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EXTENDED ABSTRACT

Maternal Age Range and Parity as Predictors of Total Nucleated Cell Count in Umbilical Cord Blood

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SUMMARY

Umbilical cord blood (UCB) has been utilised as a source of stem cells for transplant and regenerative medicine purposes. The efficacy of stem cells in UCB can be predicted by total nucleated cell count (TNCC). This retrospective observational study investigated the association of maternal age and number of pregnancies with TNCC in UCB stored at National Blood Centre (NBC), Kuala Lumpur. The highest mean TNCC were found in 26-30 years age group and in primigravida. However, statistical analysis showed no significant association between maternal age and number of pregnancies with TNCC. In this study, we conclude that TNCC in UCB is not associated with maternal age and number of pregnancies. Further studies looking at other parameters such as gestational age, race and sex are warranted to look at factors determining UCB quality in Malaysian population.

Keywords: Maternal age, Number of pregnancy, Total nucleated cell count, Umbilical cord blood

INTRODUCTION

UCB has been utilized as a source of stem cell for transplant and regenerative medicine purposes. The efficacy of stem cells in UCB can be predicted by the number of TNCC. Higher TNCC is associated with a better outcome for patients who receive UCB transplantation and higher yield of stem cell for other purposes in regenerative medicine (1). The factors that influence the TNCC in UCB are not fully understood (2). This study investigated the association of maternal factors such as age and number of pregnancies with TNCC in UCB which has been stored at the NBC, Kuala Lumpur.

MATERIALS AND METHODS

This study was a retrospective analysis of data from 386, randomly selected cord blood donation stored in NBC from 2006 to 2022. The UCB were collected in maternity units of four hospitals under the Ministry of Health. The cord blood units were then sent to Cord Blood Bank in NBC for screening test, processing, and storage. The data included were maternal age, number of pregnancies, and the TNCC of UCB unit. Haematology analyser with flow cytometric principle was used to quantify TNCC per microlitre (µL) of the UCB unit. Data entry and statistical analysis were performed using Statistical Package for Social Sciences (SPSS) version 25 for window-software. A p-value of <0.05 was considered statistically significant.

RESULTS AND DISCUSSION

After inclusion and exclusion criteria 386 donations with complete data on maternal age and TNCC were included. However only 215 donations with complete data on number of pregnancy were included. The mean age was 29.1 ± 4.7 SD years (range, 18-47) while the mean number of pregnancies was 2.5 ± 1.7 SD (range, 1-12). Mean TNCC for the whole study was 1400.35 ± 452.07 SD (range, 656.90-3103.73). To determine the association with TNCC, maternal age was then categorised into 18-25, 26-30, 31-35 and more than 35 years. For number of pregnancies, only 5 and more were group together while the other numbers were analysed separately. When comparing among maternal age group, the highest mean TNCC, 1420.32 cells/µL was observed in the 26-30 age group. The analysis of mean TNCC by number of pregnancy showed that donation from first pregnancy yielded the highest mean TNCC (1580.28 cells/µL). However, from one-way ANOVA tests revealed there were no significant difference in mean TNCC between maternal age group (P=0.921) and between number of pregnancy (P=0.691) as shown in Table I.

Table I: Association between independent variables and mean TNCC

		Mean TNCC (SD) (cell/µL)	*t* stastistic (df)	*P*-value[a]
Age Group (years) n=386	18-25	1383.95 (430.63)	0.164 (3, 381)	0.921
	26-30	1420.32 (484.38)		
	30-35	1388.09 (418.84)		
	>35	1397.50 (477.50)		
Number of Pregnancy (times) n=216	1	1580.29 (485.46)	0.561 (4, 210)	0.691
	2	1564.46 (488.29)		
	3	1490.19 (383.22)		
	4	1458.25 (397.56)		
	≥ 5	1506.05 (435.75)		

[a] One-way ANOVA

CONCLUSION

Higher TNCC in UCB units has been associated with higher stem cell yield and better transplantation outcome. This study analysed whether the maternal age and number of pregnancies would influence the TNCC. The results of this study suggest that both have no association with TNCC in the stored UCB. These results are similar with previous studies which found both factors were not significant (4-6). However, we observed that younger maternal age (range 26-30 years) has higher mean, as suggested by other study done in Indonesia (3). Further research with larger sample size is needed to confirm the findings of this study. It is suggested in the future to include other donor factors which may influence the TNCC in order to improve the donor selection with higher TNCC.

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EXTENDED ABSTRACT

Autologous Eye Drop in University Malaya Medical Centre for Ocular Surface Disorders

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SUMMARY

Autologous eye drops (AED) are customized eye drops that are prepared from a patient's own blood serum or plasma. These eye drops offer a therapeutic option in a second-line treatment when standard treatment modalities fail for various ocular surface disorders (OSD) including epithelial defects caused by Stevens–Johnsons syndrome (SJS). The preparation of AED requires strict criteria in terms of the facilities for production, specialized equipment requirement and maintenance of sterility during the production chain. The use of AED in our patient demonstrated substantial improvement in symptoms and gradual closure of the epithelial defect.

Keywords: Autologous Eye Drop, AED, Ocular surface disease, OSD, Steven-Johnsons Syndrome, SJS

INTRODUCTION

Autologous eye drops (AED) are customized eye drops prepared from a patient's own blood serum or plasma. These eye drops offer therapeutic option for various OSD including epithelial defects which present a significant clinical challenge particularly when a conventional therapy fails and causes non-healing epithelial defects (1). Conditions that attribute to this include SJS, severe dry eye disease, herpetic infections, neurotrophic keratopathies or keratoconjunctivitis sicca (example; Sjugren's syndrome). The use of AED was first reported in 1975 (2) and has gained popularity in 1990s as a second-line treatment of OSD.

Here, we present our first case of autologous eye drop preparation in University Malaya Medical Centre (UMMC). We received a request for autologous eye drop production for a 56-year-old man with a non-healing epithelial defect in both eye. The patient has history of SJS causing epithelial defect and had undergone standard treatment modalities (1) including moisturizing eye drops, oral prophylaxis antibiotic, epithelial debridement and tarsorrhaphy which showed no significant improvement. These therapies are limited in supplying the neurotrophic factors, vitamins, and immunoglobulin necessary for the health of the ocular surface. Hence AED was suggested for him. The preparation of AED requires specialized equipment and facility which our center was able to comply (3).

MATERIALS AND METHODS

The patient/donor was assessed and met the blood collection criteria (1,3) and fit to donate one unit of blood. A sterile blood collection process was employed to obtain 350mls of donor's blood using sterile blood bag that meets ISO 9000 standards. The collection bag contains Citrate Phosphate Dextrose Adenine (CPDA-1) anticoagulant4. Post collection, blood bag was centrifuged by using soft spin at 20°C-24°C to obtain platelet rich plasma (PRP) (3) which will be used as AED. This centrifuged blood bag was placed on a manual plasma extractor and PRP was extracted into a satellite bag. The extracted PRP was connected to a sterilized tygon tube, which was ten feet in length tubing by using a sterile docking device (Figure 1). Tygon tubing

Figure 1: Collage of images describing the preparation of autologous serum e1ye drop. (A) Blood bag collected from donor; (B) Blood bag centrifuged to separate PRP and red cell; (C) PRP extracted in a satellite bag leaving red cells in primary bag; (D) Extracted PRP allowed to flow; (E) Tygon tube is sealed in 1 inch size each; (F) All tube sealed and frozen to -25°C.

containing PRP was sealed in one inch's segment and frozen to -25°C. Once the virology and serology results were negative (3), the eye drops was dispensed to the patient.

RESULTS AND DISCUSSION

The ophthalmologist prescribed eight times a day usage of AED for three months in our patient. The prepared AED has shelf life of twelve months from the blood collection date. The frozen AED is thawed overnight at 4°C before daily use. Over the course of four weeks, the patient demonstrated substantial improvement in symptoms and gradual closure of the epithelial defect. Many case reports and randomized controlled clinical trials have outlined favorable outcome with the use of AED (4,5). As corneal epithelial defect healing is a complex process, the rationale behind using AED is based on the beneficial properties present in the plasma which is similar to natural tear (1). The composition of autologous plasma enriched with growth factors such as epidermal growth factor (EGF), transforming growth factor-beta (TGF-β), fibroblast growth factor (FGH) and platelet-derived growth factor (PDGF) gives an ideal microenvironment for corneal epithelial healing, cell migration and tissue regeneration. However, the safety concern associated with AED is the potential for microbial growth during preparation and storage, which can serve as a breeding ground for microorganisms. This microbial contamination poses a substantial risk of infection in patients who have a compromised ocular surface. Hence, adherence to standard procedure using a sterile manner when preparing this eye drops is important. Patient receiving AED should be compliant towards appropriate care and instructions given in order to minimize risk of contamination (5).

CONCLUSION

This is our first experience of preparing AED using PRP in a sterilized tygon tube. In view of availability of resources and advanced processing facility in our center, we aim to establish user friendly AED utilizing autologous serum as an alternative to treatment for OSD in future.

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EXTENDED ABSTRACT

Differentiation of Cord Blood-derived Induced Pluripotent Stem Cells for Regenerating the Ocular Surface

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SUMMARY

Contemporary corneal regenerative strategies have focused on stem cell therapy to repopulate the cornea epithelial cells with the aim to restore the integrity and function of the cornea. Human induced pluripotent stem cells (hIPSC) have the potential to become autologous therapy for the cornea due to ease of source access from adult fibroblast, peripheral blood or the skin. A relative smaller transplantable tissue is required to regenerate the corneal surface. However, a standardized protocol is necessary to increase the efficiency to be a regenerative therapy. In this study, a modified protocol from previous investigations has successfully promoted cornea epithelial-like cells formation.

Keywords: Ocular surface regeneration, Cornea stem cells, Human induced pluripotent stem cells, Cord blood stem cells

INTRODUCTION

The ocular surface is constituted of the cornea, limbus and conjunctiva. The corneal limbal stem cells which are located at the cornea periphery are important for maintenance of the structure and the function of the eye. A disorder known as limbal stem cell insufficiency (LSCD) is brought on by damage to the ocular surface, namely to the cornea and limbus (1). Innovative tissue-engineered tools using ex vivo expansion of cornea epithelial cells or mesenchymal stem cells have been transplanted as a potential treatment. However, the downside of the techniques limits its use to unilateral case and possible immune rejection occurring in allogeneic transplantation. hIPSCs have tremendous potential as an alternative unlimited autologous source of bio-replacement tissue for multiple investigative purposes in the cornea. Here we present data on modifying signalling factors involved from culture maintenance and differentiation and maturation of IPSC-derived cornea epithelia-like cells formation.

MATERIALS AND METHODS

Cord blood human IPSCs (Gibco) were maintained in matrigel-coated plates with miltenyi medium for two days. Rho kinase inhibitor, Y27632 was added for the first 24 hours. Briefly, four Groups with various combination of small molecules were added from Day 0 to Day 3. All the groups were treated with the complete medium and changed daily. Retinoic Acid (RA) (1 µM), Bone Morphogenetic Protein-4 (BMP4) (25 ng/ml) and Epidermal growth factor (EGF) (10ng/ml) was added to the maintained hIPSC. The growth and expansion of progenitors were maintained by supplementing complete media daily. The basal medium of low glucose DMEM/F12 (1:1) is supplemented with N2, B27, non-essential amino acids, glutamine and penicillin/streptomycin solution which consist of essential nutrients needed for the optimal cell growth as a substitute to serum. Cells in cultures, plates, and flasks were observed using an inverted phase contrast microscope (Figure 1). Immunocytochemistry and flow cytometry were performed on D10 and D20 for characterisation.

