

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/364841263>

# THE CONDITIONED MEDIUM-RAT BONE MARROW DERIVED MESSENCYHMAL STEM CELL (CM-RATBMMSC) CAN INDUCE THE DIFFERENTIATION ABILITY OF NEURAL STEM AND PROGENITOR CELLS (NPCS)

Article in *Asian Journal of Microbiology Biotechnology and Environmental Sciences* · December 2018

CITATIONS

4

READS

132

4 authors, including:



Ariyani Noviantari

National Research and Innovation Agency (BRIN)

29 PUBLICATIONS 30 CITATIONS

[SEE PROFILE](#)



Uly Alfi Nikmah

National Research and Innovation Agency

16 PUBLICATIONS 26 CITATIONS

[SEE PROFILE](#)



Ratih Rinendyaputri

National Research and Innovation Agency

33 PUBLICATIONS 106 CITATIONS

[SEE PROFILE](#)

## Asian Journal of Microbiology Biotechnology & Environmental Sciences Editorial Advisory Board

### Chief Editors

Dr. P.K.Wong : Professor, Deptt. of Biology, Chinese University of Hong Kong, Hong Kong  
and Dr. R.K.Trivedy, Ex. Prof. & Head, Deptt. of Environmental Sciences, University of Pune, Pune, India

### Associate Editors

Dr. Sadhana Sharma, Prof. & Head, Deptt. of Biochemistry, AIIMS, Patna, India,  
Dr. Namrata Sharma, AIIMS, New Delhi,  
Dr. Theesha Bahoun, Univ. of Mauritius, Mauritius,  
Dr. C.Visvanathan, AIT, Thailand and  
Dr. Azni H. Idris University of Putra Malaysia, Malaysia

### EDITORIAL ADVISORY BOARD

1. Dr. Hiroshi Tsuno, Japan 2. Dr. Jiro Koyama, Japan 3. Dr. Clem Adokpayi, Nigeria 4. Dr. C.D. Nwani, Nigeria 5. Dr. D.J. Lee, Taiwan 6. Dr. Zidan Abduldiem Bashir, Malaysia 7. Dr. S.M. Talebi, Iran 8. Dr. G. Khittoo, Mauritius 9. Dr. Rao Bhamidimari, New Zealand 10. Dr. Chee Kong Yap, Malaysia 11. Dr. Y. Anjaneyulu, U.S.A 12. Dr. A.H. Subratty, Mauritius 13. Dr. Sani Mashi, Nigeria 14. Dr. B. Leenanon, Thailand 15. Dr. Kawsar Ahmed, Bangladesh 16. Dr. (Ms.) Liqa Raschid, Sri Lanka 17. Dr. Jonas Contiero, Brazil 18. Dr. Shyam Bhagwant, Mauritius 19. Dr. K.P. Chong, Malaysia 20. Dr. J. Rotimi, Nigeria 21. Dr. Duangrat Inthorn, Thailand 22. Dr. Asgar Ali, Malaysia 23. Dr. S.A. Abbasi, Puducherry, India 24. Dr. W. Fuchs, Austria	25. Dr. V. Jirku, Czech Republic 26. Dr. Mark L.D. Lopez, Phillipines 27. Dr. G. Suresha, Saudi Arabia 28. Dr. Mohd. Nural Anwar, Bangladesh 29. Dr. Margaret Greenway, Australia 30. Dr. A.R. Ghosh, Burdwan, India 31. Dr. Anju Singh, Mumbai, India 32. Dr. Rashid Noor, Dhaka, Bangladesh 33. Dr. B.B. Ayade, Nigeria 34. Dr. Reda, Elabayoumi, Egypt 35. Dr. T. Koliopoulos, Greece 36. Dr. A.K. Kumaraguru, Madurai, India 37. Dr. Sesha Shrinivas Vutukuru, Hyderabad, India 38. Dr. A.K. Dixit, Mumbai, India 39. Prof. (Dr.) D.P. Singh, Lucknow, India 40. Dr. Hassan Moffadel, Sudan 41. Dr. U.S. Bagade, Mumbai, India 42. Dr. Okezie LA. Rouma, U.K. 43. Dr. Wilson S. Tisera, Kupang, Indonesia 44. Mr. Pavan Kumar Pindi, Mahabubnagar 45. Dr. Mohd. Adnan University of Ha'il, Saudi Arabia 46. Dr. M.H. Sayadi, Iran 47. Prof. Christian Paul P. Dela Cruz, Philippines 48. Dr. Rislika Putri Istanli, Indonesia
---	--

[Back to AJMBES Journal Details](#)

**ASIAN JOURNAL OF MICROBIOLOGY, BIOTECHNOLOGY AND  
ENVIRONMENTAL SCIENCES  
(VOL. 20, December Suppl., 2018)**

**CONTENTS**

S1-S5 ANALYSIS OF TISSUE RESPONSE AFTER SUBCUTANEOUS IMPLANTATION OF DEMINERALIZED FREEZE-DRIED BOVINE CORTICAL BONE MEMBRANE  
—DANANG PRIYO UTOMO, DAVID B. KAMADJAJA, FIKA RAHAYU, PRATIWI SOESILOWATI AND ACHMAD HARIJADI AND R. SOESANTO

S6-S9 BONE FORMATION IN RAT'S CALVARIAL DEFECT AFTER APPLICATION OF DEMINERALIZED FREEZE-DRIED BOVINE CORTICAL BONE MEMBRANE  
—GANENDRA ANUGRAHA, DAVID B. KAMADJAJA, R. SOESANTO AND ACHMAD HARIJADI

S10-S13 EFFECT OF ALPHA TOCOPHEROL SUPPLEMENTATION ON EXPRESSION OF PLATELET DERIVED GROWTH FACTOR IN HUMAN BONE MARROW MESENCHYMAL STEM CELLS (IN VITRO STUDY)  
—ISNOE SURYANDANU, ANDRA RIZQIawan, EDUWARD, PURWATI AND COEN PRAMONO

S14-S17 INFLAMMATORY RESPONSE IN RAT'S DORSUM AFTER SUBCUTANEOUS IMPLANTATION OF DEMINERALIZED FREEZE DRIED BOVINE CORTICAL BONE MEMBRANE  
—LULUK YULIANANI, DAVID B. KAMADJAJA, NURUL MAULIDAH AND PRATIWI SOESILOWATI

S18-S21 CELLULAR VIABILITY STUDY OF ASCORBIC ACID SUPPLEMENTATION IN RABBIT BONE MARROW MESENCHYMAL STEM CELL CULTURE (IN VITRO STUDY)  
—RISKA DIANA, ANDRA RIZQIawan, OKKY PRASETIO, PURWATI AND COEN PRAMONO

