Gmail **Purwati Sumorejo <purwatisumorejo@gmail.com>**

KOREKSI JURNAL SYMPOSIUM STEM CELL AND BIOMEDICAL SCIENCES

2 messages

Symposium Stemcell Universitas Airlangga <symposiumstemcell@stemcell.unair.ac.id> Wed, Nov 7, 2018 at 7:54 PM
To: purwatisumorejo@gmail.com

Selamat Malam Bapak/Ibu
Jurnal anda telah kami koreksi. Revisi anda kami tunggu hingga Kamis, 8 November 2018 pukul 15.00. Terimakasih

--
Warm regards,

Symposium Stem Cell and Biomedical Sciences
Stem Cell Research and Development Center Universitas Airlangga
Office : (031) 5950930
Surabaya, Indonesia.

2 attachments

Purwati 2 (UNAIR) (koreksi LBA 7 Nov).doc
110K

Purwati 1 (UNAIR) (koreksi LBA 7 Nov).doc
2188K

Purwati Sumorejo <purwatisumorejo@gmail.com> Thu, Nov 8, 2018 at 10:20 AM
To: symposiumstemcell@stemcell.unair.ac.id

Yth. Tim Jurnal Symposium Stem Cell and Biomedical Sciences

Berikut terlampir jurnal kami yang telah direvisi sesuai koreksi dari tim jurnal.
Terima kasih

Best regards,
Purwati
[Quoted text hidden]

2 attachments

Purwati 2 (UNAIR) (koreksi LBA 7 Nov).doc
110K

Purwati 1 (UNAIR) (koreksi LBA 7 Nov).doc
2188K

ELECTROSPUN FIBERS AS A WOUND DRESSING MATERIAL USING COMBINATION OF CELLULOSE ACETATE/COLLAGEN SEEDING STEM CELL

Purwati, Bagus Satrio Nurwito And Hendita Nur Maulida

Commented [D1]: Nama yang menjadi corresponding author diberi tanda *
Nama penulis diberi tanda angka untuk keterangan asal instansi

Stem Cell Research and Development Center, Universitas Airlangga, Surabaya, Indonesia

Adjunct Associate Professor, Department of Biotechnology, Asia University, Taichung, Taiwan

Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

Commented [D2]: Nama instansi diberi tanda angka untuk keterangan instansi asal penulis

***Corresponding Author:** Purwati. Email: purwatisumorejo@gmail.com

Keywords: wound dressing, electrospun fibers, electrospinning, cellulose acetate, collagen, stem cell

Abstract

Wound healing is a complex tissue regeneration process that the body undergoes as a response to wound openings or missing cellular structures as a result of various types of traumatic injury. Because of the ability of materials to induce a high immune response or limited donor tissues, the skin repair and regeneration methods using allografts and autografts cannot be widely used. Many research have shifted into tissue engineering approaches using scaffolds. To achieve the goal of tissue reconstruction, scaffolds must meet some specific requirements include biocompatibility, biodegradability, and mechanical properties. Our study aimed to fabricate composite cellulose acetate-collagen (CA/Collagen) scaffolds by electrospinning and determine the appropriate compositions of CA:Collagen for obtaining

skin substitutes as wound dressing through investigating the morphological of stem cell seeded on electrospun CA/Collagen membranes. High proliferation of mesenchymal stem cells on electrospun CA/collagen 75:25 (wt.%) confirmed the capability of CA/collagen 75:25 nanofibers as a tissue-engineered scaffold, while the electrospun CA/collagen 75:25 can be a potential low-adherent wound dressing.

Introduction

Wound healing is a complex tissue regeneration process that the body undergoes as a response to wound openings or missing cellular structures as a result of various types of traumatic injury (Mulugeta *et al.,* 2018). In adult humans, optimal wound healing involves: (1) rapid hemostasis; (2) appropriate inflammation; (3) mesenchymal cell differentiation, proliferation, and migration to the wound site; (4) suitable angiogenesis; (5) prompt re-epithelialization (re-growth of epithelial tissue over the wound surface); and (6) proper synthesis, cross-linking, and alignment of collagen to provide strength to the healing tissue (Gosain *et al.,* 2004; Mathieu *et al.,* 2006). To facilitate effective wound healing, a wound site is typically covered with a sterile dressing material to avoid infection and to promote the healing process.

In the last decade, several skin repair and regeneration from xenografts, allografts and autografts have been used for wound healing, such as human amnion or chorion membrane. However, because of the ability of materials or antigen to induce a high immune (antigenicity) response or limited donor tissues, the skin repair and regeneration methods mentioned above cannot be widely used (Boyce, 2011; Schulz *et al.,* 2005). Many research have shifted into tissue engineering approaches. This technique is an interdisciplinary field of study that emerges by applying the principles of biology, chemistry and engineering science to tissue regeneration (Hoerstrup *et al.,* 2004). The approach used in tissue engineering is

more to the use of biomaterials, cells or combination of both, and suitable biochemical and physico-chemical factors to restore, maintain and improve biological functions.

The most important thing in skin tissue engineering is the construction of scaffolds. Scaffolds are materials that have been engineered to cause desirable cellular interactions to contribute to the formation of new functional tissues, that serves in infiltration and physical support to guide cell differentiation and proliferation into targeted functional tissues (Mertsching *et al.,* 2009). To achieve the goal of tissue reconstruction, scaffolds must meet some specific requirements. Ideal scaffolds for skin tissue engineering applications must have good biocompatibility, suitable microstructure such as average pore size of 63-150 μm and porosity values above 90%, biodegradation can be controlled and suitable mechanical properties (O'Brien *et al.,* 2005; Newman *et al.,* 2013). Many different biomaterials have been investigated and being already employed as skin tissue engineering. Examples of these materials are cellulose acetate and collagen.