RESULTS AND DISCUSSION

Retinoic Acid, BMP4 and EGF in a cocktail preparation showed significant upregulation of the peripheral as well a nuclear corneal markers. Putative limbal stem cells marker (ΔNp63) and PAX6, marker of corneal protein and neuroectodermal of the developing eye and lens respectively, demonstrated a significantly higher expression (Figure 2). This cocktail combination (RBE) was utilized for further differentiation to obtain quantitative corneal marker expression by flow cytometry (Figure 3).

Our differentiation protocol led to changes in morphology as well as expression of the cornea epithelial stem cell markers and the terminally differentiated epithelial differentiation markers, CK3 and

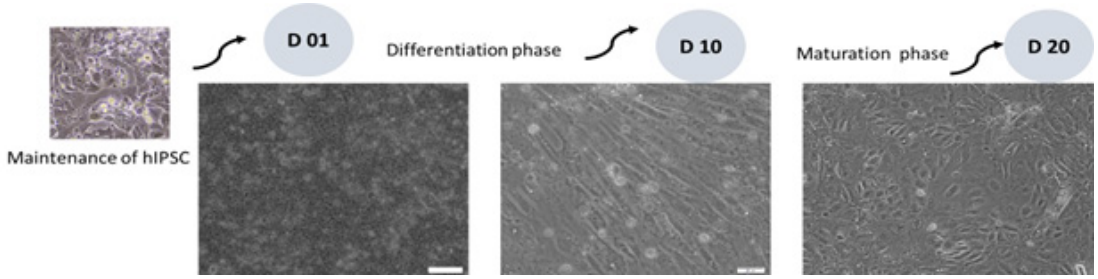


Figure 1: Phase contrast microscopy depicting the two-stage differentiation phase (Day 10) and maturation phase (Day 20) from maintenance of human cord blood-iPSC (Day 1) in the development of cornea epithelia-like cells. Scale bar=20 µm.

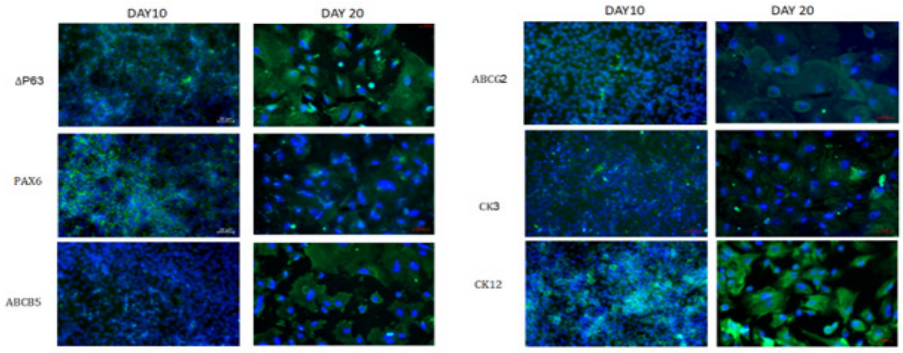
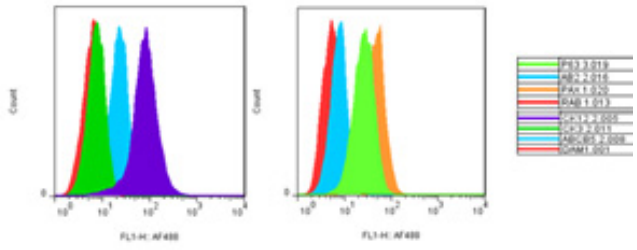


Figure 2: Characterisation of corneal markers expression at differentiation phase (D10) and increased expression shown at maturation stage (D20) for ΔNP63, PAX6, ABCB5, ABCG2 and CK3/CK12 by modulations of signalling factors. Scale bar = 20µm, green – Alexa fluor, Blue = DAPI.

CK12. The two-stage protocol involves differentiation stage to induce endodermal lineage formation and surface ectoderm specification using small molecules as previously described (2,3,4). The second stage was designed to refine for corneal epithelial differentiation and allowing it to acquire native corneal epithelial maturation stage using collagen IV, culture media and specific morphogens.



Corneal markers	Mean % of Positive cells (Gated on DAM/DAR 488)
ABCG2	41.9
PAX6	78.9
P63	77.4
ABCB5	89.4
K3	5.2
CK12	96.9

Figure 3: Mean percentage of corneal markers expression of differentiated cells by flow cytometry

CONCLUSION

A two-staged protocol by modifying the signalling factors followed by promoting cellular maturation in appropriate culture conditions was developed in this study. IPSC-derived cornea epithelial-like cells were successfully characterised by increased corneal markers expression. We further aim to develop standard methods for efficient corneal epithelial differentiation to be clinically applied for corneal regenerative medicine.

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EXTENDED ABSTRACT

Experience of Harvesting Lymphocyte for CAR-T Cell Therapy in University Malaya Medical Centre During the COVID Pandemic

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SUMMARY

Chimeric Antigen Receptor (CAR)-T cell therapy is a personalised immunotherapy where a patient's T cells are genetically modified to target cancer cells. This case study describes the first lymphocyte harvesting experience for CAR-T cell therapy, harvested at the University Malaya Medical Centre (UMMC) in Malaysia. Due to travel restrictions caused by COVID-19, patient was unable to travel abroad and hence the T cells were collected in UMMC and sent to a specialized facility in Singapore for genetic modification and expansion before being infused back into the patient. The study also discusses the limitations associated with CAR-T cell therapy, including production failures, scalability challenges, high costs, adverse events, vein to vein time, patient eligibility, and healthcare practitioner challenges. The conclusion highlights the financial viability and the importance of learning and improving CAR-T cell therapy for better patient care in Malaysia.

Keywords: CAR-T, Apheresis, Chimeric Antigen Receptor T-cell Lymphocyte

INTRODUCTION

CAR-T cells are a form of personalised immunotherapy. Patient's T cells are genetically modified outside the body to express chimeric antigen receptors that recognise cancer cells in various haematological malignancies including lymphoma, leukaemia and multiple myeloma (1,2). This modification takes place in a specialised facility, where the modified T cells are then multiplied and infused back into the patient. The patient's own T cells subsequently target and attack the cancer cells (3).

A 6 year old boy was diagnosed with relapsed pre B acute lymphoblastic leukemia (ALL) and underwent apheresis to collect T cells for CAR-T engineering. Prior to this, he had received multiple rounds of chemotherapy and undergone a stem cell transplant at National University Hospital (NUH) in Singapore. Due to the failed transplant and evidence of MDR1 RNA positive, CAR-T cell therapy was planned.

MATERIALS AND METHODS

We present the first case of lymphocyte harvesting for CAR-T cell done in University Malaya Medical Centre. This procedure is usually performed in Singapore, but due to travel restrictions, patient had to undergone harvesting locally and CAR-T infusion in our centre.

Prior to harvesting, infectious disease screening was conducted and all yielded negative results. Additionally a bone marrow aspirate was performed prior to procedure to assess the progression of disease and the bone marrow sample was sent along with the lymphocyte harvested. Harvesting was performed under sedation. Considering the patient's weight of 18kg, priming was necessary to prevent significant hemodynamic changes caused by the extracorporeal volume of apheresis machine. Priming was done using a leucodepleted and irradiated whole blood unit before the procedure. The total blood volume taken was calculated as 80mls/kg.

RESULTS AND DISCUSSION

The T cells were collected using an OPTIA machine with an IDL set. The collected samples were sent via a courier to NUH in Singapore twice. The first harvest took place on the September 15, 2022, but unfortunately it failed due to leaking of product when arrived at manufacturing facility, resulting in the inability to proceed with CAR-T cell generation. The second harvest occurred two months later , and this time it went smoothly. The collected lymphocyte product , with a total volume of $15.2x10^6$ mononuclear cells and a volume of 189mls , was sent to NUH in Singapore. Both products were transported with strict cold chain with products maintained at room temperature and took 6 hours to reach NUH.

At NUH, the T cells were genetically modified to express a chimeric antigen receptor (CAR) targeting CD19. Following modification, the T cells were expanded to desired dose and then infused back into a patient.

The complex manufacturing process of generating autologous CAR T cells carries limitations and risks. The personalized nature of this therapy increases the likelihood of production failures, causing delays and denying access to patients. Meeting commercial demand and expanding geographically while maintaining product quality and clinical equivalence poses scalability challenges. The high risk cost of CAR-T cell therapies and complicated payer policies further limit access. As a result, the queue of patients awaiting insurance clearance continues to grow (4).

There are also several limitations directly affect patients receiving CAR-T cell therapy. The treatment is associated with life-treating adverse events, including cytokine release syndrome (CRS) and CAR T – related enchephalopthy syndrome (CRES) , necessitating close patient monitoring and adherence to safety protocols. The manufacturing process results in a significant wait time, known as vein-to-vein time, which can be challenging for patients with rapidly progressing diseases. Furthermore, eligibility for CAR-T cell therapy is restricted to heavily pretreated patients, limiting the pool of qualified individuals. Healthcare practitioners face challenges with complex patient referral pathways, the accreditation of speciality centres and uncertainty regarding the placement of CAR T cells in treatment practice (5).

CONCLUSION

Outsourcing CAR-T cell engineering to Singapore whilst harvesting and infusing here in Malaysia is a financially viable alternative. It also emphasize the need of local CAR-T manufacturing facility to further improve the care our cancer treatment.

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EXTENDED ABSTRACT

Granulocyte Transfusion (GTX) for severe neutropenic patient, which acquired using Pooled Granulocytes Concentrates (PGC) method in University Malaya Medical Centre (UMMC)

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SUMMARY

Granulocyte transfusion (GTX) is an adjunctive treatment for neutropenic patients with severe and uncontrolled infections. Granulocytes can be prepared by pooled granulocytes concentrates from whole blood buffy coats (PGC) or apheresis granulocytes concentrates (AGC) methods. PGC method is associated with lesser donor risk and its yield is not inferior to AGC method. In this case, a paediatric patient with Acute Myeloid Leukaemia (AML) was given a GTX that was prepared using the PGC method. The GTX was successful in improving the patient's condition and the patient was able to be discharged three weeks later.

Keywords: Granulocyte transfusion (GTX), Pooled granulocytes concentrates (PGC), Treatment of severe neutropenia, Granulocyte product

INTRODUCTION

Granulocyte transfusion (GTX) may be considered the oldest form of cellular therapy whereby infusion of buffy coat preparation to treat neutropenia was initially reported back in 1934 (1). Nowadays aggressive chemotherapy and bone marrow or hematopoietic stem cell transplantation are being utilised to treat an increasing range of clinical diseases (2). The most common side effect of these therapies is neutropenia, when the Absolute Neutrophil Count (ANC) drops less than 1.5 x 10^9/L, infection is likely to occur if ANC is less than 0.5 x 10^9/L (1). GTX can be used as adjunctive treatment in neutropenic patients with severe and uncontrolled infections where several studies provide evidence for its safety and efficacy (2, 3, 4). However, GTXs are not without risks to the patient, including febrile reaction, allo-immunisation, and a potentially fatal complication is transfusion-related acute lung injury (TRALI) (3). Granulocytes can be prepared by pooled granulocytes concentrates from whole blood buffy coats (PGC) or apheresis granulocytes concentrates (AGC) methods. PGC method in acquiring granulocyte product is associated with lesser donor risk and its yield is not inferior to AGC method (1). Our department received an urgent request for granulocyte transfusion for a paediatric case of Acute Myeloid Leukaemia (AML) presented with neutropenic fever post chemotherapy.