S22-S25 EFFECT OF ALPHA TOCOPHEROL SUPPLEMENTATION ON TUMOR NECROSIS FACTOR-  
ALPHA EXPRESSION IN HUMAN BONE MARROW MESENCHYMAL STEM CELL (IN VITRO STUDY)  
—WAYAN SUTRESNA YASA, NI PUTU MIRA SUMARTA, FREDY MARDIYANTORO, PURWATI AND COEN PRAMONO

S26-S29 CORRELATION BETWEEN THE FACIAL GROWTH PATTERN AND THE SMILE ARC IN MALE AND FEMALE PATIENTS IN THE DEPARTMENT OF ORTHODONTICS, SPECIALIST CLINIC OF DENTAL AND ORAL EDUCATION HOSPITAL, UNIVERSITAS AIRLANGGA  
—IRA ANGGAR KUSUMA, VISKASARI PINTOKO KALANJATI AND ABDURACHMAN

S30-S33 SMILE AND ORAL HEALTH  
—ACHMAD HENDRA HARTAWAN WAWAN, VISKASARI PINTOKO KALANJATI AND ABDURACHMAN

S34-S37 THE EFFECT OF VITAMIN C ON THE CEREBRAL CORTEX NEURONS OF RATS EXPOSED BY PRENATAL NOISE STRESS  
—LUH GDE EVAYANTI, VISKASARI PINTOKO KALANJATI AND ABDURACHMAN

S38-S42 STEM CELL FROM HUMAN EXFOLIATED DECIDUOUS TEETH (SHED) VERSUS HUMAN UMBILICAL CORD BLOOD MONONUCLEAR CELLS (CBMNC) TRANSPLANTATION IN NEURAL DAMAGE REDUCTION IN RAT MODEL OF CEREBRAL ISCHEMIA  
—YETTY RAMLI, SALIM HARRIS, AHMAD SULAIMAN AL WAHDY, NANDINI PHALITA LAKSMI, MOHAMMAD KURNIAWAN, MASAGUS ZAINURI, RATIH RINENDYAPUTRI AND PUSPUTA EKA WUYUNG

S43-S47 ELECTROSPUN FIBERS AS A WOUND DRESSING MATERIAL USING COMBINATION OF CELLULOSE ACETATE/COLLAGEN SEEDING STEM CELL  
—PURWATI, BAGUS SATRIO NURWITO AND HENDITA NUR MAULIDA

S48-S54 CLINICAL OUTCOME OF INTRAVENTRICULAR IMPLANTATION AUTOLOGOUS ADIPOSE DERIVED NEURAL PROGENITOR CELLS IN PARKINSON  
—PURWATI, ASRA AL FAUZI AND PRASTIYA I. GUNAWAN

S55-S61 THE CONDITIONED MEDIUM-RAT BONE MARROW DERIVED MESSENCYHMAL STEM CELL (CM-RATBMMSC) CAN INDUCE THE DIFFERENTIATION ABILITY OF NEURAL STEM AND PROGENITOR CELLS (NPCS)  
—RATIH RINENDYAPUTRI, ARIYANI NOVANTARI, VISTA BUDIARIATI, ULY ALFI NIKMAH AND MASAGUS ZAINURI

S62-S65 CYTOTOXICITY STUDY OF FREEZE-DRIED BOVINE BONE XENOGRAFT IN HUMAN BONE MARROW MESENCHYMAL STEM CELL  
—YENI D. LESMAYA, DAVID B. KAMADJAJA, WISNU M. WARDANA AND PURWATI

S66-S73 FIBRIN GLUE (FG) ENCAPSULATED LIMBAL MESENCHYMAL STEM CELLS (LMSCS) DECREASE BLEB FIBROSIS AREA AFTER TRABECULECTOMY THROUGH TGF- $\beta$  AND MMP-9 MODULATION  
—EVELYN KOMARATHI, YUYUN RINDIASTUTI, EDDYANTO, HELEN SUSILOWATI, ERYK HENDRIANTO, GATUT SUHENDRO AND FEDIK A. RANTAM

S74-S78 THE EFFECT OF EXTREME LOW FREQUENCY-PULSE ELECTROMAGNETIC FIELD EXPOSURE IN THE HEALING PROCESS OF SPRAGUE DAWLEY MOUSE DELAYED UNION FEMUR FRACTURE: STUDY OF RUST RADIOLOGY SCORE  
—ISMAIL HADISOEBROTO DILOGO, ANDIKA D. DJAJA AND RONALD H. TENDEAN

*(Continued on Inside Back Cover)*

**ASIAN JOURNAL OF MICROBIOLOGY, BIOTECHNOLOGY AND  
ENVIRONMENTAL SCIENCES**  
**(VOL. 20, December Suppl., 2018)**

**CONTENTS**

*(Contents Continued from Back Cover)*

S79-S82 OPTIMIZATION OF HEMATOPOIETIC STEM CELLS CULTURE SUPPLEMENTATION IN SERUM FREE MEDIUM: A REVIEW  
—YOSAFAT LAMBANG PRASETYADI, BERYL ALODIA, RADIANA DHEWAYANI ANTARIANTO AND IMELDA ROSALYN SIANIPAR

S83-S87 THE COMPUTATIONAL STUDY REVEALS THE IMMUNOMODULATORY AND ANTIMICROBIAL EFFECTS OF VERNONIA AMYGDALINA EXTRACT  
—LIDWINA TRI KRISTANTI SETIAWAN, JUSAK NUGRAHA, WAHYU DEWI TAMAYANTI AND DIDIK HUSWO UTOMO

S88-S92 EFFECT OF FREEZE-DRIED BOVINE BONE XENOGRAFT ON TUMOR NECROSIS FACTOR- ALPHA SECRETION IN HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS  
—AHMAD K.M. HUMIDAT, DAVID B. KAMADJAJA, CHRIST BIANTO, ANINDITA Z. RASYIDA, PURWATI AND ACHMAD HARIJADI

S93-S96 BIODEGRADATION STUDY OF DEMINERALIZED FREEZE DRIED BOVINE CORTICAL BONE MEMBRANE AFTER SUBCUTANEOUS IMPLANTATION IN RAT'S DORSUM  
—ASTRID B.U. PURBA, DAVID B. KAMADJAJA, AKHSANAL FAUZI, IKHRAM KHARIS, ANDRA RIZQIawan AND COEN PRAMONO

S97-S100 BIOCOMPATIBILITY STUDY OF DEMINERALIZED FREEZE DRIED CORTICAL BONE MEMBRANE  
—NICCO MARANTSON, DAVID B. KAMADJAJA, R. HANDITO SATRIYO SUSILAWAN AND ENY WAHYUNI