Polysaccharides are natural biopolymers available from plant sources and they show excellent biocompatibility (Miao *et al.,* 2011). Cellulose, the most abundant renewable polysaccharide in the form of woven cotton gauze, has been utilized for many years as wound dressings (Elham *et al.,* 2014). While collagen is known as the most promising materials in tissue engineering application because of its biocompatibility and biodegradability. Scaffold from collagen has a homeostatic effect, antigenicity and can increase cell growth and cell adhesion (O'Brien *et al.,* 2005).

Nanofibrous membranes due to their porous structure, high surface area and structural similarity to the native extracellular matrix (ECM) can serve as an excellent functional skin substitute for deep wounds. Among the different methods of nanofiber fabrication, electrospinning is a simple, cost-effective and versatile technique for generating nanofibers of polymers as wound dressings (Elham *et al.,* 2014; Zahedi *et al.,* 2010). In recent study,

cellulose acetate (CA) was combined with polyurethane to fabricate a composite scaffold as wound dressing by electrospinning (Liu *et al.*, 2012). However, very few reports are available on the application of electrospun CA composite scaffolds with natural polymers such as collagen for tissue regeneration (Powell *et al.*, 2008).

Our study aimed to fabricate composite CA/Collagen scaffolds by electrospinning and determine the appropriate compositions of CA:Collagen for obtaining skin substitutes as a wound dressing through investigating the morphological of stem cell seeded on electrospun CA/Collagen membranes. Stem cells are unique types of cells that are undifferentiated. So the main focus of creating these constructs is to be able to safely deliver these stem cells, and create a structure that is physically and mechanically stable so that these stem cells can differentiate.

Materials and Methods

Materials

Cellulose acetate (CA; white powder; Mr ~29,000; acetyl groups ~40%;), acetone and formic acid were all purchased from Sigma-Aldrich, collagen type 1 from snapper scales was purchased from the National Nuclear Energy Agency of Indonesia. Mesenchymal stem cell was derived from rat tissues. Dulbecco Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), and penicillin streptomycin solution were purchased from Gibco.

Electrospinning

The CA/Collagen solutions were prepared in three different weight ratios, including 95:5, 85:15 and 75:25 (wt.%). To achieve beadless fibers, CA were dissolved in aceton with the concentration of 10% (w/v). After stirring for 1 h, each solution was loaded into a 5-mL syringe attached to a 23G blunted stainless steel needle at a flow rate of 0.01 mL/h. A high

voltage of 14 kV was applied to the tip of the needle. The fibers were placed on a flat aluminum foil-wrapped collector kept at a distance of 10 cm from the needle tip. Nanofibrous membranes were dried in a room temperature for at least 24 h to ensure the solvent residuals vaporized completely.

Cell Culture

Mesenchymal stem cells (MSCs) were derived from rat tissue using aspiration and separation on Histopaque-1.077 (Sigma). Harvested cells were cultured in Dulbecco's Modified Eagles Medium containing 1.0 g/L glucose. MSCs characterization were performed by analyzing the expression of 90+, CD34- and CD105+ by using DAB immunostaining and FACS (BD). Confluent stem cells were seeded on the scaffolds placed in a 24-well plate and tissue culture at a density of 5000 cells per well.

Membrane Morphology

The morphology of CA/collagen seeded stem cell on electrospun scaffolds was observed by scanning electron microscopy (SEM). After 7 days of cell seeding, samples were washed with PBS and fixed with 3% glutaraldehyde. After being washed with distilled water, scaffolds were dehydrated through ethanol solutions. Subsequently, the samples were treated with hexamethyldisilazane and air-dried in a fume hood. Completely dried specimens were sputtercoated with gold. The SEM images were analyzed with image analysis software to determine average fiber diameter.

Results

The morphology of electrospun nanofibers was investigated by SEM and Figure 1 shows the SEM micrographs of electrospun scaffolds. The interaction between mesenchymal stem cells and electrospun nanofibrous scaffolds were evaluated after 7 days of cell culture and the

results are shown in Figure 2. The cells were found to attach on all the electrospun nanofibers, but the compositional variations of the scaffolds caused some differences in the extent of proliferation such that the scaffold containing the highest collagen content showed the stretching of the cells across the nanofibrous substrate.

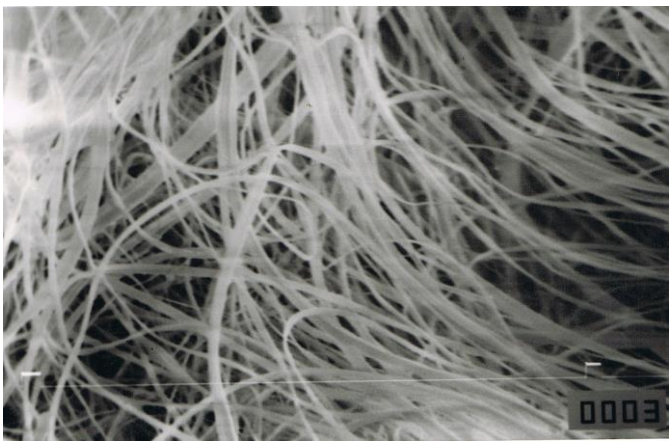

Figure 1. Morphology of electropun CA/Collagen (75:25 wt.%) using scanning electron microscopy (SEM)