MATERIALS AND METHODS

Granulocyte product acquired through PGC method. Regular blood donors with the same blood group as the patient were enumerated in a list (six donors) by the paediatric team and the donation date and time was decided. A specific batch in donor management system designated for these donations, and expedition of serological infective screening and Nucleic Acid Tests (NAT) done by liaising with the microbiology department. Figure 1 illustrates some processing steps of granulocytes concentrates. Whole blood donations were centrifuged at ambient temperature (20 to 24 degrees Celsius) using soft spin centrifugation and the buffy coat layer containing granulocytes were carefully separated into a satellite bag using a manual blood separator (1). Subsequently, cross-matching granulocyte product with patient's plasma was done, and cross-matched compatible granulocyte product was stored on the workbench without agitation at ambient temperature. Granulocyte product was then labelled and underwent irradiation with minimum dose 25Gy by gamma irradiator prior to issuing (3).

RESULTS AND DISCUSSION

GTX was given on top of continuing administration of antimicrobials and Granulocyte colony-stimulating

Figure 1: Collage of images describing the preparation of pooled granulocyte concentrate. (A) Donations of whole blood that used for PGC; (B) Tubes sent for serological infective screening and NAT; (C) Separation of blood components done via soft spin; (D) Buffy coat layer separated using manual separator into satellite bag; (E) Dose calculation worksheet; (F) Granulocyte product irradiated and labelled for issuing

factor (G-CSF). PGC has advantage over AGC as for apheresis a single donor is utilised for harvesting an immense amount of granulocyte. Hence, the apheresis donor is required to be subjected to a mobilisation regime via G-CSF that carries risk of adverse effects to the donor (5). Pooling method (PGC) eliminates this by employing multiple donors for harvesting the amount required for the appropriate dose for the patient. Main drawback of PGC is the increased risk of alloimmunisation. Irradiation prior infusion is paramount to substantially reduce the risk of Transfusion Associated Graft vs Host Disease (TA-GvHD) (3), by rendering lymphocytes incapable of dividing or attacking host tissues. Transfusion of granulocytes was done within 24 hours from collection (as the lifespan of granulocytes around 24 hours) and infused through a standard integral blood filter (170-240micron). It is vital for GTX to use the correct infusion set, as microaggregate filters (10micron) or leucodepletion bedside filters (4micron) may arrest the granulocytes on the filters resulting in inadequate doses of GTX given to the patient. Dosage achieved from pooling of 6 buffy coats typically yields approximately 5.5 x 10^9 granulocytes/unit, where the patient required a dose of 0.3 x 10^9 granulocytes/kg (3). As the patient weighs 18kg, the minimal dose required was deemed successfully achieved. Subsequently, patient showed clinical improvement and was able to be discharged three weeks post GTX. Patient was discharged home with oral medications (antibiotic, antifungal, and antipyretics), and advised for home care monitoring.

CONCLUSION

The mode of collection for granulocyte products must be considered, either PGC or AGC (1). GTX can cause multiple adverse effects to the patient (2, 3, 5). It's worth noting the efficacy is limited to case reports (4,5). Hence it remained being utilised sparingly in view of these and should be decided upon case-by-case basis.

ACKNOWLEDGEMENT

The authors would like to thank all the dedicated individuals that contributed to this case report and want to declare that there was no conflict of interest in this study.

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EXTENDED ABSTRACT

Mitogenic Effects of Induced Magnetic Field on Human Umbilical Cord-Derived Mesenchymal Stem Cells

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SUMMARY

This study examines the impact of induced magnetic fields on human umbilical cord-derived mesenchymal stem cells (hUC-MSCs). It investigates the growth kinetics, population doubling time (PDT), and expression of pluripotent markers (OCT4, Rex1, Sox2, and Nanog) in hUC-MSCs under direct and indirect magnetic field exposure. The results indicate that an MF with an intensity of 21.6 mT enhances the in vitro proliferation of hUC-MSCs. These findings hold potential to advance regenerative medicine applications.

Keywords: hUC-MSCs, Magnetic field, Growth kinetics, Population doubling times, Pluripotent markers

INTRODUCTION

Human umbilical cord-derived mesenchymal stem cells (hUC-MSCs) are highly promising for regenerative medicine. Lately, non-invasive methods utilizing induced magnetic fields have gained significant attention as a way to influence the growth of cells (1). In this study, the focus is on investigating the impact of induced magnetic fields on the growth kinetics and PDT of hUC-MSCs. Early-passage hUC-MSCs were subjected to predetermined MF conditions, and their growth kinetics were assessed on cell viability (2). This study also analyzes the expression of pluripotent markers such as *OCT4, REX1, SOX2*, and *NANOG* using reverse transcription-polymerase chain reaction (RT-PCR) (3). This study's findings could improve regenerative medicine by advancing our understanding of how induced magnetic fields affect hUC-MSCs.

MATERIALS AND METHODS

This study utilized hUC-MSCs, which were divided into three groups: direct exposure (DE), positioned between magnets with an MF intensity of 21.6 mT; indirect exposure (IE), placed in the upper compartment of the DE group; and negative control (NC), kept in a separate incubator without MF exposure. Samarium-cobalt (SmCo5) was used to induce a magnetic field with uniform distribution over the cell cultures. The growth kinetics of hUC-MSCs were investigated, and the cell counts were determined using the trypan blue exclusion test at various time points. The population doubling time (PDT) was calculated using the Patterson Formula. RT-PCR analysis was performed to assess the expression of pluripotent markers, using specific primers for *GADPH, REX1, SOX2, OCT4*, and *NANOG*. The band intensity was quantitated using the Image J™ software, normalized against the reference gene (*GADPH*), and presented as fold change (4).

RESULTS AND DISCUSSION

Figure 1(a) and 1(b) show the growth kinetics of the hUC-MSCs, indicating the effect of direct and indirect MF exposure on cell viability. Meanwhile, figure 1(c) illustrates the PDT of the MF-exposed (DE and IE) as well as the control (NC) hUC-MSCs from passage 1 to passage 6. As shown in figure 1(a), a similar growth pattern was observed, starting with the lag phase, followed by the phase of exponential growth between day 4 and 10, and eventually reaching the stationary phase for both the cells in direct and control groups. In figure 1(b), a comparison of the cell count at day 4 (duration of the lag phase) and day 10 (exponential phase peak) was made between DE, IE, and NC groups. Meanwhile, figure 1(c) demonstrates that the PDT decreased as the cells expanded up to passage 4, after which it plateaued until the 6th passage.

Figure 2(a) shows the RT-PCR product on agarose gel-

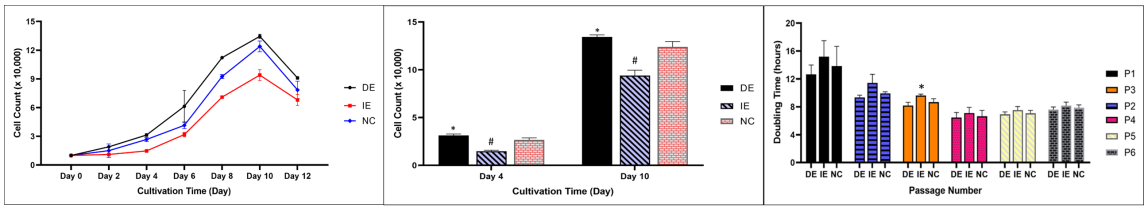

Figure 1: (a) and (b) shows the growth kinetics of the hUC-MSCs. (c) shows the population doubling time. * shows a statistically significant increase in cell count compared to the NC while # shows a statistically significant decrease compared to the NC ($p < 0.05$).

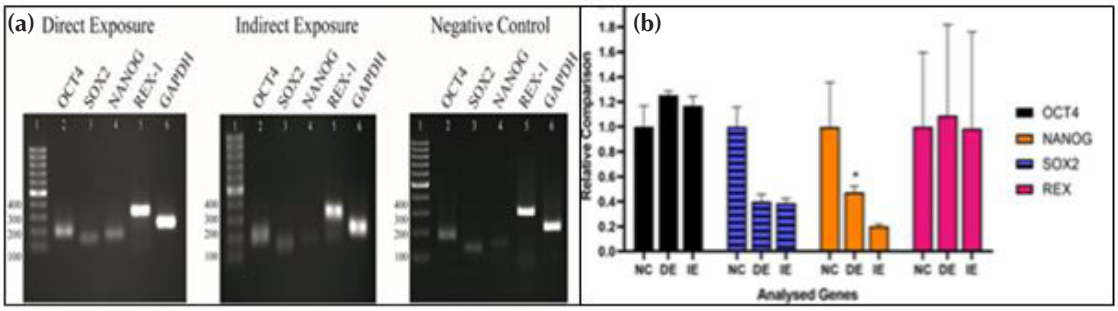

Figure 2: (a) shows the intensity of the markers expressed in undifferentiated MSCs. (b) shows fold change showing the upregulation of OCT4 in both the DE and IE groups with expression levels higher in the DE group relative to the IE group. #The SOX2 gene was significantly downregulated in the DE and IE groups, *while NANOG was significantly upregulated in DE (2.73-fold change) relative to IE groups ($p<0.05$)

electrophoresis in undifferentiated MSCs. Meanwhile, Figure 2(b) illustrates the fold change, indicating the upregulation of *OCT4* in both the DE and IE groups, with higher expression levels observed in the DE group compared to the IE group. Additionally, as depicted in Figure 2(b), the *SOX2* gene was significantly downregulated in both the DE and IE groups, whereas *NANOG* was significantly upregulated in the DE group (2.73-fold change) relative to the IE group.

CONCLUSION

The current study demonstrated that an MF with an intensity of 21.6 mT stimulated in vitro proliferation and enhanced the propagation of hUC-MSCs. The increased gene expression of NANOG amongst other associated pluripotent markers such as OCT4, SOX2, and REX1, may indicate a potential adverse event.

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EXTENDED ABSTRACT

Generation and Characterisation of Mesenchymal Stem Cells Expressing Trail

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SUMMARY

Adipose-derived Mesenchymal Stem Cells (ADMSCs) expressing TNF-related apoptosis inducing ligand (TRAIL) (ADMSC- TRAIL) have the ability to eliminate lung cancer cells. Hypothesis: TRAIL expressing MSCs from other sources could be more efficient in cancer killing ability. Aim: To generate AD, Umbilical Cord (UC) and Bone Marrow (BM) -derived MSCs expressing TRAIL and characterize these cells post-modification. Results: Higher concentration of TRAIL was detected from putative UCMSC-TRAIL than putative BM and AD- MSC-TRAIL. The putative AD and UC MSC-TRAILs exhibited MSC differentiation potential and MSC surface markers expression. The apoptotic efficacy of these engineered MSCs can be compared in future studies.