S101-S106 THE COMBINATION OF PLATELET RICH PLASMA WITH SKIN NEEDLING OR SUBCISION FOR POSTACNE SCARING: A SERIAL CASE STUDY  
—ENDRA YUSTIN, ARI KUSUMAWARDANI, SUCI WIDHIATI AND INDAH JULIANTO

S107-S112 OSTEOPGENIC POTENTIAL OF BOVINE CORTICAL BONE MEMBRANE AFTER IMPLANTATION IN RAT'S CALVARIA CRITICAL-SIZED DEFECTS  
—ADI RIZAL SOLEH, DAVID B. KAMADJAJA, NUGROHO SETYAWAN AND NI PUTU MIRA SUMARTHA

S113-S116 EARLY HEALING PHASE IN RAT'S CALVARIAL CRITICAL-SIZE DEFECT AFTER IMPLANTATION OF BOVINE CORTICAL MEMBRANE  
—EKO WICAKSONO SUBAGIO, DAVID B. KAMADJAJA, DIANIZA AFIKANINGTYAS, ZEFRY ZAINAL ABIDIN, PRATIWI SOESILOWATI AND COEN PRAMONO

S117-S120 THE EFFECT OF ALPHA TOCOPHEROL SUPPLEMENTATION ON THE EXPRESSION OF VASCULAR ENDOTHELIAL GROWTH FACTOR ON HUMAN BONE MARROW MESENCHYMAL STEM CELL (IN VITRO STUDY)  
—IVAN TANTRA, NI PUTU MIRA, LISKA BARUS, REZA PAHLEVI, PURWATI AND COEN PRAMONO

S121-S123 EFFECT OF DIFFERENT CONCENTRATION OF ALPHA TOCOPHEROL SUPPLEMENTATION ON HUMAN BONE MARROW MESENCHYMAL STEM CELL VIABILITY (IN VITRO STUDY)  
—JEFRY WAHYUDI S., NI PUTU MIRA SUMARTA, GATOT BAYDOWI AND COEN PRAMONO D.

S124-S126 EFFECT OF ALPHA TOCOPHEROL SUPPLEMENTATION ON FGF EXPRESSION IN HUMAN BONE MARROW MESENCHYMAL STEM CELLS (IN VITRO STUDY)  
—REZA AL FESSI, ANDRA RIZQIawan, ELISSA CHAIRANI, ELLEN SATYA PRATIWI, PURWATI AND COEN PRAMONO

S127-S130 THE EFFECT ON FREEZE-DRIED BOVINE BONE XENOGRAFT ON THE SECRETION OF INTERLEUKIN-1 IN HUMAN PERIPHERAL BLOOD MONONUCLEAR CELLS CULTURE  
—ARDIAN JAYAKUSUMA AMRAN, DAVID B KAMADJAJA, I KOMANG SUTRIADI TEGUH KELANA PUTRA NUGRAHA, PRADIKA ADHIATSA AND PURWATI

S131-S133 EFFECT OF FREEZE DRIED BOVINE BONE XENOGRAFT PARTICLES ON RUNX-2 EXPRESSION IN HUMAN BONE MARROW MESENCHYMAL STEM CELL  
—MAHARDIKA YOGA P, DAVID B KAMADJAJA, BAGUS T. UTOMO, PURWATI AND ACHMAD HARIJADI

S134-S137 EXPRESSION OF TRANSFORMING GROWTH FACTOR- $\beta$ 1 AND OSTEOCALCIN IN RAT CALVARIA DEFECT AFTER APPLICATION OF BOVINE CORTICAL BONE MEMBRANE  
—NURUL FARIZAH, DAVID B KAMADJAJA, ADELINE WIBOWO, PRATIWI SOESILOWATI AND ACHMAD HARIJADI

## THE CONDITIONED MEDIUM-RAT BONE MARROW DERIVED MESSENCYHMAL STEM CELL (CM-RATBMMSC) CAN INDUCE THE DIFFERENTIATION ABILITY OF NEURAL STEM AND PROGENITOR CELLS (NPCS)

RATIH RINENDYAPUTRI<sup>\*1</sup>, ARIYANI NOVIANTARI<sup>1</sup>, VISTA BUDIARIATI<sup>2</sup>,  
ULY ALFI NIKMAH<sup>1</sup> AND MASAGUS ZAINURI<sup>1</sup>

<sup>1</sup>Center For Research and Development of Biomedical and Basic Health Technology, National Institute Health Research and Development, Ministry of Health Republic of Indonesia

<sup>2</sup>Department of Anatomy, Physiology and Pharmacology, Faculty of Veterinary Medicine, Bogor Agricultural University, West Java, Indonesia

(Received 25 September, 2018; accepted 15 November, 2018)

**Key word :** Conditioned medium /CM, mesenchymal stem cell/MSC, Rat bone marrow derived MSCs, Neural stem and progenitor cells/NPCs

**Abstract**— Mesenchymal stem cell (MSC) and conditioned medium-MSC (CM-MSC) are potential therapeutic agents for the treatment of neurogenerative diseases. Conditioned medium-mesenchymal stem cell (CM-MSC) contained growth factors that can protect neuronal cells from cell death and induce differentiation of neural stem/progenitor cells (NPCs). **Aims** : To investigate the capability of conditioned medium rat bone marrow-derived mesenchymal stem cells (CM-rat BMMSC) induces in vitro differentiation of NPCs into astrocytes and neuron. **Methods** : We assessed the effects of CM-ratBMMSC to induce differentiation of NPCs by culturing cells in serum-free medium DMEM/F12 (FM), CM 100%, CM 50% and neuro basal medium with supplement (NM) for 4 days. We examined the effects on differentiation by assessing the expression of A2B5, PSANCAM, and beta-tubulin by flowcytometry, and the expressions of glial fibrillary acidicprotein (GFAP) and neuronal nuclei (NeuN) by immunocytochemistry (ICC). **Results**: CM-ratBMMSC induced differentiation of NPC to astrocytes and neurons which indicated by the expression of GFAP, beta-tubulin and NeuN. Our findings showed that growth factors or neurotrophic factors such as basic fibroblast growth factor (bFGF) and nerve growth factor (NGF) in CM-ratBMMSC induces NPCs differentiations that may happen via phosphoinositide 3-kinases (PI3K)/protein or kinase B (Akt) signalling pathway. **Conclusions** : Growth and neurotrophic factors from CM-rat BMMSC such as bFGF and NGF increased the differentiation ability of NPCs into astrocytes (GFAP) and neurons (NeuN).