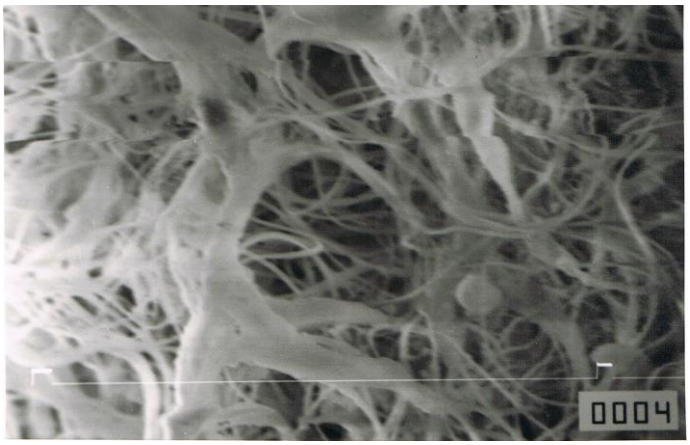

Figure 2. Morphology of electropun CA/Collagen (75:25 wt.%) after 7 days of cell culture using scanning electron microscopy (SEM)

Discussion

Wound healing is a dynamic process consisting of four continuous, overlapping, and precisely programmed phases. The events of each phase must happen in a precise and regulated manner. Interruptions, aberrancies, or prolongation in the process can lead to delayed wound healing or a non-healing chronic wound (Guo *et al.,* 2010). Most chronic wounds are ulcers that are associated with ischemia, diabetes mellitus, venous stasis disease, or pressure. Non-healing wounds affect about 3 to 6 million people in the United States, with total cost estimated at more than $3 billion per year (Gou *et al.,* 2010; Mathieu *et al.,* 2006; Menke *et al.,* 2007).

Trauma at the epidermis layer could be healed through re-epithelialization without any skin grafting and wound dressing is recommended for such applications. Healing process of deep dermal injuries owing to the lack of remaining cell sources at the site for regeneration takes a long time and the process remains complicated (Chong *et al.,* 2007). Tissue engineering such as wound dressing is a promising method to provide functional alternatives to allografts and autografts for skin regeneration, without restrictions involving donor site limitations, disease transmission or risk of immunological rejection (Venugopul *et al.,* 2006).

Dressing selection should be based on its ability to 1) provide or maintain moist environment, 2) enhance epidermal migration, 3) promote angiogenesis and connective tissue synthesis, 4) allow gas exchange between wounded tissue and environment, 5) maintain appropriate tissue temperature to improve the blood flow to the wound bed and enhances epidermal migration, 6) provide protection against bacterial infection, 7) should be non-adherent to the wound and easy to remove after healing, 8) must provide debridement action to enhance leucocytes migration and support the accumulation of enzyme and 9) must be sterile, non-toxic and non-allergic (Selvaraj *et al.,* 2015).

Selection of material compositions is required. The dermal matrix consists of collagen (mainly type I and III) and elastic fibers surrounded by the ECM made up of proteoglycans. Therefore, a nanofibrous architecture composed of a combination of polysaccharide-protein might be an ideal choice for treatment of skin injuries. Various researchers have develop scaffolds of collagen/chitosan, silk fibroin/chitosan, or collagen/hyaluronic acid by electrospinning for skin tissue engineering. Therefore, we considered the development of electrospun skin substitutes using cellulose acetate or CA (a linear organic polysaccharide) and collagen (Elham *et al.,* 2014).

Fibers obtained from electrospinning are considered as ideal dressing materials for non-healing wounds since the method is versatile and can deliver various biological agents long-term to local tissues at the wound site (Mulugeta *et al.,* 2018). Electrospun nanofibrous membranes serve as a biomimetic fibrous structure of the native dermis, provide good support for wound healing and increase the rate of epithelialization and dermal organization (Elham *et al.,* 2014). In this study, the obtained nanofibrous membranes from scanning electron microscopy had an average fiber diameter and pore size that might not only provide sufficient space for efficiently cell housing and exchanging of nutrient and metabolic waste between the membrane and environment but also engage in presenting excess exudate absorbability and oxygen permeability.

The epithelium of the skin has a remarkable ability of self-renewal over the lifetime and also produces daughter cells that differentiate into one or multiple lineages. Although epidermal stem cells in the basal layer, as an endogenous source of stem cells, can regenerate skin, but these cells are not sufficient to provide perfect repair after deep and extensive skin damage. Thus, exogenous supply of stem cells in traumatic conditions may be one of the novel therapeutic strategies to achieve perfect skin repair (Suman *et al.,* 2017). In this study, the stem cells were found to attach on all the electrospun nanofibers.

Once a wound occurs, mesenchymal stem cells (MSCs) mobilize to the wound site, where they manage cell proliferation and migration during the inflammation phase of cicatrization. MSCs influence the wound´s ability to progress beyond the inflammatory phase and not regress to a chronic wound state. The mechanism of action of these cells is that they directly attenuate inflammatory response so that they decrease secretion of the proinflammatory cytokines while increasing the production of antiinflammatory cytokines. These anti-inflammatory properties make them particularly beneficial to chronic wounds by advancing the wound past a chronic inflammatory state into the next stage of healing. Furthermore, MSCs secreted several growth factors so these cells promote dermal fibroblast proliferation, angiogenesis and collagen deposition (Suman *et al.,* 2017; Menendez *et al.,* 2014).

This study approves the feasibility of electrospun CA/Collagen scaffolds seeded stem cells for skin treatment especially for wound dressing and shows the importance of compositional designing to provide appropriate features of the target application. Further research is needed to find out how the scaffold mechanism in wound dressing able to deliver stem cells to the injured area. It is also needed another study to determine the mechanical properties of the scaffold and its biodegradability.

