11	75300	98	99	10
8	65 /M	-	-	-	-	-	-	-	-
9	36 /M	62900	96	98	14	64500	96	97	10
10	28 /F	78600	98	99	12	81500	99	98	11

Discussion

Schwann cells have been extensively investigated in various *in vitro* and pre-clinical studies. Several studies for treatment of peripheral nerve and spinal cord injuries have revealed promising results of Schwann cell transplantation for future clinical applications (3,7,14,15). Schwann cell cultivation dates back to 1940 and various methods have been suggested to achieve maximum cell count and purity (16). Most of published protocols utilized different growth factors, animal derived materials, and mitogens to achieve higher yield and purity. These protocols are not suitable for clinical applications and can increase the risk of disease transmission and may induce undesirable genomic changes (5). To eliminate fibroblast contamination some protocols have used growth inhibitors such as: cholera toxin, mitomycin, or cytosine arabinoside in early 24 to 48 hours of cultivation which could have some potential risks for patients (17-19). In the current study, except for one sample the others cultivated successfully. The exact reason of failure was not determined but it may be due to donor aging. Despite in previous studies Schwann cells cultivated from donors above 60 years, they reported considerably lower yield, viability and purity in comparison with younger donors. In contrast to our study, the mentioned studies used mitogens, growth factors, and coated culture plates that may be the reason for difference (16,20). Our fasting method resulted in

about 95% purity that is similar to previous studies (90% to 98%) that used fibroblast growth inhibitors or adhering factors such as collagen, laminin, and fibronectin (1,12,13,19). In our previous study the fasting method also resulted in 98% purity, therefore it seems to be a safe and cost-effective method for fibroblast depletion. Although, human serum and other blood derivatives (plasma, platelet lysate, PRP, and albumin) have been successfully used for human cell cultures (6,8-11), the application of HAS to culturing human Schwann cells was reported by the authors for the first time (21). Our previous study was a feasibility assessment and in current study the effects of animal and human serum were compared. Furthermore, some changes were made in previous culture protocol to omit progesterone, trypsin and ascorbic acid in order to increase safety and decrease final costs. For cell detachment, we replaced porcine trypsin with TrypLE™ solution that is free of animal-derived components. There was no significant difference between two groups in final cell count but, cells in second group reached at 100% confluency faster than first group. Similarly, previous studies have demonstrated that bone marrow and adipose-derived mesenchymal stem cells grow faster in human serum than FBS (6,10). In conclusion, this study revealed the role of HAS in shortening the time of cell culture with no undesirable effects on cell count, viability, and purity. On the other hand up to 5 sub-cultures no chromosomal changes were seen. The 8th sub-culture did not yield any metaphase chromosome that may be due to decline of cell replications. In our previous trials (4,15) Schwann cells

were transplanted at first sub-culture, so it is feasible and safe to increase the number of transplantable cells up to 5 sub-cultures. In the current study we established safer protocol for Schwann cell culture with lower costs that is suitable for clinical cell transplantation trials.

Acknowledgements

The authors would like to acknowledge Tehran University of Medical Sciences for supporting this project (Grant number; 21195). We also thank Mozhgan Hassanpour-Goudarzi, Maryam Kavousi, Bahman Delalat, Seyed Amirhossein Tavakoli, Seyed Kazem Hosseini and Hassan Bahrami-Nasab for their considerable assistance.