### INTRODUCTION

Mesenchymal stem cell (MSC) is multipotent adult stem cells and plastic adherent stromal cells which can differentiate into bone, cartilage and adipose tissue. (Tondreau *et al.*, 2004) It can be obtained from dental pulp, bone marrow and wharthon's jelly. (Inoue *et al.*, 2013)(Tondreau *et al.*, 2004; Zhang *et al.*, 2017) Mesenchymal stem cell (MSC) has a promising prospect as a regenerative medicine for neurodegenerative diseases. These cells can transdifferentiate into mesodermal lineage such as neural lineage (Ahmedy *et al.*, 2015). Moreover, previous studies reported that MSC has an ability to

differentiate into neuronal cells and also can secrete growth factors or cytokines for neurological function improvement. (Tondreau *et al.*, 2004; Ahmedy *et al.*, 2015; Kurozumi *et al.*, 2005) MSC secretes neurotrophic and anti inflammation factor so that it reduced ischemic damage in stroke and spinal injury (SCI) in animal model. (Kurozumi *et al.*, 2005);(Cantinieaux *et al.*, 2013) Currently, it is believed that not only the cells can be used as therapeutic agents but also the conditioned medium (CM) derived from MSC. (Pawitan, 2014)

Conditioned medium (CM) is a medium from specific cells culture such as stem cell culture that contained factors/molecules secreted by the cells to

\*Corresponding author's email: ratihr79@yahoo.com

the extracellular space. (Vizoso *et al.*, 2017; Pawitan 2014) Conditioned medium contains soluble factors, such as cytokines interleukin 10 (IL10) and tumor necrosis factor (TNF); growth factors such nerve growth factor (NGF), brain derived nerve factor (BDNF), insulin growth factor (IGF); and basic-fibroblast growth factor (bFGF) which plays a role for neuroprotection and neurogenesis. (Woodbury and Ikezu, 2014; Nakajima *et al.*, 2017); (Nguyen *et al.*, 2010); (Sun *et al.*, 2018). The advantages of CM application compared to the cells itself are it is safe, it can be mass produced, and it is more easy to transport and package (Pawitan, 2014)

The preclinical studies of MSC and CM-MSC for neurodegenerative diseases such as stroke and SCI in animal model had been successfully done, but it is important to explore various factors that promote and initiate neuronal cell regeneration and prove whether CM MSC can maintain and induce NPCs differentiation or not. Because of these reasons, we did in vitro studies to investigate whether CM-rat BMMSC and its growth factor contents can maintain and induce differentiation of NPCs into neuronal cells.

## METHODS

### Isolation and Culture of rat BMMSC

Wistar rats (12-week-old males) were used. Bone marrow was collected from femurs and tibias. Bone marrow was extruded by inserting a 22-gauge needle into the shaft of the bone and flushed out with 1 mL complete culture medium consist of DMEM/F12+ glutamax (Gibco), 10% fetal bovine serum (FBS; Gibco) and 1% antibiotic-antimycotic (Gibco 15,240,062, Carlsbad, USA) incubated at 37°C with 5% CO<sub>2</sub>. At 24 h after initial plating, the cells were washed twice with phosphate-buffered saline (PBS) to remove non adherent cells. The next day, medium must be replaced and every 2 days the culture medium was changed for 7 days. First passage was done with 0,05% trypsin EDTA (Gibco) and cells replated (6x10<sup>5</sup>cells/mL) in 25TC flask.

### Collection the CM-rat BMMSC

Conditioned Medium-rat BMMSC collected from 2<sup>nd</sup> passage of rat BMMC. After 2 days culture with complete culture medium, the flasks were washed twice with PBS and the medium was changed with 2 ml serum-free culture medium (FM; consisting of

DMEM/F12 + glutamax and 1% antibiotic-antimycotic) for 24 hours. CM was collected and centrifuged at 1500 rpm for 5 minutes continued at 3000 rpm for 3 minutes. The CM was filtered with 0,22µm size of pore and further used for NPCs culture.

### Isolation and Culture of Neural Stem and Progenitor Cells (NPCs)

NPCs were isolated from Wistar rat embryos on embryonic day 17 (E17). Pregnant female Wistar rat was euthanized by intraperitoneal injection of ketamine xylazine cocktail (91 mg/kg ketamine + 9.1 mg/kg xylazine) 0.1mL/100 g body weight. Lower abdomen was sprayed with 70% alcohol. The uterus was exposed by medial cutting through the skin. All fetuses were removed from the uterus then placed in sterile dissection solution (HBSS containing 0.3% glucose). Whole brains were isolated from the foetuses and dissected into small pieces then rinsed 3 times with dissection medium. Dissected tissues were centrifuged at 300 x g for 2 minutes then supernatant was discarded. Digestion process of brain tissues to obtain the cells were done using Neural Tissue Dissociation Kit (T) (Miltenyi Biotec). All the procedures were based on manufacturer protocols. Resuspended cells were seeded in coated dishes (1% poly D-lysine (PDL) and 1% gelatin for 24 hours) at cell density of 5x10<sup>4</sup> cells/cm<sup>2</sup> in 24 wells plates. After 4 days culture, cells were characterized by immunohistochemistry and flowcytometry.

The medium used in this study was neurobasal medium (NM) consisted of MACS® neuro medium (Miltenyi Biotec) containing 2% MACS NeuroBrew-21(Miltenyi Biotec), 1% antibiotic antimycotics (100x) (GibcoTM) and 1% GlutaMax® (GibcoTM). Conditioned medium 100% (CM100%) was CM-rat BMMSC only, while CM50% was CM-rat BMMSC and NM (1:1).

### Flowcytometry

In this study, the rat BMMSCs were characterized with CD29<sup>+</sup>-FITC,CD90<sup>+</sup>-APC and CD34-PE (Biolegend). The NPCs were characterized using A2B5<sup>-</sup>-APC, PSANCAM<sup>+</sup> -PE-A (Miltenyi Biotec) and beta tubulin-FITC (Biolegend) markers by flowcytometry at fourth day of culture. Staining process was done according to the instruction of staining kit. Flowcytometry process and analysis were done using BD Accuri™ C6 Plus flowcytometer.