Acknowledgements

We would like to acknowledge the support of all laboratory technicians in Stem Cell Research and Development Centre, Airlangga University; and Cell and Tissue Bank, Dr. Soetomo General Hospital, Surabaya, Indonesia.

Potential Conflict of Interest

The authors declare that there is no conflict of interest.

References

Boyce ST. 2011. Design principles for composition and performance of cultured skin substitutes. *Burns* 27: 523–533.

Chong EJ, Phan TT, Lim IJ. 2007. Evaluation of electrospun PCL/gelatin nanofibrous scaffold for wound healing and layered dermal reconstitution. *Acta Biomater*; 3(3): 321–330.

Elham Vatankhah, Molamma P Prabhakaran, Guorui Jin, Laleh Ghasemi Mobarakeh, Seeram Ramakrishna. 2014. Development of nanofibrous cellulose acetate/gelatin skin substitutes for variety wound treatment applications. *Journal of Biomaterials Applications*:Vol 28(6) 909–921.

Gosain A, DiPietro LA. 2004. Aging and wound healing. *World J Surg*; 28(3):321-6.

Hoerstrup S and Joseph P. Vacanti. 2004. Tissue Engineering of a Trileaflet Heart Valve—Early In Vitro Experiences with a Combined Polymer. *Mary Ann Liebert inc.*

Liu X, Lin T, Gao Y. 2012. Antimicrobial electrospun nanofibers of cellulose acetate and polyester urethane composite for wound dressing. *J Biomed Mater Res Part B*; 100B(6): 1556–1565.

Mathieu D, Linke J-C, Wattel F. 2006. Non-healing wounds. In: Handbook on hyperbaric medicine, Mathieu DE, editor. Netherlands: *Springer*, pp. 401-427.

Menendez Yolanda, Alvarez-Viejo Maria, Ferrero-Gutierrez Amaia, Perez-Basterrechea Marcos, Perez Lopez Silvia, Escudero Dolores, Otero-Hernandez Jesús. 2014. Adult Stem Cell Therapy in Chronic Wound Healing. *J Stem Cell Res Ther* 4:162.

Menke NB, Ward KR, Witten TM, Bonchev DG, Diegelmann RF. 2007. Impaired wound healing. *Clin Dermatol*; 25(1):19-25.

Mertsching H, Schanz J, Steger V, Schandar M, Schenk M, Hansmann J, Dally I, Friedel G, Walles T. 2009. Generation and transplantation of an autologous vascularized bioartificial human tissue. *Transplantation*; 88: 203-10.

Miao JJ, Pangule RC, Paskaleva EE. 2011. Lysostaphinfunctionalized cellulose fibers with antistaphylococcal activity for wound healing applications. *Biomaterials*; 32(36): 9557–9567.

Mulugeta Gizaw, Jeffrey Thompson, Addison Faglie, Shih-Yu Lee, Pierre Neuenschwander, Shih-Feng Chou. 2018. Electrospun Fibers as a Dressing Material for Drug and Biological Agent Delivery in Wound Healing Applications. *MDPI Bioengineering*:5,9.

Newman Peter, Minett Andrew Ellis-Behnke R, Zreiqat Hala. 2013. Carbon nanotubes: their potential and pitfalls for bone tissue regeneration and engineering. *Nanomedicine: Nanotechnology, Biology and Medicine*. 9 (8): 1139–1158.

O'Brien FJ, Harley BA, Yannas IV, Gibson L. 2005. The effect of pore size on cell adhesion in collagen-GAG scaffolds. *Biomaterials* 26: 433–441.

Powell HM, Boyce ST. 2008. Fiber density of electrospun gelatin scaffolds regulates morphogenesis of dermal-epidermal skin substitutes. *J Biomed Mater Res Part A*; 84A(4): 1078–1086.

S. Guo, L.A. DiPietro. 2010. Factors Affecting Wound Healing. *Dent Res* 89(3):219-229.

Schulz JT, Tompkins RG, Burke JF. 2005. Artificial skin. *Annu Rev Med* 51: 231–244.

Selvaraj Dhivya, Viswanadha Vijaya Padma, Elango Santhini. 2015. Wound dressings – a review. *Biomedicine*; 5(4): 22.

Suman Kanji, Hiranmoy Das. 2017. Advances of Stem Cell Therapeutics in Cutaneous Wound Healing and Regeneration. *Mediators Inflamm*; 5217967.

Venugopal JR, Zhang YZ, Ramakrishna S. 2006. In vitro culture of human dermal fibroblasts on electrospun polycaprolactone collagen nanofibrous membrane. *Artif Organs*; 30(6): 440–446.

Zahedi P, Rezaeian I, Ranaei-Siadat SO. 2010. A review on wound dressings with an emphasis on electrospun nanofibrous polymeric bandages. *Polym Advan Technol*; 21(2): 77–95.

CLINICAL OUTCOME OF INTRAVENTRICULAR IMPLANTATION AUTOLOGOUS ADIPOSE DERIVED NEURAL PROGENITOR CELLS IN PARKINSON

Purwati, Asra Al Fauzi And Prastiya I. Gunawan

Stem Cell Research and Development Center, Universitas Airlangga, Surabaya, Indonesia

Department of Internal Medicine, Faculty of Medicine, Universitas Airlangga – Dr. Soetomo Teaching Hospital, Surabaya, Indonesia

Adjunct Associate Professor, Department of Biotechnology, Asia University, Taichung, Taiwan

Department of Neurosurgery, Faculty of Medicine, Universitas Airlangga – Dr. Soetomo Teaching Hospital, Surabaya, Indonesia

Department of Paediatrics and Child Health, Faculty of Medicine, Universitas Airlangga – Dr. Soetomo Teaching Hospital, Surabaya, Indonesia