Keywords: Mesenchymal stem cells, TRAIL, Genetic engineering

INTRODUCTION

TRAIL has been the subject of interest in cancer therapy due to its ability to target cancer cells while sparing normal cells (1). However, several clinical trials have shown that recombinant human TRAIL has transient serum half-life, low bioavailability (2) and unable to reach tumour sites. One possible solution is using MSCs as a vector to deliver TRAIL to cancer cells. Studies have shown that MSCs expressing TRAIL (MSC-TRAIL) were able to eliminate lung cancer cells efficiently. However, the MSC-TRAIL obtained in the previous study were from the adipose tissue (3,4). This raises the possibility of utilising other sources of MSCs, and whether MSC-TRAIL from other sources could be more potent in inducing apoptosis. Therefore, we aim to generate adipose (AD), bone marrow (BM) and umbilical cord (UC)-derived MSC expressing TRAIL and to characterize these cells post-modification, so the evaluation of the apoptotic efficacy of these engineered MSCs can be performed.

MATERIALS AND METHODS

Umbilical cord MSCs were donated by Rajesh Ramasamy, Pathology Department, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia. Human adipose-derived MSCs were purchased from American Type Culture Collection while human bone marrow-derived MSCs were purchased from Centre for Tissue Engineering and Regenerative Medicine, Faculty of Medicine, Universiti Kebangsaan Malaysia. The vectors pLVX-EF1α-TRAIL-mCherry and pLVX-EF1α-empty vector (EV)-mCherry have been described by Fakiruddin et al, 20194. The lentivirus was produced by transfecting 293FT with the Lentiviral plasmids using Lipofectamine3000 (Thermofisher) to generate LV-TRAIL-mCherry and LV-EV-mCherry. Then, the LVs were transduced into respective MSCs with polybrene. The mCherry fluorescent was compared between TRAIL-transduced AD-, BM- and UC-derived MSCs to determine percentage of positively-transduced cells. The TRAIL expression from the engineered MSCs was quantified through flow cytometry and ELISA (Raybiotech). The TRAIL-transduced AD and UC-MSCs were then characterised through FACS analysis of CD markers and differentiation assay kit (Gibco, Thermo Fisher Scientific).

RESULTS AND DISCUSSION

Analysis of transduction efficiency by flow cytometry exhibited higher percentage of mCherry-positive cells

detected in putative UCMSC-TRAIL (93.86 ± 0.21 %) compared to putative ADMSC-TRAIL (37.32 ± 3.01 %) at 48 hours post-transduction. Interestingly, the percentage of mCherry-positive cells detected in putative ADMSC-TRAIL significantly increased (88.16 ± 0.33 %) at 72 hours post-transduction. Generally, the TRAIL expression analysis shows that the putative AD-, UC- and BM- MSC-TRAIL exhibited higher TRAIL expression than the control cells. The highest concentration of TRAIL protein was detected in the protein lysate of putative UCMSC-TRAIL (15874.67 ± 626.26 pg/mL) compared to putative BMMSC-TRAIL (9101.333 ± 1059.05 pg/mL) and putative ADMSC-TRAIL (5292.33 ± 290.36 pg/mL), empty vector (MSC-EV) and wild-type MSCs using ELISA. However, significantly higher TRAIL protein was also detected in the conditioned medium of putative BMMSC-TRAIL (1602.667 ± 315.04 pg/mL) compared to putative ADMSC-TRAIL (462 ± 6.56 pg/mL) and putative UCMSC-TRAIL (1354.67 ± 40.19 pg/mL) using ELISA (Figure 1). The high expression of TRAIL in protein lysate from the engineered MSCs implies that the TRAIL was expressed as a cellular or membrane bound protein, and less as a soluble protein. Moreover, all the transduced MSCs retained their original characteristics post-transduction, such as morphology and ability to adhere to plastic. Furthermore, the putative ADMSC-TRAIL and putative UCMSC-TRAIL were able to differentiate into adipocytes, osteocytes, and chondrocytes (Figure 2), and maintained their MSC surface markers expression (CD44, CD90, CD105, and CD73) as analyzed by flow cytometry (Figure 3), indicating that the MSC-TRAILs maintained their multipotent characteristics post-transduction.

CONCLUSION

In conclusion, these analyses verified that the ADMSC-TRAIL, BMMSC-TRAIL and UCMSC-TRAIL have been generated. To further compare the potency of TRAIL-transduced MSCs in cancer therapy, functional assays incorporating the MSC-TRAIL and NSCLC cell lines can be performed in the future.

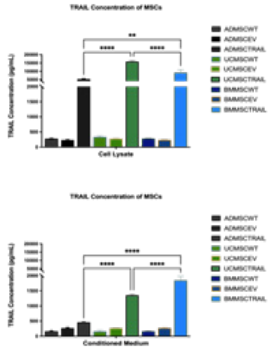

Figure 1: ELISA results of TRAIL concentration, using one-way ANOVA analysis (p<0.0001)

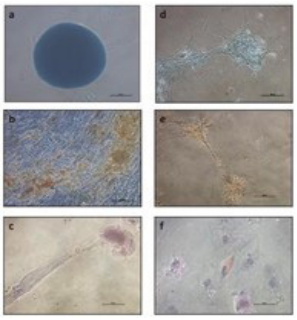

Figure 2 (a-f): MSC-TRAIL differentiation; (a-c) shows chondrogenesis, osteogenesis and adipogenesis of UCMSC-TRAIL respectively; (d-f) shows chondrogenesis, osteogenesis and adipogenesis of ADMSC-TRAIL respectively.

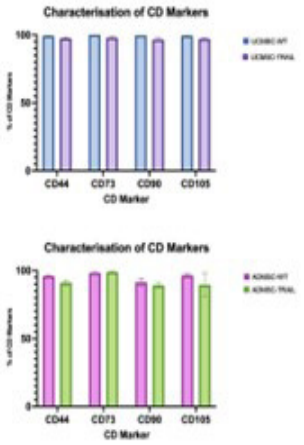

Figure 3: MSC surface markers, cluster of differentiation (CD44, CD90, CD105, and CD73) analysis from MSC-TRAIL and MSCs by flow cytometry (p > 0.01; t-test)

ACKNOWLEDGEMENT

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EXTENDED ABSTRACT

Elucidating Disease-Specific Phenotype in IPSC-Derived Neuronal Model of Mitochondrial Disease-Causing ND6-Mutation

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SUMMARY

Mitochondrial disease is a progressive neurodegenerative disorder hallmarked by dysfunction in normal mitochondrial metabolism. ND6-mutation is a pathogenic mutation causing mitochondrial disease. Limitation in understanding disease mechanism is hampered due to poor corelation between genotype and disease phenotype particularly in the absence of a tissue-specific model. In this study, induced pluripotent stem cells (iPSC)-derived neuronal cells carrying ND6-mutation was generated by reprogramming of primary human dermal fibroblasts. The iPSC-derived neuronal model developed was characterized, showing high pluripotency and differentiation potential. The developed model was used to study disease specific phenotype by demonstrating impaired mitochondrial function.

Keywords: Mitochondria, ND6, Induced Pluripotent Stem Cells, Neural Stem Cells, Neurons

INTRODUCTION

Mitochondrial disease is a multisystem disorder caused by inherited or spontaneous mutations leading to altered mitochondrial metabolism, affecting high energy dependent organs such as the brain. Mutations in mitochondrial DNA encoding the ND6 gene (m.14487T>C) is a recognized diagnostic mutation causing mitochondrial disease. Pathogenesis of disease is poorly understood due to limitations of current disease models. The discovery of iPSCs derived from adult somatic cells and cell-specific differentiation protocols has allowed researchers to create in-vitro models that are able to study disease phenotypes in organ systems that were previously inaccessible. The generation of iPSC-derived in-vitro models has allowed investigation into the pathological mechanism of diseases in a specific target. These models can help identify targets for treatment and therapeutic interventions. This study aimed to generate iPSC-derived neural stem cells (NSCs) from fibroblasts obtained from a patient carrying ND6-mutation and analyse the mitochondrial function in these NSCs and maturing neurons.

MATERIALS AND METHODS

Human dermal fibroblasts from a patient diagnosed with mutation in the ND6-gene (m.14487T>C) was reprogrammed into iPSCs by lentiviral transduction of six transcription factors, Oct4A, Sox2, Klf4, c-Myc, Nanog and Lin28. Three iPSC clones were expanded in feeder-free culture system and characterized for pluripotency by immunophenotyping and tri-lineage differentiation potential. iPSC clones were differentiated into neural stem cells (NSCs) and subsequently neurons by defined differentiation medium containing small molecules. Differentiated NSCs and neurons were characterized by immunophenotyping with neural specific markers. The effect of ND6-mutantion in iPSCs and NSCs were compared to two healthy control iPSCs and NSC clones by measuring mitochondrial function. Mitochondrial function was measured by image cytometry analysis of mitochondrial membrane potential activity measured by Mitotracker® Red CMXRos intensity and production of ATP measured by luminescence assay. Viability of NSCs undergoing maturation into neurons was measured by spectrophotometric analysis.

RESULTS AND DISCUSSION

Human dermal fibroblasts carrying the ND6-mutation (m.14487T>C) can be reprogrammed into iPSCs and highly expressed pluripotency markers Oct4A and Tra-1-81. Spontaneous differentiation of embryoid bodies formed by ND6-mutant iPSCs exhibited mixed cell morphology staining positive for alpha-fetoprotein (AFP), smooth muscle actin (SMA) and BIII-tubulin (TUJ1), markers of endoderm, mesoderm, and ectoderm derived cells respectively. ND6-iPSCs can efficiently be differentiated into NSCs expressing neural stem cell markers, Nestin and SOX2. The differentiated NSCs retained the original mutant genotype as parental cells. NSCs subjected to neural maturation protocols exhibited markers of mature neurons, expressing BIII-tubulin and MAP2. Analysis of mitochondrial function in ND6-mutant iPSCs showed impaired ATP production. Mitochondrial membrane potential was increased in ND6-mutant iPSCs compared to controls, possibly due to iPSC dependence on glycolysis rather than mitochondrial oxidative phosphorylation as a primary metabolic source of energy (1). Measurement of mitochondrial membrane potential was reduced in ND6-mutant NSCs compared to healthy controls, without significant changes in mitochondrial mass. The reduction in NSC mitochondrial membrane potential was accompanied by significant reduction in ND6-mutant NSC intracellular ATP levels compared to healthy control NSCs. This correlates with a switch from glycolysis to mitochondrial OXPHOS metabolism upon differentiation into NSCs (2). ND6-NSCs can differentiate into neurons, however preliminary observation showed a significant reduction in cell number by day 6 of maturation in ND6-mutant neurons. This indicates that ND6 mutation may play a role in neurogenesis (3).

CONCLUSION

This study shows that it is possible to generate iPSCs and iPSC-derived neuronal cells from ND6-mutant fibroblasts. The generated iPSC-derived cell models can recapitulate the biochemical defects associated with ND6-mutation and has the potential of being utilized for studying the functional impact of ND6-mutation on tissue-specific cells.