### Immunocytochemistry

Cells were characterized after 4 days of culture by immunocytochemistry with GFAP and NeuN markers. Medium was discarded from the wells then, the cells were washed twice with PBS. Fixation of the cells was done with 4% paraformaldehyde (PFA) for 15 minutes then were washed with PBS three times for 5 minutes each continued with blocking steps. Blocking steps consisted of (i) blocking of endogenous peroxidase with 3%  $H_2O_2$  in methanol (Merck K38122297) for 15 minutes, (ii) blocking of nonspecific background staining with background sniper (Starr Trek Universal HRP Detection Kit Biocare®) for 15 minutes. After blocking steps, samples were washed in PBS 3x for 5 minutes each then were incubated overnight with primary antibody, GFAP (Santa Cruz sc) and NeuN (Abcam ab104225) at 4°C. For the next process, samples were washed in PBS 3x for 5 minutes each and further incubated with secondary HRP-conjugated antibody (Trekkie Universal Link, Starr Trek Universal HRP Detection Kit Biocare®) for 15 minutes and were washed in PBS for 5 minutes followed by incubation with Trek-Avidin-HRP (Starr Trek Universal HRP Detection Kit Biocare®) for 15 minutes. Samples then were washed in PBS for 5 minutes and incubated in chromogen substrate diamino benzidine (DAB)

with the addition of substrate buffer (Starr Trek Universal HRP Detection Kit Biocare®) for 1-2 minutes and were washed with mili-q water for 10 minutes. Counterstaining was done with Hematoxylin Mayer (Biocare3570) for 1-2 minutes and final steps were washed the samples with miliq water for 5 minutes. Positive and negative control was included in every staining protocol.

### ELISA for CM-rat BMMSC

The concentration of bFGF dan NGF from CM-rat BM MSC were measured by Elabscience ELISA kit and inspection method adjusted according to the manufacturer's instructions.

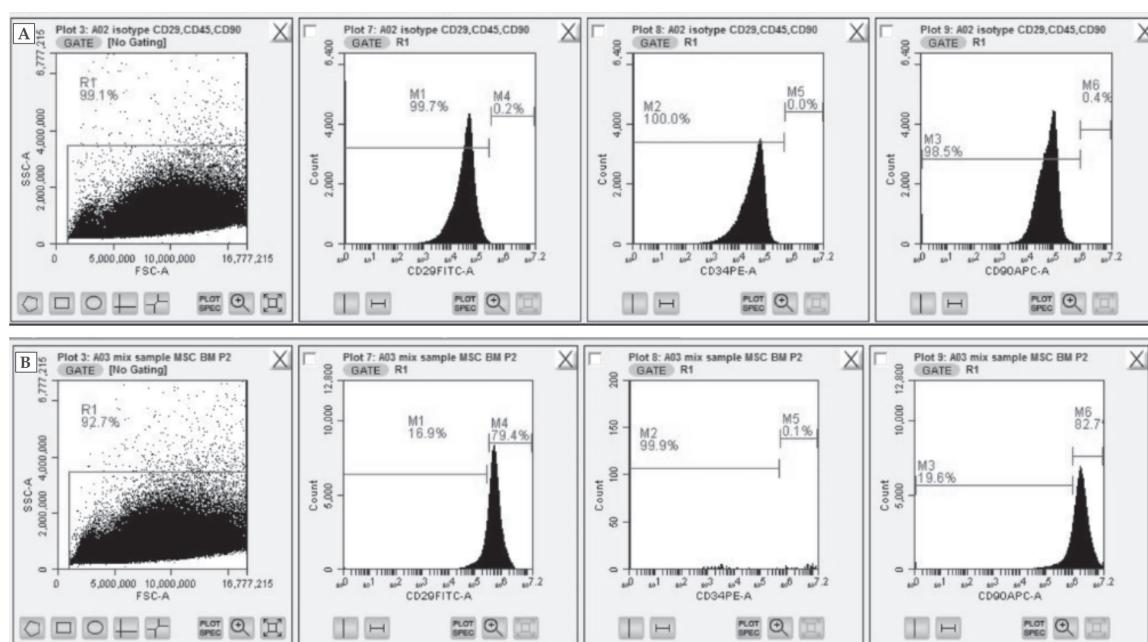
### Statistic

Data expressed as means $\pm$ SD. P values were calculated using one-way ANOVA analysis.

## RESULTS

The characteristics rat BMMSCs were analyzed by flowcytometry to determine the expression of cell surface markers. The results showed highly (e $\geq$  70%) expressed positive marker MSC (CD90 and CD29) and less than 1% of CD34 (haematopoietic specific marker) in the 2<sup>nd</sup> passage (Figure 1).

The characteristics of CM-rat BMMSC were



**Fig. 1.** Flow cytometry of CD29<sup>+</sup>CD90<sup>+</sup>CD34<sup>-</sup> rat BMMSCs after 5 days culture at passage 2 in which their conditioned medium (CM) will be collected and utilized to induce NPCA. Isotype of CD29, CD90 and CD34 < 0.5%. B. Percentage of CD29 and CD90 > 70% .

analyzed by ELISA to determine the secretion of growth factors such as bFGF and NGF. The result showed that CM100 from the 2<sup>nd</sup> passage of rat BMMSC contained both of growth factor as shown in Table 1.

**Table 1.** Concentration of bFGF and NGF of CM-rat BMMSC

Sample	bFGF (pg/mL)	NGF (pg/mL)
FM	0	0
CM100	625,823	16,83
NM	0	12,5

Characteristics of NPCs were analyzed by flowcytometry to determine the expression of cell surface markers after 4 days of culture with CM50 and NM with different extracellular matrix. There were less than 50% double expression of PSANCAM and beta-tubulin while the expression of PSANCAM were more than 50% when the cells cultured in CM50 on plates coated with PDL (Figure 2). However, culturing cells in NM on plates coated with gelatin showed that double expression of PSANCAM and beta-tubulin were less than 50% as similar as the percentage of the expression of PSANCAM only (Figure 3).

Characterizations by immunocytochemistry with GFAP and Neu-N markers were done after 4 days culture in 37°C and 5% CO<sub>2</sub>. Cells that grew in CM100 were qualitatively positive for NeuN markers as shown in Figure 4.

Quantification of positive expressions of NeuN and GFAP markers showed high percentage in all groups (FM, CM100, and NM) with significant differences between free-serum medium and NM or CM100 medium (Table 2).