***Corresponding Author:** Purwati**.** Email: purwatisumorejo@gmail.com

Commented [D1]: Nama penulis yang menjadi corresponding author diberi tanda *.
Nama penulis diberi tanda angka untuk keterangan asal instansi

Commented [D2]: Nama instansi diberi keterangan angka untuk menerangkan asal instansi penulis

Keywords: Parkinson's disease, stem cell therapy, adipose tissue, neural progenitor cells, intraventricular implantation

Abstract

Parkinson's disease (PD) involves the malfunction and death of vital nerve cells in the brain, is a chronic and progressive movement disorder. Supportive medications and surgery may conducted, but no optimal results have been obtained. The main goal of this study was to

investigate the effectiveness of the intraventricular implantation of adipose derived neural progenitor stem cells in post-Parkinson's disease patients. 12 patients were included in this study. Small adipose tissue was isolated by small lipopectomy under local anesthesia, cultured and derived become neural progenitor cells. Intraventricular implantation were performed in the operating room. The evaluation was carried out using the Unified Parkinson's Disease Rating Scale (UPDRS), include non-motor experiences and motor experiences of daily living, motor examination, and motor complications. The primary target was the UPDRS over the time period of 12 months after treatment as the end point. Descriptive statistics are provided. 10 of 12 patients (83.33%) had a significant improvement in mentation, behavior and mood, activity of daily living, and motor examination after treatment. There were no serious adverse events reported, limited to mild headaches, fever or vomiting, and all side effects resolved within few days. Because of the small sample size and non-randomised trial performed, we could not reach a definitive conclusion regarding the potential of intraventricular implantation. However, this study shows that repeated intraventricular implantation of autologous stem cells is advantageous.

Introduction

Parkinson's disease (PD) involves the malfunction and death of vital nerve cells in the brain, is a chronic and progressive movement disorder. The most obvious are shaking, rigidity, slowness of movement, difficulty with walking, thinking problems, depression, and anxiety. In 2015, PD affected 6.2 million people and resulted in about 117,400 deaths globally (NINDS, 2016; GBD, 2016). Parkinson's disease typically occurs in people over the age of 60, of which about 1% are affected. Males are more often affected (Carrol *et al.*, 2016; Kalia *et al.*, 2015). The average life expectancy following diagnosis is between 7 and 14 years (Sveinbjornsdottir, 2016). The cause of PD is generally unknown, but believed to

involve both genetic and environmental factors. Non-motor symptoms, which include autonomic dysfunction, neuropsychiatric problems, sensory, and sleep difficulties are also common (Kalia *et al.*, 2015; Jankovic, 2008). The motor symptoms of the disease result from the death of cells in the substantia nigra, a region of the midbrain. This results in not enough dopamine in these areas (NINDS, 2016). The reason for this cell death is poorly understood, but involves the build-up of proteins into Lewy bodies in the neurons (Kalia *et al.*, 2015).

Several neuroprotective agents have been developed to prevent brain tissue damage after Parkinson's disease. Initial treatment for PD is typically with the anti-parkinson medication levodopa (L-DOPA) with dopamine agonists (Samii *et al.*, 2004). As the disease progresses, these medications become less effective while at the same time they produce a complication marked by involuntary writhing movement (Sveinbjornsdottir, 2016). When oral medications are not enough to control symptoms, surgery, deep brain stimulation, subcutaneous waking day apomorphine infusion and enteral dopa pumps may be of use. This stage presents many challenging problems requiring a variety of treatments for psychiatric symptoms, orthostatic hypotension, bladder dysfunction, and more (Olanow *et al.*, 2011). Surgery to place microelectrodes for deep brain stimulation has been used to reduce motor symptoms, but it is more invasive and full of risks.

In the last 10 years, alternative approaches to restoring neural function after Parkinson's disease have been developed using the concept of neurorestoration using stem cell therapy (Bhasin *et al.*, 2011). Stem cells are multipotent progenitor cells that have been shown to have regenerative as well as imunomodulatory and growth stimulating properties. They have been shown in vitro to have the capacity to induce angiogenesis and differentiate into different cells types including cells of the nervous system. Stem cell treatment for Parkinson's disease is designed to target these neurons and help with the creation of new dopamine producing neurons. In addition, stem cells may release natural chemicals called

cytokines which can induce differentiation of the stem cells into dopamine producing neurons (Najm *et al.*, 2011; Lee YH *et al.*, 2011).

Stem cell research has the potential to significantly impact the development of disease-modifying treatments for Parkinson's disease, and considerable progress has been made in creating dopamine-producing cells from stem cells. Cell models of Parkinson's disease generated from stem cells could help researchers screen drugs more efficiently than in currently available animal models, and study the underlying biological mechanisms associated with Parkinson's disease in cells taken from people living with the disease. Young et al reported that all subjects with Parkinson's disease were honed in on the salient variables include cognition, depression, sleep, and adjustment, and showed an improvement using stem cell (Young HE *et al.*, 2013).

The main goal of this study was to investigate the effectiveness of the intraventricular implantation of adipose derived neural progenitor stem cells in post- Parkinson's Disease patients and evaluate using the Movement Disorder Society-Unified Parkinson's Disease Rating Scale (MDS-UPDRS) before and after stem cell implantation. The MDS-UPDRS has four parts, namely, I: Non-motor Experiences of Daily Living; II: Motor Experiences of Daily Living; III: Motor Examination; IV: Motor Complications (Goetz *et al.*, 2007). Adipose tissue-derived stem cells are considered to be ideal for application in regenerative medicine, e.g. Parkinson's disease. They can be easily and repeatable harvested using minimally invasive techniques with low morbidity. Adipose tissue-derived stem cells are multipotent and can differentiate into various cell types of the tri-germ lineages, including osteocytes, adipocytes, neural cells, vascular endothelial cells, cardiomyocytes, pancreatic β-cells, and hepatocytes. Interestingly, adipose tissue-derived stem cells are characterized by immunosuppressive properties and low immunogenicity. Their secretion of trophic factors

enforces the therapeutic and regenerative outcome in a wide range of applications (Laura *et al.*, 2016).