ACKNOWLEDGEMENT

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EXTENDED ABSTRACT

Development of Lung Organoid: The Whole Lung Cell vs AT2 Cell Isolation Methods

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SUMMARY

The lung serves as the primary respiratory organ, with gas exchange facilitated by vital structures known as alveoli. Alveoli consist of two distinct cell types: alveolar type 1 cell (AT1), characterized by a flat, simple squamous shape, and alveolar type 2 cell (AT2), which is cuboidal and covers approximately 3-5% of the surface area. Defective regeneration of the epithelium is believed to be associated with various lung diseases, as suggested by certain theories. To gain a better understanding of lung development and the behavior of its cells, as well as to comprehend lung disorders, innovative techniques such as the advancement of novel 3D organoid culture have been employed. The objective of this study was to establish suitable methodologies for investigating the behavior of lung stem cells. By doing so, we aimed to enhance our understanding of the mechanisms that maintain tissue equilibrium and the changes that occur in the lung as a result of diseases.

Keywords: Lung organoid, 3D organoid culture, Alveolar type 2 cell, Stem cell

INTRODUCTION

The lung serves as the primary respiratory organ, and the essential site for gas exchange within the lung is the alveoli. Alveoli consist of two distinct cell types: alveolar type 1 cell (AT1) - simple squamous cells covering approximately 95-97% of the alveolar surface area, and alveolar type 2 cell (AT2) - cuboidal cells occupying about 3-5% of the surface area. AT2 cells play a crucial role by secreting surfactant, an active substance that reduces surface tension and prevents alveolar collapse during expiration (1). Consequently, it has been suggested that improper renewal of the epithelium is associated with various lung illnesses.

Recent advancements have significantly improved our understanding of the environments conducive to stem cell thriving and the influence of essential signaling regulators on the preservation and specialization of these cells. These discoveries have led to the development of novel three-dimensional (3D) culture techniques that facilitate the formation of organoids, self-organizing structures closely resembling natural physiological conditions, driven by stem cells (2). An innovative method for investigating lung development in the laboratory setting involves the use of lung organoids, which hold great promise in advancing our comprehension of lung disorders.

The most advanced *in vitro* systems for replicating critical aspects of organogenesis rely on pluripotent stem cell-based organoid cultures, enabling researchers to simulate and explore both the development and diseases of the human lung (3). Therefore, it is crucial to establish suitable methods for studying the behavior of lung stem cells to enhance our understanding of the mechanisms maintaining tissue balance and the changes that occur in the lung due to disease.

MATERIALS AND METHODS

The mice were humanely euthanized, and their lungs were immediately dissected using sterile tools. To initiate the digestion process, a 1ml cocktail of enzymes was injected into the trachea before collecting the lung. The lung tissue was then cut and minced and placed into 3ml of enzyme solution. The minced lung tissue was incubated in a rotator at 37 °C for 30 minutes. To halt the digestion, 4ml of 10% FBS (Fetal Bovine Serum) was added to the lung tissue, and the sample was strained through a 100um cell strainer before being centrifuged at 400G for 5 minutes. After resuspension, 2ml of RBC (Red Blood Cell) buffer was added and incubated for 2 minutes to remove red blood cells. Subsequently, 8ml of DMEM/F12 (Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12) was added to the sample, which was then strained through a 40um cell strainer and centrifuged at 400G for 3 minutes. The cell pellet was collected and used for cell counting. The resulting sample was divided into two parts for further experimentation namely (a) Lung organoid culture using the entire lung cell population and (b) Lung organoid culture involving the isolation of AT2 cells.

Prior to proceeding with the AT2 cell isolation, the sample was divided for AT2 and fibroblast staining. Antibody staining with Sftpc antibody was used to identify AT2 cells, and CD140a antibody was used for fibroblast staining. After the staining incubation was completed, the sample

underwent FACs (Fluorescence-Activated Cell Sorting) to isolate the targeted cells. The isolated cells were then used to proceed with the organoid culture.

RESULTS AND DISCUSSION

This study aimed to compare two different 3D organoid methods: the whole lung cell method and the AT2 cell isolation method. The number of formed organoids was counted and presented in Figure 1, showcasing the organoid culture conditions from both methods.

The study observed a significant difference ($p<0.0007$) in the number of organoid formations between the two groups. Further analysis showed that the number of organoids on day 7 and day 14 in each group also displayed significant differences, with p-values of <0.0181 and <0.024, respectively (Figure 2).

According to our findings, the whole lung cell population method demonstrated a higher number of organoid formations compared to the AT2 isolation method. This discrepancy can be attributed to the fact that the whole lung cell population includes various cell types such as endothelial, immunological, and fibroblastic cells. This suggests that cell-cell communication plays a critical role in organoid formation under this culture condition. Cell-cell communication involves the transmission of signals and data among cells, promoting their arrangement within the 3D organoid structure (4,5). On the other hand, the AT2 cell isolation method specifically focuses on studying AT2 cells, which are responsible for producing surfactant, preventing lung collapse, and playing a vital role in lung regeneration and repair. However, our study found a lower number of organoid formations in the AT2 isolation method compared to the whole lung cell method. We believe that the interplay and interactions among different cell types are crucial for successful organoid formation. These cellular interactions allow the exchange of information in response to changes in the organoid's microenvironment. Previous research has indicated that the mitotic activity of lining cells is 1% per day, and they can differentiate into AT2 and AT1 cells in response to alveolar wall damage (1). Thus, utilizing a diverse range of cell types in the whole lung cell method stimulates intercellular communication, leading to the production of growth factor mixtures that promote rapid organoid maturation and development. However, the isolation of AT2 cells method remains valuable for studying specific details and understanding their functions, gene expression patterns, signaling pathways, and potential therapeutic targets in a more focused manner.

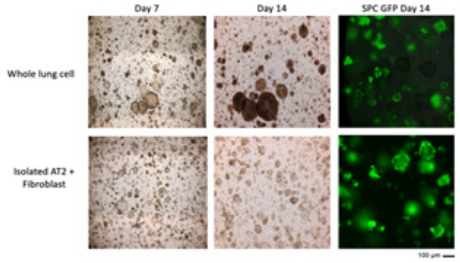

Figure 1: Comparison of lung organoid culture image between two different methods which are the whole lung cell method and AT2 and fibroblast isolation methods starting Day 7 and Day 14. The formation of organoid can be seen on day 7 in both methods. The images also showed the SPC GFP staining that tagging on AT2 cell on Day 14

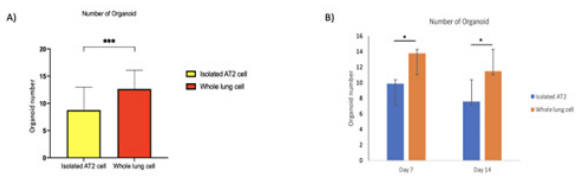

Figure 2: (A) The bar chart showing significantly different in the total number of organoids formed between isolated AT2 cell and whole lung cell groups ($p<0.0007$). (B) The bar chart of the lung organoid number showing significantly different on day 7 ($p<0.0181$) and day 14 ($p<0.0241$) respectively.

CONCLUSION

In conclusion, according to our study, one benefit of using whole lung cells to create organoids is that it allows researchers to collect various organoid morphologies and phases at the same time and under the same growing circumstances. However, the approach for isolating AT2 cells can also be utilised to thoroughly examine and comprehend their roles, their behaviour, gene expression patterns and signalling networks in certain condition. Hence, organoids are structures created from stem cells that mimic the structure and function of real organs. These unique characteristics make organoids a valuable tool in various fields such as developmental biology, molecular biology, and health-related studies like pharmacology, disease progression, and therapy, among others.

ACKNOWLEDGEMENT

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EXTENDED ABSTRACT

CRISPR-Cas9 transfection of guide RNAs targeting on *MMP2* and *MMP9* reduced migratory activities in cutaneous squamous cell carcinoma cell line

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SUMMARY

Cutaneous squamous cell carcinoma (SCC) is the second most common form of skin cancer. Matrix metalloproteinase (MMP)-2 and MMP9 proteins play critical roles in tumour progression of cutaneous SCC. This study aimed to determine whether *MMP2* and *MMP9* genes are suitable gene targets for anti-cancer therapy for cutaneous SCC. Two guide RNAs (gRNAs) targeting each of the MMP2 and MMP9 genes were transfected into human cutaneous SCC cell line A431 using Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas9. The migratory activities ($p<0.0001$) of A431 cells were found to be reduced significantly. Quantitative polymerase chain reaction (RT-qPCR) also revealed downregulation of the mRNA expression levels of cancer-promoting genes *VEGF-A* and *vimentin*.

Keywords: CRISPR-Cas9, MMP2, MMP9, matrix metalloproteinase, skin cancer

INTRODUCTION

Cutaneous squamous cell carcinoma (SCC) is the second most prevalent type of skin cancer, accounting for approximately 20% of all skin malignancies (1). It is the leading cause of death due to non-melanoma skin cancer annually. The prognosis for patients with metastatic cutaneous SCC is unfavourable, necessitating a more effective and precise approach, such as targeting genes associated with cancer metastasis. Matrix metalloproteinases (MMPs) are enzymes that contribute to cancer progression and are considered significant therapeutic targets (2). Among these, MMP2 and MMP9 proteins play critical roles in the advancement of cutaneous SCC, including tumour progression, angiogenesis and invasion (3,4). However, there is limited research on targeted inhibition of *MMP2* and *MMP9* at the genetic level. Therefore, this study aimed to investigate the potential of *MMP2* and *MMP9* genes as targets for anti-cancer therapy for cutaneous SCC.

MATERIALS AND METHODS

Two guide RNAs (gRNAs) were designed to target each of the *MMP2* and *MMP9* genes. These gRNAs were then transfected into the A431 human cutaneous SCC cell line using the Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-Cas9 system. Following transfection, the cells were incubated for 48 h. To assess the migratory activities of the cells, a scratch assay was performed. A scratch wound was created in the middle of A431 cell monolayer prior to transfection. The initial and final wound widths were measured using ImageJ software and the rate of cell migration was calculated. In addition, the expression levels of cancer-promoting genes *VEGF-A* and *vimentin* were evaluated by performing quantitative polymerase chain reaction (RT-qPCR) 48 h after transfection. All the data obtained was normalised to house-keeping gene *beta-actin* and the results were calculated using comparative CT method ($2^{-\Delta\Delta Ct}$).

RESULTS AND DISCUSSION

The migratory activity of cells was observed under the phase contrast microscope (Figure 1A-1J). Scratch assay results showed strong migratory activity in non-transfected A431 cells (Figure 1F). In contrast, other transfected A431 cells showed lower potential to migrate and heal the wound after 48 h of CRISPR transfection (Figure 1G-J). The rates of cell migration significantly decreased ($p<0.0001$) in A431 cells after CRISPR transfection of gRNAs targeting on MMP2 and MMP9 genes, relative to that of non-transfected cells (Figure 1K). The rates of cell migration for non-transfected cells were 11.86 μm/h while the rates for MMP2-1-

, MMP2-2-, MMP9-1- and MMP9-2-transfected cells were 3.91 µm/h, 5.82 µm/h, 5.68 µm/h, and 6.28 µm/h, respectively.