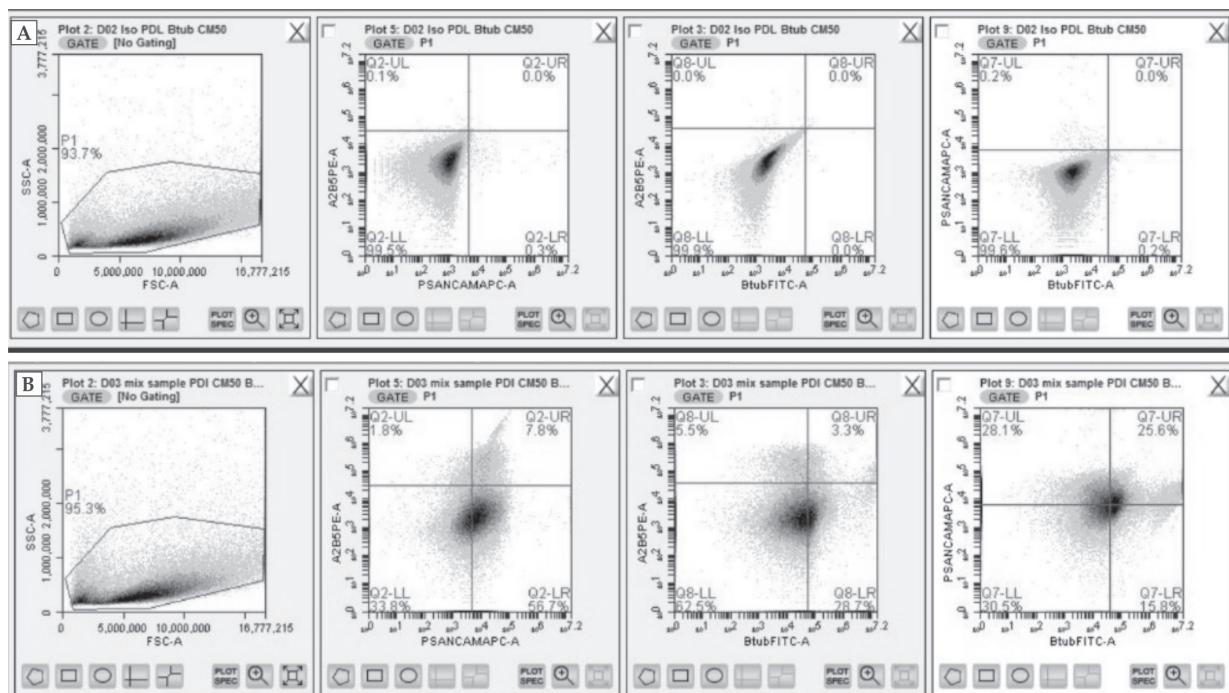
**Table 2.** Percentage of NeuN and GFAP of NPCs

Medium -coating	% NeuN	% GFAP
FM- PDL	43.88 ±2.5 <sup>a,b,c</sup>	28,66 ±2.7 <sup>a,b,c</sup>
NM – PDL	63.28 ±2.57 <sup>a,b</sup>	67.79 ±3.84 <sup>a,b</sup>
CM100 - PDL	61.16 ±4.11 <sup>a,c</sup>	68.77 ±4.37 <sup>a,c</sup>

\* a, b, c Values with different letters within a column indicates significant differences

## DISCUSSION

Isolation and culture method for rat BMMSC in this research were very simple but can produce high purity of MSC (Figure 1) even though separation method would increase positive surface markers of

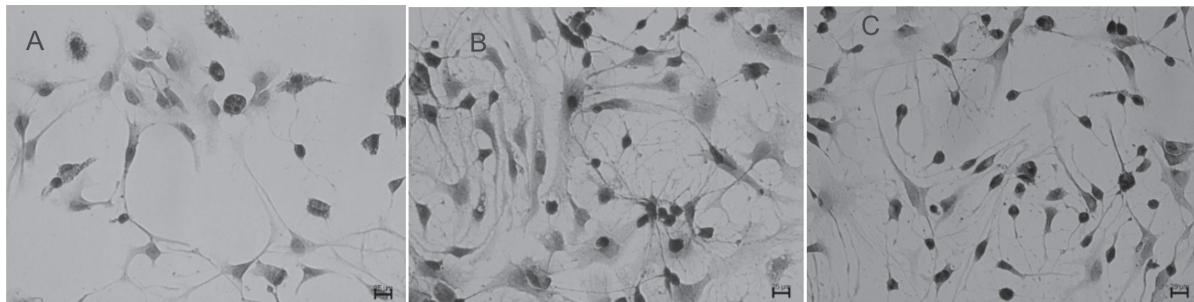


**Fig. 2.** CM-RatBMMSCs affected the cell differentiation to be predominantly a PSANCAM and Beta Tubulin (marker for neuron) after 4 days culture of NPC with CM50 and poly-D-lysine as extracellular matrix . A. Isotype of A2B5, PSANCAM and beta Tubulin < 0,5%. B. The purity of PSA NCAM<sup>+</sup> and Beta tubulin<sup>+</sup> > 20% and A2B5- <10%.

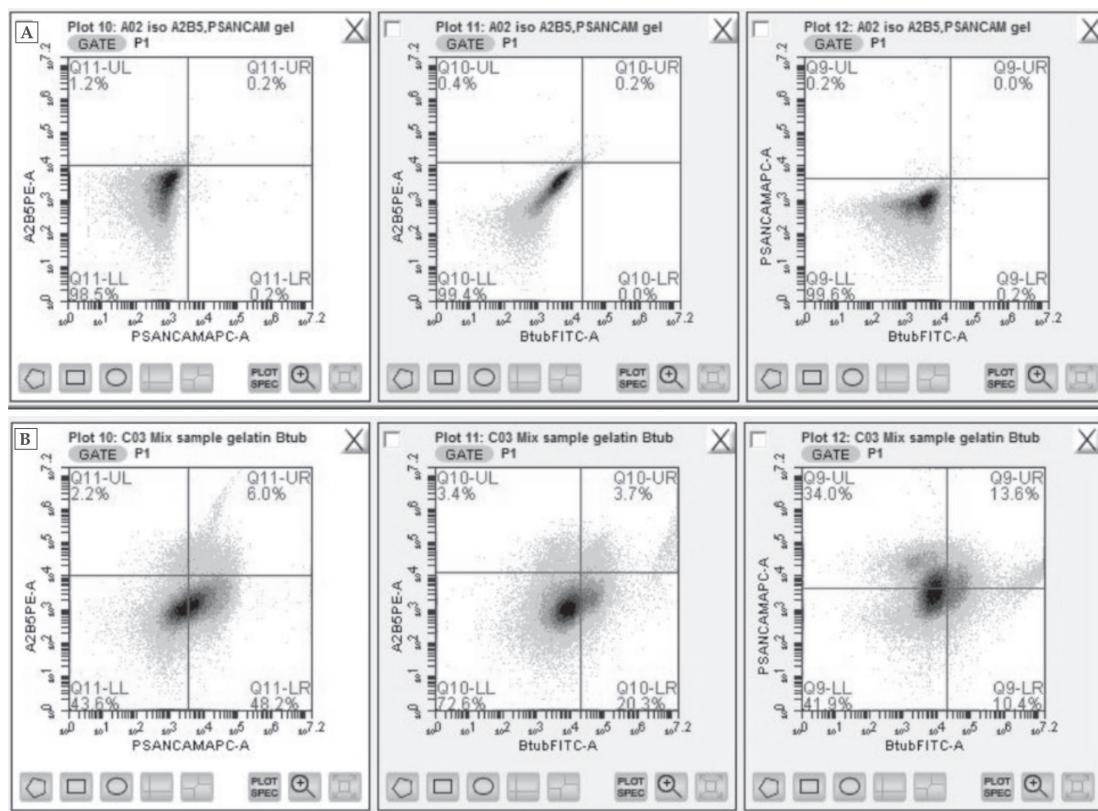
MSC (CD73, CD105, CD90, CD29). (Zhang and Chan 2010) Simple technique to separate MSC and haematopoietic stem cells (HSC) by washing with PBS after 24h plating were effective to eliminate HSC that could not attach to the petridish. This method was done by Li *et al.*, (2013) who reported direct adherent was better than density gradient centrifugation (Li *et al.*, 2013) Isolation technique will affect the purity of MSC culture in which