Materials and Methods

Subjects

This study was following the regulatory guidelines of the country. The patients were included if they had confirmed by two neurologists. Prior to the study, informed consent documents, details of the medical treatment and other necessary approval documents were delivered to all patients after full explanation of the procedure and the safety issues involved.

Twelve patients were included in this study. The evaluation was carried out using the Unified Parkinson's Disease Rating Scale (UPDRS). The scales include (1) non-motor experiences of daily living (13 items), (2) motor experiences of daily living (13 items), (3) motor examination (18 items), and (4) motor complications (6 items). The primary target was the UPDRS over the time period of 12 months after treatment as the end point. Descriptive statistics are provided. The following inclusion and exclusion criteria were used for the patient (Table 1).

Table 1. Inclusion and exclusion criteria

Inclusion Criteria	Exclusion Criteria
- Parkinson's disease patients severe - Aged 40 to 80 years - Parkinson's Subjects will not currently be experiencing dementia (DSM-IV criteria) - MMSE 20 or greater - No active infection/disease	- Subjects with severe hepatic impairment, COPD, galactorrhea and/or prolactin sensitive tumors - Parkinsonism due to Parkinson's-plus diagnoses or to medication - Subjects with a communicable disease, include HIV, Hepatitis

	- Subjects having deep brain stimulation

Procedure and Implantation Techniques

Isolation and intraventricular implantation of adipose derived neural progenitor stem cells were performed in the operating room. Autologous adipose tissue isolation was performed under local anaesthesia, and aspiration was performed with a sterile procedure.

Neural progenitor cells was derived from autologous adipose tissue. Small adipose tissue was isolated by small lipopectomy under local anesthesia after isolation then cultured and derived become neural progenitor cells for around 3 weeks. Before used, neural progenitor cells was validated. Characterization of neural progenitor cells by expression of L-Dopa with immunocytochemistry and expression of Notch using flow cytometry (NPCP technique by Purwati).

Under general anaesthesia, patients were conditioned in a supine position. The hair was shaved just behind the right frontal hairline, and the area was washed with antiseptic solution. A mark was made on the right Kocher point. A 2.5-cm wide linear incision was made in layers through the periosteum. The process was continued by creating a burr hole in the calvaria and a small dural incision. An Ommaya reservoir was inserted into the ventricle, then a maximum of 5 cc cerebrospinal fluid was slowly aspirated through the Ommaya reservoir with a wing needle. Stem cells were transplanted with the same wing needle (2×10^6 cells in 3cc normal saline) and flushed with 2cc normal saline. The surgical wound was then sutured layer by layer.

For booster implantation, the same procedures were performed without the open procedure or general anaesthesia one month after the first implantation. Hair did not need to be shaved, disinfection with povidone-iodine was performed at the skin and stem cell

injection was carried out with the same dose using wing needle no. 25 through the subcutaneous transplanted Ommaya reservoir. Booster implantation was done twice at one-month intervals.

Results

There were 12 subjects in this study, and all subjects were male. The youngest patient was 53 years old, and the oldest was 77 years old. Ten of the 12 patients had a significant improvement after stem cell therapy (83.33%) according to their improvement in mentation, behavior and mood, activity of daily living, and motor examination. Further details on patient characteristics and improvements are shown in Table 2.

Table 2. Data progress of post-treatment patients

No.	Gender (M/F)	Age (years old)	48-Weeks Evaluation		Significant Improvement
			Pre-UPDRS*	Post-UPDRS*	
1.	M	65	4	2	• Activity of daily living improved → speech, handwriting, eating, cutting food • Hallucinations and delusions decreased • Motor examination improved → facial expression, rigidity, finger taps
2.	M	68	4	3	• Activity of daily living improved → sleep problems, cognitive impairment, speech • Motor examination improved → finger taps, hand movements

3.	M	53	3	2	• Activity of daily living improved → daytime sleepiness, eating, handwritting • Motor examination improved → facial expression, arising from chair, finger taps, hand movements
4.	M	77	4	4	-
5.	M	73	4	2	• Activity of daily living improved → speech, handwriting, eating, cutting food, dressing • Hallucinations and delusions decreased Motor examination improved → facial expression, rigidity, finger taps, hand movements, balance walking
6.	M	68	3	2	• Activity of daily living improved → sleep problems, cognitive impairment, speech • Motor examination improved → facial expression, finger taps, hand movements
7.	M	70	4	3	• Activity of daily living improved → daytime sleepiness, eating, handwritting • Motor examination improved → facial expression, finger taps, hand movements
8.	M	66	3	2	• Activity of daily living improved → sleep problems, cognitive impairment, pain and other sensations, speech

					• Motor examination improved → facial expression, finger taps, hand movements
9.	M	74	3	2	• Activity of daily living improved → sleep problems, depressed mood • Motor examination improved → facial expression, finger taps, hand movements
10.	M	66	3	2	• Activity of daily living improved → eating, cognitive impairment, speech, hanwritting • Motor examination improved → facial expression, finger taps
11.	M	68	4	4	-
12.	M	66	3	2	• Activity of daily living improved → daytime sleepiness, eating, handwritting • Motor examination improved → facial expression, arising from chair, finger taps, hand movements

* MDS-UPDRS Score:

0 = Normal

1 = Slight

2 = Mild

3 = Moderate

4 = Severe

Discussion

Stem cells, including adipose tissue-derived stem cells, have emerged as a key element of regenerative medicine therapies due to their ability to differentiate into a variety of different cell lineages. Their capacity of paracrine secretion of a broad selection of cytokines, chemokines, and growth factors make them highly clinically attractive. Adipose tissue-derived stem cells have been shown to have the capacity as anti-apoptotic, anti-inflammatory, immunomodulatory, anti-scarring effects, and proangiogenic, which make these cells promising candidates for cellular therapy in regenerative medicine (Laura *et al.,* 2016; Bertolini *et al.,* 2012).