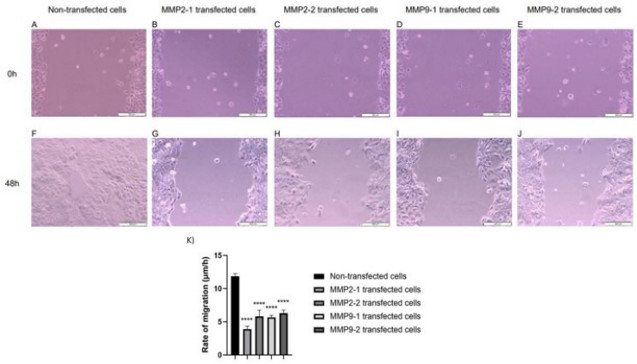

Figure 1: Cell migration assay of A431 cells after CRISPR transfection for 48 h. (A-E) In the middle of the cell monolayer, a scratch wound was created at the time of CRISPR transfection. (F) Non-transfected cells showed smallest wound gap after 48 h. (G-J) All transfected cells showed bigger wound gaps after 48 h. The scale bars denote 200 µm. K) The rate of A431 cells migrating towards neighbouring cells and healing the wound gap in 48 h were analysed using ImageJ software and represented in µm/h. The data were expressed as mean values ± Standard Error of the Mean (S.E.M) (n=9). Statistical analysis using one-way analysis of variance (ANOVA) and the post hoc Tukey's multiple comparison test was performed to compare rate of migration between transfected and non-transfected groups. **** p<0.0001

VEGF-A and vimentin are markers of epithelial to mesenchymal transition (EMT) involved in cancer cell invasion and metastasis. The expression of VEGF-A mRNA was downregulated by log2 fold change of 0.23 in MMP2-1-transfected cells and upregulated by log2 fold change of 0.35 in MMP2-2-transfected cells. Whereas, MMP9-1- and MMP9-2-transfected cells demonstrated decreased *VEGF-A* gene expression by $\log_2$ fold change of 1.27 and 0.7, respectively (Figure 2A). The mRNA expression of vimentin was upregulated by log2 fold change of 1.46 in MMP2-1-transfected cells and downregulated by log2 fold change of 0.56 in MMP2-2-transfected cells. Downregulated expressions of vimentin were observed in both MMP9-1- and MMP9-2-transfected groups, with log2 fold change of 1.02 and 1.54, respectively (Figure 2B).

CONCLUSION

CRISPR-mediated inhibition of *MMP2* and *MMP9* genes suppressed migratory capacity of cutaneous SCC cell line A431. This study demonstrated the potential of CRISPR treatment as a non-invasive approach that direct targets the DNA level of oncogenes, offering promising

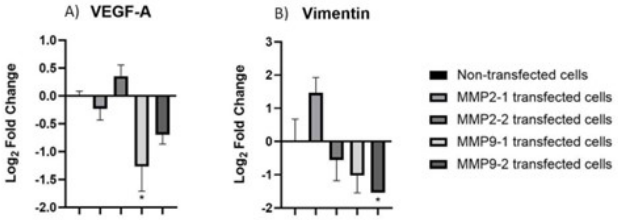

Figure 2: Relative fold changes of mRNA expression of cancer promoting genes (A) *VEGF-A* and (B) *vimentin*. Results were calculated using comparative CT method ($2^{-\Delta\Delta Ct}$) where beta-actin house-keeping gene was used as endogenous control to normalise the Ct values obtained from qPCR. Data were displayed as mean log2 fold change ± Standard Error of the Mean (S.E.M) (n=3). Statistical analysis was performed using Mann-Whitney U-test between control group (non-transfected cells) and each of the experimental groups (transfected cells) individually. *p<0.05

prospects for managing skin cancer and other types of metastatic malignancies.

ACKNOWLEDGEMENT

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EXTENDED ABSTRACT

From Womb to Brain: Unravelling Neuro-Enhancement Effect of *Centella asiatica* on Transdifferentiation of Full-Term Amniotic Fluid Stem Cells into Neural Stem Cells

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SUMMARY

An efficient protocol that could enhance the generation of quality non-brain-sourced neural stem cells (NSCs) for the treatment of neurodegenerative diseases (ND) is essential. This study aims to evaluate the effect of Centella asiatica (CA), which has been consumed traditionally as memory tonic, in enhancing the transdifferentiation potential of full-term amniotic fluid stem cells (AFSCs), a highly neurogenic stem cell, into NSCs. Treatment with CA extract was found to increase the expression of NSC protein markers, generate more good quality neurospheres and enhance the expression of selected neuronal markers. These findings strongly suggest that CA has the potential to enhance NSCs transdifferentiated from AFSCs, demonstrating the remarkable ability of non-brain-sourced stem cells derived from merely discarded fluid to transform into brain cells.

Keywords: Full-term amniotic fluid stem cell, neural stem cell, *Centella asiatica*, neuro-enhancement

INTRODUCTION

The incidence of neurodegenerative diseases (ND) is now increasing globally. Neuronal dysfunction and progressive neuronal cell death are among the main causes in most ND cases (1). An increasing number of studies have demonstrated that neural stem cells (NSCs) serve as a good source for neuro-transplantation as the means to treat ND. However, due to the limited sources of brain NSCs, treatment via neuro-transplantation has become very challenging (2). A recent study showed that highly potent stem cells residing in the amniotic fluid of full-term gestation could differentiate into neural lineage (3). Their neurogenic property has made them potential cells to generate NSCs. Establishing an efficient method with the addition of appropriate enhancers is therefore essential. Centella asiatica (CA) extract has been shown to contain high content of triterpenoids as its most abundant phytoconstituent, also known as centelloids, which give the extract its neuroprotective and neuroregenerative properties (4), making CA a candidate enhancer worth exploring. The study aimed to unravel the neuroenhancement effect of ethanolic extract of CA (EECA) on the transdifferentiation potential of full-term rat AFSCs line (R3), into NSCs.

MATERIALS AND METHODS

R3 was propagated in a stem cell culture medium (ESM) prior to MTT assay to determine the best dosage of EECA for treatment. The presence of R3 in the culture was confirmed by the expression of pluripotency-associated markers, Oct-4, Nanog and Sox2. In this study, R3 was treated with EECA at concentrations of 1 and 10 µg/mL and with 5µM of dibutyryl camp (dBcAMP) as positive control and subjected to undergo NSC induction using monolayer adherent culture technique. The transdifferentiation of R3 into NSCs was evaluated based on the expression of NSC-specific markers (Nestin and Sox2). The generation of NSCs was further confirmed by the ability of the cells to form neurospheres, the multicellular aggregates of NSCs in low attachment plates for 72 hours. The generated neurospheres were characterised based on the number and diameter. At post-72 hours, the neurospheres from each treatment were dissociated and subjected to neural differentiation for nine days. The NSC-derived neurons derived from each experimental group were characterised using selected markers specific for immature (Tuj1) and mature (MAP2) neurons.

RESULTS AND DISCUSSION

Good quality of R3 was present in the culture, as high expressions of Oct-4, Nanog and Sox2 were observed quantitatively, proving the stemness of the stem cells. Through MTT assay, non-detrimental concentrations of EECA on R3 were determined at 1 and 10 ug/mL. EECA has more neuroenhancement effect as elevated expression of NSC protein markers (Nestin, Sox2, and Sox1) were detected (Figure 1a). Good morphology of neurospheres, with the presence of irregular boundaries and microspike was observed, validating the success in transdifferentiating R3 into NSCs (Figure 1b). A higher percentage of good quality and proliferative neurospheres ranging between 50-150 µm was observed in the EECA-treated group, suggesting the neuro-enhancement effect of the extract (Figure 1c).

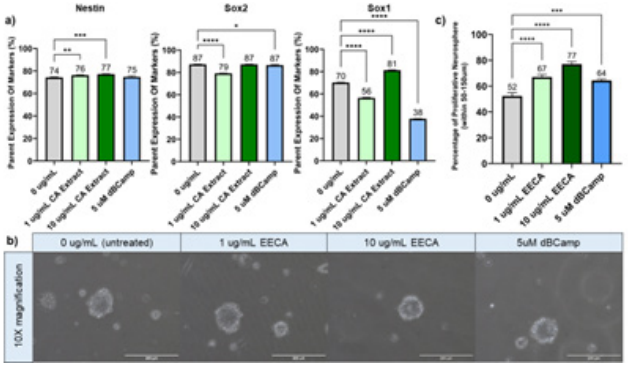

Figure 1: Characterisation of R3-derived NSCs. a) Expression of NSC protein markers (Nestin, Sox2, and Sox1) by flow cytometry on transdifferentiated NSCs. b) Morphological assessment of generated neurospheres after 48 hours cultured in 100 mm uncoated bacteriological petri dishes showing c) the proliferative size (50-150 µm). The scale bars are 200µm. Data are presented as mean ± STD, from three independent experiments, n=3. * indicates p<0.001 (One-way ANOVA and Dunnett's test).**

The generation of neurospheres with appropriate size is crucial for an adequate supply of nutrients and metabolites for the viability and differentiation of neural progenitor cells (5). They also expressed Nestin and Sox1 indicating the presence of neural stem/progenitor cells in the neurospheres (Figure 2a). Enhanced expression of Tuj1 and MAP2 were observed in NSCs-derived neurons from EECA-treated groups (Figure 2b). These results suggest the prospective neuro-enhancement effect of EECA in inducing good-quality NSCs from full-term AFSCs, a fluid that is merely discarded upon delivery.

CONCLUSION

These finding highly suggests the prospective

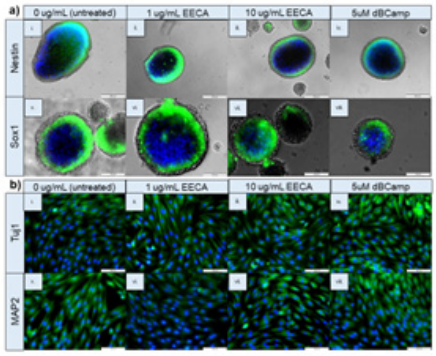

Figure 2: Expression of a) NSC protein markers (Nestin and Sox1) by neurospheres generated from R3-derived NSCs and b) neuronal markers (Tuj1 and Map2) by NSC-derived neurons according to group of treatment. The scale bars are 200 µm.

application of EECA as a potential NSCs enhancer by promoting neurogenesis. Furthermore, it also signifies the capabilities of full-term AFSCs, a non-brain-sourced stem cell, from a fluid that is merely discarded to transdifferentiate into brain cells for therapeutic applications.

ACKNOWLEDGEMENT

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EXTENDED ABSTRACT

Enhancing the Population of CD133+ Cancer Stem Cells from Non-Small Cells Lung Cancer Cell lines Through Spheroid Generation

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SUMMARY

Lung cancer's poor prognosis is primary influenced by cancer stem cells (CSCs). They are highly resistant to chemotherapy and are believed to repopulate the tumour. Magnetic activated (MACS) and fluorescence activated (FACS) cell sorting are the two most common methods for the CSCs isolation. Alternatively, a 3-dimensional stem cell culture method could be used. This method resulted in the formation of spheroid. Four generation of spheroids were established and characterized. Significant increase in the CD133+ population was observed across subsequent generations, which had higher chemotherapy resistance. They exhibited self-renewal capacity and differentiation ability, mirroring the characteristics of the original tumour.