expected has a correlation with optimal growth factor secretions in CM. In the other hand, supplement in culture medium was important to increase MSC proliferation as reported by previous study that addition 11% FBS for culturing of rat BMMSC was better than 10% FBS. (Li *et al.*, 2013)

Conditioned medium (CM)-MSC that contained some growth factors were potential for inducing differentiation into some types of cell from



**Fig. 4.** Growth factors in CM100 induces neurogenesis *in vitro*. Immunocytochemistry of NPC showed positif expression of NeuN (marker for neuron) after 4 days culture. (A) serum-free culture medium (DMEM/F12+glutamax), (B) neurobasal medium (NM), (C) CM100. Scale bar = 25 $\mu$ m.



**Fig. 3.** CM-RatBMMSCs affected the cell differentiation to be predominantly a PSANCAM and Beta Tubulin (marker for neuron) after 4 days culture of NPC with neurobasal medium and gelatin as extracellular matrix. A. Isotype of A2B5, PSANCAM and Beta tubulin < 0,5%. B. The purity of PSA NCAM<sup>+</sup> and Beta Tubulin<sup>+</sup> < 15% and A2B5- < 10%.

endodermal, ectodermal and mesodermal lineage. Growth factors such as bone morphogenic 4 (BMP4) and bFGF in CM-MSC can initiate differentiation of embryoid body (EB) into mesodermal lineage (osteogenic and chondrogenic) by increasing Wnt3 expression (Lee *et al.*, 2014).

In this study, we successfully cultured NPCs in CM100 and CM50. This indicated that various growth factors in the CM-ratBMMSC were able to maintain NPCs growth and proliferation and also its differentiation. Based on immunoassay we found that CM-ratBMMSC contained specific growth factor, bFGF and NGF, in which the concentration of both growth factors was higher compared to other mediums used in this study (FM and NM) (Table 1).

Basic fibroblast growth factor (bFGF) and NGF can be secreted by MSC adipose derived or other tissue (Clauser *et al.*, 2013). Basic fibroblast growth factor (bFGF) is one of the proteins that play a role in neurogenesis both in differentiation and proliferation (Woodbury and Ikezu, 2014). This growth factor usually added in cocktail growth factor and neurotrophic factor such as retinoid acid, FGF, insulin growth factor (IGF) to differentiate MSC to neuronal (Ahmedy *et al.*, 2015). Our result showed that CM50 can also be used for culturing of NPCs (Figure 2 and 3). It induced NPCs differentiations which were shown by positive expression of beta-tubulin marker after 4 days culture in both of gelatin and PDL coated culture plates. Gelatin and PDL used in this study were different extracellular matrix (ECM) that support NPCs culture. These results were also interesting secondary findings of this study that revealed there were differences between PDL and gelatin coating on neuronal cultures.

Extracellular matrix (ECM) is one of supporting factors for NPCs culture. Choosing appropriate coating agent is very important for neuronal cultures. Poly-D-lysine (PDL) were strongly modulates the adhesion and morphogenesis of primary hippocampal neurons (Sun *et al.*, 2012) We found that NPCs population and neuron (PSANCAM and beta-tubulin markers) in PDL-coated was better than gelatin (Figure 2 and 3). This result was similar to previous study reported by Kim *et al.* (2011). They suggested that PDL has cell adhesion properties for cell growth and morphology, so that the number of neuronal cells, neurites per neuronal cell will be higher. They also reported that the neuronal cells on PDL bounded surfaces survived for longer time (Kim *et al.*, 2011).

Our experiments that used three different mediums (FM, CM100 and NM) showed positive expressions of astrocytes marker (GFAP) and neuron (NeuN) (Figure 4 and Table 2). The number of cells with positive expressions of GFAP and NeuN from CM100 culture were higher than other groups which indicated there were specific substance in CM-rat BMMSC that play role in the neurogenesis in vitro. ELISA results confirmed this hypothesis in which there were higher concentration of bFGF and NGF in that CM compared to FM and NM.

Fibroblast growth factor (FGF) has neurogenesis and neuroprotection effect for NPCs culture. Besides, nerve growth factor (NGF) and FGF in CM-ratBMMSC may activate phosphoinositide-3-kinase–protein kinase B/Akt (PI3K/Akt) pathway that is very important for NPCs activity. This pathway controls neurogenesis such as proliferation, migration and differentiation of NPCs. (Koh and Lo, 2015) In addition, FGF will also binds FGF receptor (FGFR). Signalling effects from that receptor binding not only important for neurogenesis but also for synaptic formation, neuroglia interactions, inflammation, and amyloidosis and also gives neuroprotective effects in hippocampal region by suppressing autophagy via mTOR pathway and inhibiting apoptosis by preventing p53 (Woodbury and Ikezu 2014); (Sun *et al.*, 2018)

Conditioned medium from MSC may not only contain growth factors but also cytokine, chemokine and extracellular vesicles that potential for neurodegenerative therapy (Cantinieaux *et al.*, 2013); (Li *et al.*, 2018); (Henry *et al.*, 2018) Extracellular vesicles (EVs) such as exosomes and microvesicles which contained a variety of cargo including proteins, lipids, DNA and various RNA can modulate signaling pathway to treat ischemic stroke (Li *et al.*, 2018) Growth factor, neurotrophic factor and EVs induced neuro restorative, increasing neurogenesis, synaptogenesis, anti-inflammatory and angiogenesis, suppressing apoptosis and inflammation (Henry *et al.*, 2018; Manuel *et al.*, 2017). The findings of this research, using CM-ratBMMSC in NPCs culture, may open up the opportunities of cell-free therapy for neurodegenerative disease.