Brain is control center of the body. This organ has a wide range of responsibilities from coordinating our movement to manage on emotion, the brain does it all. For almost hundred years, it has been a mantra of biology that brain cell do not regenerate so need to add new neuron when the brain injured. In this study, the source of neural progenitor cells we used from autologous adipose tissue by small lipopectomy, because neural progenitor cells high expressed from adipose derived compared with from bone marrow derived, with expression of Notch and L-Dopa (Brito *et al.*, 2012; Purwati *et al.,* 2017).

There is no standardised dose for stem cell therapy associated with the route of administration and the type of disease. For example, an overly high dose in intraparenchymal implantation can affect the nutrition of grafted cells and, if given intravascularly, cause micro-emboli and vessel occlusion (Wang L *et al.,* 2004). In this study, we used the dose of 2 x 10^7 stem cells with the intraventricular route applied directly into the intracranial space. This route makes the dose adjustment is more flexible, because it can be controlled by reducing the ventricular fluid if necessary based on the transplant dose. The risk of increased intracranial pressure and mass effects of the body can also be avoided. This dose was administered in 3 ml of fluid to avoid highly concentrated doses and excess fluid volume. No

complications, such as signs of increased intracranial pressure, infections or seizures were observed.

The ventricular system has thin walls composed of ependymal cells. The permeable properties of ependymal cells make it quite effective for the treatment of certain medicines, including stem cell therapy targeting the brain parenchyma (Bordey *et al.*, 2006; Kazania *et al.*, 2009). On the lateral ventricle, the ventricular walls are surrounded by the subventricular zone (SVZ), which continuously produces new neurons (Rosenbaum *et al.*, 2007). The location of the neurogenic niche area is very close to the lateral ventricle, which explains why the administration of stem cells through the intraventricular route is an effective method for stem cell therapy in this study. The lateral ventricles are easy to access, enabling direct stimulation of the SVZ. Moreover, cerebrospinal fluid is the endogenous regulatory factor of neuronal differentiation in neural regeneration, where the plexus choroideus produces substances during brain development or the regeneration process after brain injury (Falcao *et al.*, 2012).

The results in all subjects showed no decrease in neurological status and no complications associated with the actions and effects from stem cells. Some possible side effects that could be observed after treatment are increased intracranial pressure, seizures, infection and rejection reaction by the body. However, this study demonstrated that this technique is safe and reported no complications. One other advantage, the presence of the reservoir, facilitates repeated injections when applying booster therapy.

There are several effective mechanisms of action involved, including neural cells regeneration, neurons direct stimulation, and trophic paracrine mediators. There is evidence that growth factors like stem cell may help improve brain regeneration (Palisano *et al.*, 2006). Adipose tissue may generate neurons and other supportive cells. Transplanted adipose-derived neural progenitor cells infiltrate the brain and may help regenerate new elements or

combat the neurodegenerative process, fibrosis, and oxidative insults. Neuroprotection may involve release of several neurotrophic factors, that work through paracrine and/or-autocrine interactions.

The Unified Parkinson's Disease Rating Scale (UPDRS) is a comprehensive questions assessment of both motor and non-motor symptoms associated with Parkinson's Disease (Goetz *et al.*, 2007). The advantages of the UPDRS include its wide utilization, its application across the clinical spectrum of Parkinson's disease, its nearly comprehensive coverage of motor symptoms, and its clinimetric properties including reliability and validity. There is currently no cure for Parkinson's disease, several treatments have focused on relieving the symptoms. Current treatments include the use of oral preparations of L-3,4-dihydroxyphenylalanine (L-DOPA) and dopamine receptor agonists, apomorphine in more serious cases, continuous intestinal infusion of L-DOPA, and deep brain stimulation (DBS) in subthalamic nucleus and globus pallidus by using surgically implanted electrodes (Parisa *et al.*, 2015).

The underlying pathogenesis of Parkinson's disease is not fully understood, that's why developing new disease modifying therapies remains difficult. The ultimate idea is to “neuroprotect” and, in so doing, to interfere with the underlying pathogenic mechanism of nigral cell death and/or rescue damaged but still viable cell neurons. The motor and non-motor symptoms of this disease presumably would be arrested and possibly reversed if stem cells were utilized (Young HE *et al.*, 2013). In this study, autologous adipose-derived neural progenitor cells have the potential to revolutionize the treatment of disease by targeting dysfunctional tissues and to repair damaged tissues without the use of immunosuppressive therapy, thereby making new treatments possible without significant adverse side effects.

Acknowledgements

We would like to acknowledge the support of all laboratory technicians in Stem Cell Research and Development Centre, Airlangga University; and Cell and Tissue Bank, Dr. Soetomo General Hospital, Surabaya, Indonesia.

Potential Conflict of Interest

The authors declare that there is no conflict of interest.

References

Bertolini F, Lohsiriwat V, Petit JY, Kolonin MG. 2012. Adipose tissue cells, lipotransfer and cancer: a challenge for scientists, oncologists and surgeons. *Biochim Biophys Acta*;1826(1):209-14.