References

1. Casella GT, Bunge RP, Wood PM. Improved method for harvesting human Schwannn cells from mature peripheral nerve and expansion in vitro. Glia 1996;17(4):327-38.
2. Fehlings MG, Vawda R. Cellular treatments for spinal cord injury: the time is right forclinical trials. Neurotherapeutics 2011;8(4):704-20.
3. Oudega M, Xu XM. Schwann cell transplantation for repair of the adult spinal cord. J Neurotrauma 2006;23(3-4):453-67.
4. Saberi H, Moshayedi P, Aghayan HR, et al. Treatment of chronic thoracic spinal cord injury patients with autologous Schwann cell transplantation: an interim report on safety considerations and possible outcomes. Neurosci Lett 2008;443(1):46-50.
5. Arjmand B, Emami-Razavi SH, Larijani B, et al. The implementation of tissue banking experiences for setting up a cGMP cell manufacturing facility. Cell Tissue Bank 2012:13(4):587-96.
6. Shahdadfar A, Frønsdal K, Haug T, et al. In vitro expansion of human mesenchymal stem cells: choice of serum is a determinant of cell proliferation, differentiation, gene expression, and transcriptome stability. Stem Cells 2005;23(9):1357-66.
7. Aghayan HR, Arjmand B, Norouzi-Javidan A, et al. Clinical grade cultivation of human Schwann cell, by the using of human autologous serum instead of fetal bovine serum and without growth factors. Cell Tissue Bank 2012; 13(2):281-5.
8. Bieback K, Hecker A, Kocaömer A, et al. Human alternatives to fetal bovine serum for the expansion of mesenchymal stromal cells from bone marrow. Stem Cells 2009;27(9):2331-41.
9. Fekete N, Gadelorge M, Fürst D, et al. Platelet lysate from whole blood-derived pooled platelet concentrates and apheresis-derived platelet concentrates for the isolation and expansion of human bone marrow mesenchymal stromal cells: production process, content and identification of active components. Cytotherapy 2012;14(5):540-54.
10. Kocaoemer A, Kern S, Klueter H, et al. Human AB Serum and Thrombin Activated Platelet Rich Plasma Are Suitable Alternatives to Fetal Calf Serum for the Expansion of Mesenchymal Stem Cells from Adipose Tissue. Stem Cells 2007;25(5):1270-8.
11. Koellensperger E, von Heimburg D, Markowicz M, et al. Human Serum from Platelet-Poor Plasma for the Culture of Primary Human Preadipocytes. Stem Cells 2006;24(5):1218-25.
12. Lopez T, De Vries G. Isolation and serum-free culture of primary Schwann cells from human fetal peripheral nerve. Exp Neurol 1999;158(1):1-8.
13. Haastert K, Mauritz C, Chaturvedi S, et al. Human and rat adult Schwann cell cultures: fast and efficient enrichment and highly effective non-viral transfection protocol. Nat Protoc 2007;2(1):99-104.
14. Wiliams RR, Bunge MB. Schwann cell transplantation: A repair strategy for spinal cord injury? Prog Brain Res 2012;201(1):295-312.
15. Saberi H, Firouzi M, Habibi Z, et al. Safety of intramedullary Schwann cell transplantation for postrehabilitation spinal cord injuries: 2-year follow-up of 33 cases. J Neurosurg Spine 2011;15(5):515-25.
16. Moretto G, Kim SU, Shin DH, et al. Long-term cultures of human adult Schwann cells isolated from autopsy materials. Acta neuropatholo 1984;64(1):15-21.
17. Rutkowski JL, Tennekoon GI, McGillicuddy JE. Selective culture of mitotically active humanSchwann cells from adult sural nerves. Ann Neurol 1992;31(6):580-6.
18. Bunge MB, Wood PM. Realizing the maximum potential of Schwann cells to promote recovery from spinal cord injury. Handb Clin Neurol 2012;109(1):523-40.
19. Vleggeert-Lankamp CLAM, Pêgo AP, et al. Adhesion and proliferation of human Schwann cells on adhesive coatings. Biomaterials 2004;25(14):2741-51.
20. Boyer PJ, Tuite GF, Dauser RC, et al. Sources of Human Schwann Cells and the Influence of Donor Age. Exp Neurol 1994;130(1):53-5.
21. Arjmand B, Aghayan S, Shabanzadeh A, et al. Assessment of Schwann cell purity cultured in autologous human serum for spinal cord injury repair. KAUMS J(FEYZ) 2010;14(2):107-11.