## CONCLUSION

Growth and neurotrophic factor such as bFGF and

NGF from CM-rat BMMSC increased the differentiation ability of NPCs into astrocytes (GFAP) and neurons (NeuN).

#### ACKNOWLEDGEMENT

The author would like to thank the head of the Center For Research and Development of Biomedical and Basic Health Technology, National Institute Health Research and Development, Ministry of Health Republic of Indonesia and research team who supports this research.

#### REFERENCES

Ahmedy, E. 2015. Neurogenic Differentiation of Bone Marrow-derived Mesenchymal Stem Cells Using Neural Induction Medium/: A Morphological and Histochemical Study. *American Journal of Bioscience and Bioengineering*. 3(4-1) : 43–50.

Cantinieaux, D. 2013. Conditioned Medium from Bone Marrow-Derived Mesenchymal Stem Cells Improves Recovery after Spinal Cord Injury in Rats/: An Original Strategy to Avoid Cell Transplantation. *PLOS ONE*. 8 (8) : 1–15.

Clauser, L. 2013. Adipose-derived stem cells secrete neurotrophic factors. *Annal of Oral and Maxillofacial Surgery*. 1 (2) : 1–5.

Henry, N. 2018. Cell-Based and Exosome Therapy in Diabetic Stroke. *Stem Cell Translational Medicine*. 7 : 451–455.

Inoue, T. 2013. Stem Cells from Human Exfoliated Deciduous Tooth-Derived Conditioned Medium Enhance Recovery of Focal Cerebral Ischemia in Rats. *Tissue Engineering*. 19 : 24–29.

Kim, Y.H. 2011. Enhancement of neuronal cell adhesion by covalent binding of poly- d -lysine. *Journal of Neuroscience Methods*. 202 : 38–44.

Koh, S. and Lo, E.H., 2015. The Role of the PI3K Pathway in the Regeneration of the Damaged Brain by Neural Stem Cells. *JCN Open Access*. 11 (4) : 297–304.

Kurozumi, K. 2005. Mesenchymal Stem Cells That Produce Neurotrophic Factors Reduce Ischemic Damage in the Rat Middle Cerebral Artery Occlusion Model. *Molecular Therapy*. 11 (1) : 96–104. Available at: <http://dx.doi.org/10.1016/j.ymthe.2004.09.020>.

Lee, T. 2014. Mesenchymal Stem Cell-Conditioned Medium Enhances Embryonic Stem Cells and Human Induced Pluripotent Stem Cells by Mesodermal Lineage Induction. *Tissue Engineering*. 20 (7&8) : 1306–1313.

Li, X., Zhang, Y. and Qi, G. 2013. Evaluation of isolation methods and culture conditions for rat bone marrow mesenchymal stem cells. *Cytotechnology*. 65 : 323–334.

Li, Y. 2018. Extracellular vesicles in mesenchymal stromal cells/: A novel therapeutic strategy for stroke (Review). *Experimental and Therapeutic Medicine*. 15: 4067–4079.

Manuel, G.E., Johnson, T. and Liu, D. 2017. Therapeutic angiogenesis of exosomes for ischemic stroke. *Int J Physiol Pathophysiol Pharmacol*. 9 (6) : 188–191.

Nakajima, M. 2017. Mesenchymal Stem Cells Overexpressing Interleukin-10 Promote Neuroprotection in Experimental Acute Ischemic Stroke. *Molecular Therapy: Methods & Clinical Development*. 6 (September):102–111. Available at: <http://dx.doi.org/10.1016/j.omtm.2017.06.005>.

Nguyen, T.L.X. 2010. Neuroprotection signaling pathway of nerve growth factor and brain-derived neurotrophic factor against staurosporine induced apoptosis in hippocampal H19-7 cells. *Experimental and Molecular Medicine*. 42 (8) : 583–595.

Pawitan, J.A., 2014. Prospect of Stem Cell Conditioned Medium in. *BioMed Research International*. 1–14.

Sun, D. 2018. bFGF plays a neuroprotective role by suppressing excessive autophagy and apoptosis after transient global cerebral ischemia in rats. *Cell Death and Disease*. 9 (172) : 1–14.

Sun, Y., Huang, Z. and Liu, W. 2012. Surface Coating as a Key Parameter in Engineering Neuronal Network Structures *In vitro*. *Biointerphases*. 7 (29) : 1–14.

Tondreau, T. 2004. Bone marrow – derived mesenchymal stem cells already express specific neural proteins before any differentiation. *International Society of Differentiation*. 72 : 319–326.

Vizoso, F.J. 2017. Mesenchymal Stem Cell Secretome/: Toward Cell-Free Therapeutic Strategies in Regenerative Medicine. *International Journal of Molecular Science*. 18 (1852) : 2–24.

Woodbury, M. and Ikezu, T., 2014. Fibroblast growth factor-2 signaling in neurogenesis and neurodegeneration. *J Neuroimmune Pharmacol*. 9 (2) : 92–101.

Zhang, L. 2017. Neural differentiation of human Wharton's jelly-derived mesenchymal stem cells improves the recovery of neurological function after transplantation in ischemic stroke rats. *Neural Regeneration Research*. 12 (7) : 1103–1110.

Zhang, L. and Chan, C. 2010. Isolation and Enrichment of Rat Mesenchymal Stem Cells (MSCs) and Separation of Single-colony Derived MSCs. *Journal of Visualized Experiments*. 2–5.

# Sources

ISSN

ISSN: 0972-3005 

## i CiteScore metrics for journals and serials

CiteScore metrics from Scopus are:

- Comprehensive
- Transparent
- Current and free

Use this page to find a source and view associated metrics. Use qualitative as well as quantitative metrics when presenting your research impact. Always use more than one quantitative metric. Learn more about CiteScore.



## Filter refine list

1 result

ⓘ

## Display options

Display only Open Access journals

Display only source with minimum

Documents   
(previous 3 years)

## Citescore highest quartile

Show only titles in top 10 percent

1st quartile  
 2nd quartile  
 3rd quartile  
 4th quartile

## Source type

Journals  
 Book Series  
 Conference Proceedings  
 Trade Publications

## About Scopus

[What is Scopus](#)  
[Content coverage](#)  
[Scopus blog](#)  
[Scopus API](#)  
[Privacy matters](#)

## Language

[日本語に切り替える](#)  
[切换到简体中文](#)  
[切换到繁體中文](#)  
[Русский язык](#)

## Customer Service

[Help](#)  
[Contact us](#)