Keywords: Lungs, Non-Small Cell Lung Cancer, Cancer Stem Cells

INTRODUCTION

Non-small cell lung cancer (NSCLC) is one of the most common cancers and has a concerning 5 year relative survival rate (28% according to American cancer society). A negligible percentage of heterogenic tumour are hypoxic stem cell-like subunit, known as cancer stem cells (CSCs), exist within the population of NSCLC. These cells have self-replicating and multicellular differentiating abilities, which are able to initiate and/or repopulate a tumour. The CSCs are highly resistant to conventional therapies including chemotherapy. Several other identified features of CSCs include expression of potential marker such as ALDH, CD44, CD133 surface biomarkers (1,2). One of the most studied surface markers for NSCLCs is the CD133. Isolation of the CD133+ cells could be performed using MACS or FACS. However, alternative method, such as 3-dimensional cell culturing, could be explored to isolate or enhance the population of CSCs in NSCLC cell lines.

MATERIALS AND METHODS

Cancer cell lines A549 & H460 representing two of the three main NSCLC cell lines subtypes (adenocarcinoma and large cell carcinoma, respectively) and two control cell lines; human embryonic lung epithelial cell (MRC5) and kidney epithelial cell (293FT) were used in this studies. Cell were cultured as adherent monolayer in their respective complete medium. Spheroid culture was then established for all the cell lines by seeding 1000 cells/well in a 96-well ultra-low attachment plate (CorningTM) for 14 days in serum-free medium containing RPMI-1640, 10 ng/mL fibroblast growth factor (bFGF), 1% B27, 20 ng/mL EGF, and 1% penicillin/streptomycin at 5% CO2 in 37°C. These spheroids (parent spheroid) were then counted and captured with a bright field microscope on the 14th day. Subsequently, spheroid were dissociated and subcultured for four more generation. The fourth generation spheroid cells were subjected for CD133 expression and characterization including colony formation, cell proliferation, and chemoresistance.

RESULTS AND DISCUSSION

Several studies have demonstrated spheroid formation with a slight increase in the CSCs markers. Therefore, it was recommended to characterize cells from higher generations of spheroid to see if this could further increase the CSC markers. This led to our finding, that a gradual increase in the size and CD133+ expression were observed from the four generations of spheroid compared to the parent cells of the NSCLC lines (Figure 1a). The CD133+ expression in parent A549 increased from 1.1% to 19.5%, while for H460 the increase was from 0.5% to 17.10%, in the 4th generation spheroid (Figure 1b). Several studies also reported of CSCs to have higher resistance to various chemotherapy drugs including cisplatin (3). Similarly, our studies showed a similar IC50 for A549 and H460 (9μM & 4μM) (Figure 1c) as previously reported. However, the chemoresistance was higher in the 4th generation of both cell lines (-40μM and 14μM, respectively) (Figure d). Consequently, this supports the hypothesis that CSCs might play an important role in tumour relapse, as they can survive even after treatment. In addition, our study shows that both MRC5 and 293FT cell lines did not form spheroids beyond the second and fourth generation respectively, hence we could not analyse the CD133+ expression. This coincides with Fakiruddin et al., (2019) that showed that non-CSCs heterogenous cells of the same cell lines produced no or smaller spheroids, probability due to the non-existence of CSCs (4).

Self-renewal and multi-lineage differentiation are evident abilities of CSCs. The spheroid dissociated cells were able to form heterogeneously populated colonies albeit fewer than the adherent/ parent cell lines. Additionally, the adherent (parent) cell lines had shorter population doubling time than the spheroid dissociated cell.

CONCLUSION

This study demonstrates that 3D cell culture using serum-free medium, successfully enhances the population of CD133-positive CSCSs in NSCLCs cell lines. This results could provide other laboratories with a more effective method to increase CSCs population for further research.

ACKNOWLEDGEMENT

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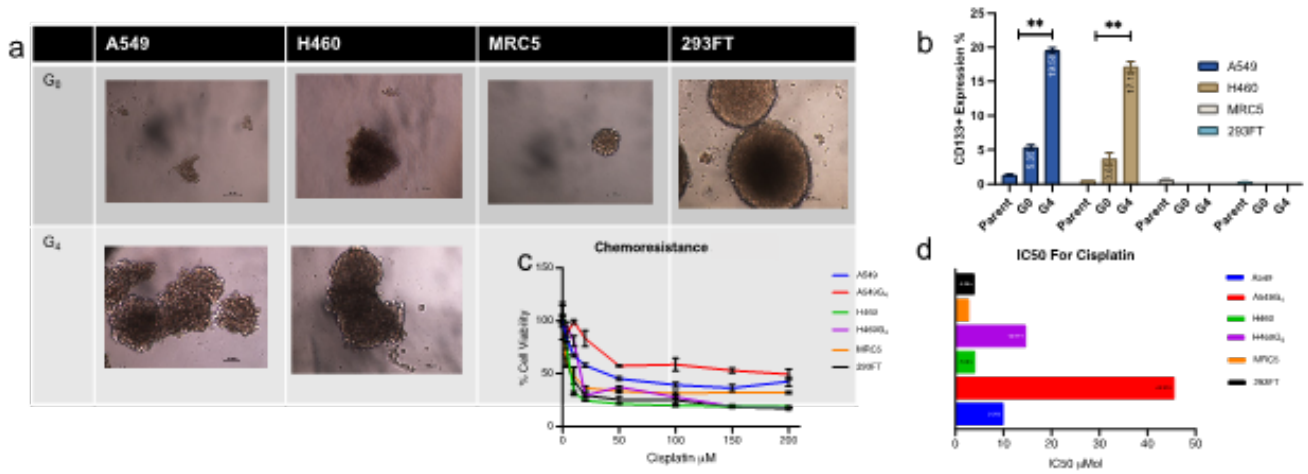

Figure 1: A thousand (1000) cells per well in ultra-low attachment plate formed a) spheroids for parent and fourth generation in NSCLC cell lines (A549 and H460) and that of normal human embryonic epithelial cell lines (MRC5 & 293FT). b) Shows flow cytometry CD133 expression in parent cell lines, parent spheroids, and the fourth generation spheroids from A549 and H460. c) Chemoresistance of the cell lines and d) their IC50 to cisplatin. Micrograph were in 4 X 10 magnification and diameter measurement was done with ImageJ. Data were analyzed in GraphPad prism

EXTENDED ABSTRACT

In Vivo Temporal Penetration of Mesenchymal Stem Cell-Derived Exosome Eye Drops Across Ocular Tissue

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SUMMARY

This study explores the potential application of extracellular vesicles (EVs) or exosomes derived from human mesenchymal stem cells (MSCs) in ophthalmology. Exosomes were isolated from MSCs' culture supernatant, and applied as eye drops to rabbits' eyes. Samples of aqueous (AH) and vitreous humour (VH) were collected at different time points for exosomes marker analysis. Our results showed that CD63 was detected in all AH and VH samples. Whereas, for CD9 and CD81, despite minimally expressed in control samples, showed varying expression in VH samples at different time points, indicating penetration and retention of exosomes in the eyes' structure.

Keywords: Mesenchymal stem cells, extracellular vesicles, exosomes, ophthalmology, penetration

INTRODUCTION

Over the past decade, mesenchymal stem/stromal cells (MSCs) have exhibited their regenerative, reparatory, and immunomodulatory capacities in cell-based therapies. However, the applicability of MSC-based therapy in ophthalmology is limited by their suboptimal biocompatibility, penetration, and delivery to the targeted ocular tissues (1). Recent studies have shown that extracellular vesicles (EVs) derived from human MSCs are one of the paracrine factors responsible for the functions of these cells, such as anti-inflammatory effect, improving angiogenesis and promoting regeneration of tissue. Investigation on the penetration rate of MSC-derived exosomes will provide initial insight on the frequency of application and quantity of exosomes that is needed to penetrate across the ocular tissue and effectively in ameliorating ocular diseases. However, due to limited research and knowledge of the subject matter, we took the initiative to study the in vivo temporal penetration of MSCs-derived exosomes across ocular surface in animal model.

MATERIALS AND METHODS

Human umbilical cords were collected with informed consent and the use of animals was approved by the Animal Care and Use Committee (ACUC), UPM (UPM/IACUC/AUP-R022/2021). MSCs were cultured-expanded and exosomes were isolated and purified from the supernatant of the culture by previous established protocol (2). The exosomes were then lyophilized and dissolved in normal saline prior to nanoparticle tracking analysis (NTA) and transmission electron microscopy (TEM). Exosomes eye drops were then applied to four rabbits' eyes and analysed at different timepoints as follows: in rabbit 1, the right eye's AH and VH was harvested after two hours from last instillation, and the left eye's AH and VH was harvested after four hours from last instillation. The AH and VH of rabbit 2's and 3's both eyes were harvested after 24 hours and 48 hours from last instillation respectively, while the fourth rabbit was served as negative control. Exosomes markers of all samples were analysed by western blot analysis.

RESULTS AND DISCUSSION

Exosomes were successfully isolated and purified from MSCs culture. NTA analysis and TEM revealed that the exosomes ranged in size from 49 nm to 144 nm, with a mean number of 2.57 x 10^{11} particles/mL (Figure 1 & 2).

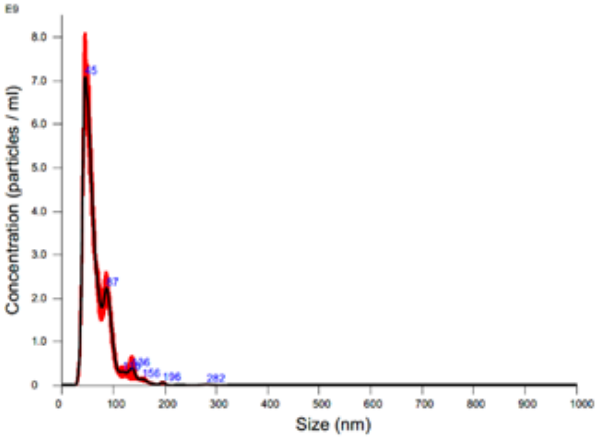

Figure 1: Size distribution of extracted exosomes by NTA.

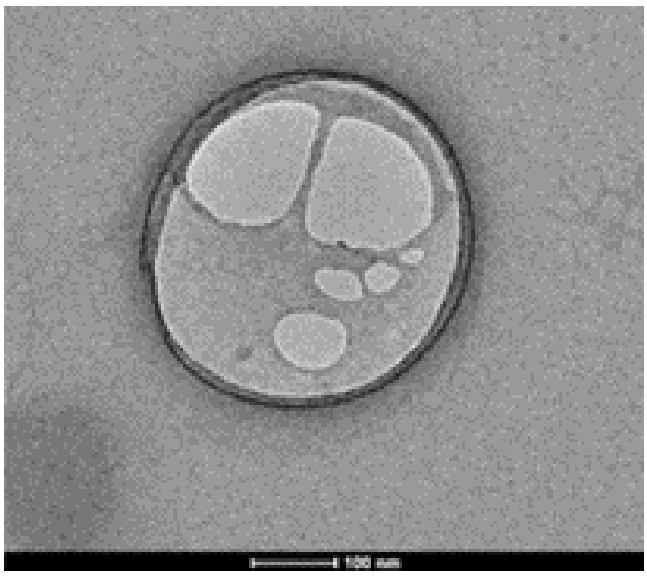

Figure 2: Morphology of extracted exosomes by TEM.