Bhasin A, Srivastava MVP, Kumaran SS, Mohanty S, Bhatia R, Bose S, Gaikwad S, Garg A, Airan B. 2011. Autologous Mesenchymal Stem Cells in Chronic Stroke. *Cerebrovasc Dis Extra*;1: 93-104.

Bordey A. 2006. Adult neurogenesis: basic concepts of signaling. *Cell cycle*;7: 722-728.

Brito C, Simao D, Costa I, Malpique R, Pereira C, Fernandes P, Serra M, Schwarz S, Schwarz J, Kremer E, Alves P. 2012. Generation and genetic modification of 3D cultures of human dopaminergic neurons derived from neural progenitor cells. *Methods*;56 (3): 452–60.

Carroll, William M. 2016. International Neurology. *John Wiley & Sons*;188.

Falcao AM, Marques F, Novais A, Sousa N, Palha JA, Sousa JC. 2012. The path from the choroid plexus to the subventricular zone: go with the flow!, *Front in cell neurosci*;6:1-8.

GBD 2015. Disease and Injury Incidence and Prevalence, Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and

injuries, 1990–2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet* 2016;388 (10053): 1545–1602.

Goetz, Christopher G, Fahn Stanley, Martinez-Martin Pablo, Poewe Werner, Sampaio Cristina, Stebbins Glenn T, Stern Matthew B, Tilley Barbara C, Dodel, Richard, Dubois Bruno, Holloway Robert, Jankovic Joseph, Kulisevsky Jaime, Lang Anthony E, Lees Andrew, Leurgans Sue, LeWitt Peter A, Nyenhuis David, Olanow C. Warren, Rascol Olivier, Schrag Anette, Teresi Jeanne A, Van Hilten, Jacobus J, LaPelle Nancy. 2007. Movement Disorder Society-sponsored revision of the Unified Parkinson's Disease Rating Scale (MDS-UPDRS): Process, format, and clinimetric testing plan. *Movement Disorders*;22 (1): 41–47.

Jankovic J. 2008. Parkinson's disease: clinical features and diagnosis. *Journal of Neurology, Neurosurgery, and Psychiatry*;79 (4): 368–76.

Kalia LV, Lang AE. 2015. Parkinson's disease. *Lancet*;386 (9996) 896–912.

Kazanis I. 2009. The subependymal zone neurogenic niche: a beating heart in the centre of the brain. How plastic is adult neurogenesis? Oppurtunities for therapy and question to be addressed. *Brain*;132: 2909-21.

Laura Frese, Petra E. Dijkman, Simon P. Hoerstrup. 2016. Adipose Tissue-Derived Stem Cells in Regenerative Medicine. *Transfus Med Hemother*;43(4): 268–274.

Lee YH, Choi KV, Moon JH, Jun HJ, Kang HR, Oh SI, Kim HS, Um JS, Kim MJ, Choi YY, Lee YJ, Kim HJ, Lee JH, Son SM, Choi SJ, Oh W, Yang YS. 2011. Safety and feasibility of countering neurological impairment by intravenous administration of autologous cord blood in cerebral palsy. *J Transl Med*;10:58.

Najm FJ, Zaremba A, Caprariello AV, Nayak S, Freundt EC, Scacheri PC, Miller RH, Tesar PJ. 2011. Rapid and robust generation of functional oligodendrocyte progenitor cells from epiblast stem cells. *Nat Methods*;8: 957–962.

NINDS. 2016. Parkinson's Disease Information Page.

Olanow, C. Warren., Stocchi, Fabrizio., Lang, Anthony E. 2011. The non-motor and non-dopaminergic fratures of PD. Parkinson's Disease: Non-Motor and Non-Dopaminergic Features. *Wiley-Blackwell*.

Palisano RJ, Cameron D, Rosenbaum PL, Walter SD, Russell D. 2006. Stability of the gross motor function classification system. *Dev Med Child Neurol*;48:424–428.

Parisa Goodarzi, Hamid Reza Aghayan, Bagher Larijani, Masoud Soleimani, Ahmad-Reza Dehpour, Mehrnaz Sahebjam, Firoozeh Ghaderi, Babak Arjmand. 2015. Stem cell-based approach for the treatment of Parkinson's disease. *Med J Islam Repub Iran*;29: 168.

Purwati, Sony Wibisono, Ari Sutjahjo, Askandar T.J, Fedik A. Rantam. 2017. Adipose-Derived Mesenchymal Stem Cells for Treatment Tertiary Failure Diabetes Mellitus Type 2. *Journal of Biomimetics, Biomaterials and Biomedical Engineering*;31: 91-95.

Rosenbaum P, Paneth N, Leviton A, Goldstein M, Bax M, Damiano D, Dan B, Jacobsson B. 2007. A report: The definition and classification of cerebral palsy. *Developmental Medicine & Child Neurology Supplement*;109: 8–14.

Samii A, Nutt JG, Ransom BR. 2004. Parkinson's disease. *Lancet*;363 (9423): 1183–93.

Sveinbjornsdottir S. 2016. The clinical symptoms of Parkinson's disease. *Journal of Neurochemistry*;139: 318–324.

Wang L, Zhang Z, Wang Y, Zhang R, Chopp M. 2004. Treatment of stroke with erythtropoetin Enhances neurogenesis and angiogenesis and improves neurological function in rats. *Stroke*;35: 1732-37.

Young HE, Hyer L, Black Jr AC, Robinson Jr JS. 2013. Treating Parkinson Disease with Adult Stem Cells. *J Neurol Disord*;1:121.