To assess the distribution of MSC-derived exosomes, AH and VH samples were extracted from the corresponding rabbit subjects. CD63, a commonly used marker for various exosome subtypes was detected in all AH, VH and control samples. However, CD9 and CD81 which were minimally expressed in the control samples, expressed in both VH samples of rabbit 1, but minimally expressed in the AH of rabbit 1's right eye and left eye

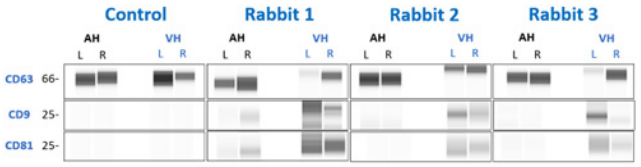

Figure 3: Western blot analysis of the AH and VH samples collected at different time point

(Figure 3). In addition, these markers were consistently expressed only in the VH but not AH of rabbit 2 and 3. Considering that the AH and VH samples from rabbit 1's right eye were collected two hours after the last instillation of exosomes eyedrops, the presence of CD9 and CD81 indicates a minimal retention time of exosomes in the eye structure. Conversely, the presence of these markers exclusively in the VH samples of the left eye suggests penetration of the exosomes to the VH after four hours. Moreover, the VH samples from rabbit 2 and 3 suggest a maximal retention time of exosomes for up to 48 hours following the last instillation (Figure 3).

CONCLUSION

Due to the limited studies available for applying exosomes in a non-invasive manner, this preliminary study is essential to elucidating the ocular penetration of exosomes, and understanding the mode of action of exosomes in regeneration of ocular tissue. The study could potentially provide a non-invasive solution to the treatment of ocular diseases in future.

ACKNOWLEDGEMENT

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EXTENDED ABSTRACT

Plasma eye drop: Usage in National Blood Centre and their indications

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SUMMARY

This article provides an overview of plasma eye drop production trend in National Blood Centre, Malaysia and analysis of its usage in various ophthalmological pathologies. The amount of usage remains inconsistent and not increasing over the years. Analysis of indications for plasma eye drop use revealed that its use is limited to a few pathologies as well. Given its regenerative potential, more research is warranted to analyse treatment outcome using plasma eye drop in various pathologies which will serve as reference for establishment of guideline for its clinical use.

Keywords: Autologous serum, ophthalmic solutions, platelet rich plasma, corneal epithelium, corneal injury

INTRODUCTION

Human serum has been a subject of interest in regenerative medicine, owing to its content of various growth factors, vitamins, cytokines and fibronectin. These substances promote tissue repair and regrowth, thereby presenting a promising alternative treatment option (1). In ophthalmology, plasma eye drop has been mostly used for corneal surface diseases which respond poorly to conventional treatment strategies or as adjunct therapy(2–5). Plasma eye drop can be prepared as platelet rich plasma or serum. Platelet rich plasma contains higher amount of growth factors as they are mostly found inside alpha granules in platelets, thus providing better regenerative properties compared to serum (1,2). Platelets are also known to interact with regulatory T cells to increase Interleukin-10 levels, thereby exerting anti-inflammatory effect which further accelerates tissue healing. Without anti-inflammatory modulation effects of platelets, serum is comparatively more pro-inflammatory and may be harmful to patients with immunological disorders (1). In National Blood Centre, plasma eye drop is produced from platelet rich plasma derived from whole blood donation. Currently, there has been no report of plasma eye drop usage trend in Malaysia and indications for its use by ophthalmologists are unanalysed.

MATERIALS AND METHODS

This study is a retrospective analysis of all plasma eye drop produced in National Blood Centre from year 2016 until 20th July 2023, which are summarised from component production unit annual statistic report. The data was crosschecked and verified with plasma eye drop form (PDN/CP/WI-21/01, ver.04) kept in duplicates by both component production unit and inventory unit. All plasma eye drop requests received by National Blood Centre and subsequently issued to clinicians are included in this study. Type of donation was identified by referring to the plasma eye drop form and crosschecked with donor unique identification barcode in the Blood Bank Information System v2.0. Patient diagnosis was identified from case referral letters attached to the plasma eye drop form. However, there were 22 forms that were not attached with any document, thus only 32 patients were identified with indication for plasma eye drop in this study.

RESULTS AND DISCUSSION

From year 2016 until 20th July 2023, there were a total of fifty-four plasma eye drop requests as shown in Figure 1. No plasma eye drop was produced in year 2021 due to no request received from clinician. Forty-six (85%) plasma eye drops were produced from autologous donation while remaining eight (15%) were produced from allogeneic blood donation. Autologous donations are preferred for plasma eye drop due to no risk of disease transmission, immunogenicity and human leucocyte antigen alloimmunisation. However, when a patient is deemed unsuitable for venesection, allogeneic donation is used instead. There were three young patients (age<17 years old) and two elderly age (age>2

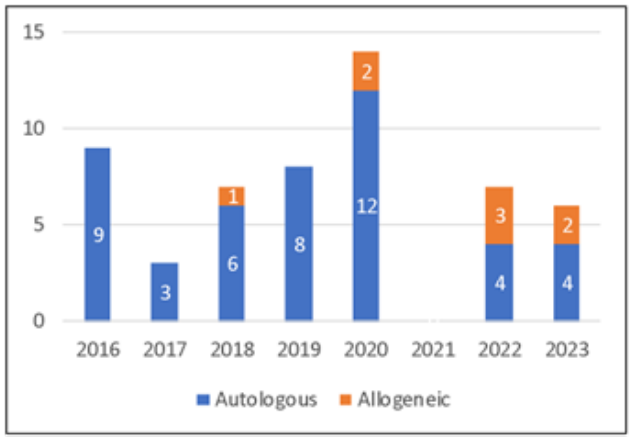

Figure 1: Plasma eye drop production in National Blood Centre, Malaysia

years old) who required allogeneic donation as they did not fulfil criteria for safe venesection. Reasons for three other allogeneic donation requests were unclear due to lack of documentation.

Chemical or thermal injury comprised the highest number of plasma eye drop indication at sixteen requests (50%). It is followed by persistent epithelial defect and secondary ocular surface disorders with seven requests (21.9%) each, and one request (3.1%) each for stickler syndrome and corneal neuropathic pain. Interestingly, there are two patients who were provided with second cycle of plasma eye drop, indicated for persistent corneal epithelial defect and chemical injury respectively (Figure 2).

The apparent low and inconsistent demand for plasma eye drop, as well as limited application should not be

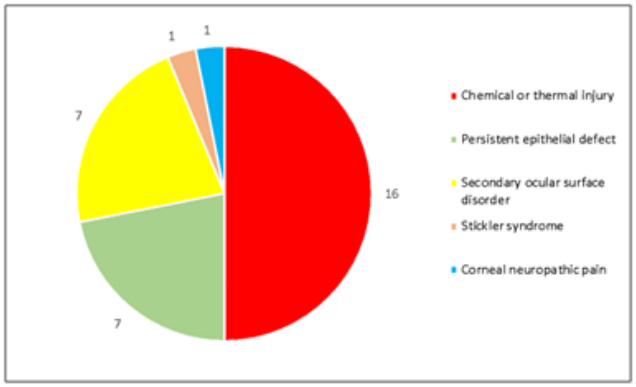

Figure 2: Indication for plasma eye drop

viewed as discouraging. Production of such solution remains limited to a few centres in Malaysia and actual national usage remains undetermined. Inaccessibility of this treatment option to most ophthalmologists also hinders wider application across the country.

CONCLUSION

The demand for plasma eye drop from National Blood Centre is inconsistent, with majority of requests intended for eye injuries. Future studies to analyse patient treatment outcome would be beneficial and serve as driving force for expansion of plasma eye drop preparation service in more blood centres.

ACKNOWLEDGEMENT

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EXTENDED ABSTRACT

Moringa oleifera Leaves Recapitulate White Blood Cells in Immunocompromised Animals and Induce Late Passage Mesenchymal Stem Cell Expansion

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SUMMARY

The Moringaceae family includes *Moringa oleifera* (MO), a tree that is widely cultivated in East and Southeast Asia. This study aimed to investigate the immunoregulatory and regenerative capabilities of a standardised 70% ethanol extract of MO leaves (MoETE) on immuno-competent and induced immuno-suppressed animal models. Additionally, in vitro analysis was conducted using umbilical cord-derived mesenchymal stem cells (UC-MSCs). Statistical analysis revealed a significant difference between the groups and days on the dependent variance WBC in both normal and immunosuppressed groups ($p < 0.05$ for α level). Supplementation with MoETE concentrations (100, 10, 1, 0.1 g/mL) for 48 hours substantially improved the proliferation and viability of UC-MSCs.Their role in modulating the immune system is still lacking.

Keywords: *Moringa oleifera*, Immunomodulatory, Mesenchymal stem cells

INTRODUCTION

The *Moringa oleifera* (MO) plant has been researched for its ability to act as an antioxidant, anti-diabetic, anti-inflammatory, and anti-cancer agent. However, its impact on the immune system requires further exploration. Therefore, this study aims to thoroughly examine the immunoregulatory and regenerative potential of a standardised ethanol extract of the plant. The aim of this study was to investigate the effects of MoETE on adaptive and innate immune cells in both normal and immunosuppressed animals. Additionally, the study aimed to observe the proliferation effect of mesenchymal stem cells treated with MoETE. The results of this research may contribute to the understanding of the biological and immunological responses to MO's therapeutic properties. The study will also highlight some important aspects of Moringa oleifera's immunomodulatory action on adaptive and innate immune cells.

MATERIALS AND METHODS

Dry powdered MO leaves were extracted with 70% ethanol using sonication extraction each at the ratio of 50 gm in 500 mL for 1 hour at room temperature. The extracts were filtered using filter paper, and the filtrate was concentrated under reduced pressure using a vacuum rotary evaporator at 40oC to obtain a crude extract. Sprague-Dawley rats (6 per group), females aged 10-12 weeks old used in this study. The animals received treatments as listed below for 28 days. A standard drug, Levamisole (50 mg/kg), was given as positive control as this is a common drug that is given to recapitulate immune responses. The dosages for MoETE were selected based on previous study conducted by Jaiswal et al. (1).

- Group N1: Administration of distilled water (negative control)
- Group N2: Administration of MoETE (150 mg/kg)
- Group N3: Administration of MoETE (300 mg/kg)
- Group N4: Administration of MoETE (600 mg/kg)
- Group N5: Administration of the standard drug (positive Control) (Levamisole 50 mg/kg/p.o/per day).

As for the immunosuppressed group, cyclophosphamide (CTX) administrated at a dose of 30 mg/kg body weight was administered orally to the mice on day 0, and 10

mg/kg on day 1 and 2 to induce immunosuppression (2). Peripheral blood from the tail of the animals was collected periodically at the end of the experiment to assess the immunomodulatory effects of MoETE. For in vitro assessment of MoETE on UC-MSCs, early passage (P3-P6) and late passage (P7-P10) were seeded at (5 x 10^3 cells/ 100 µl) per well of a 96-well plate for 24 hours to allow cell attachment. The media was discarded, and the fresh media with graded doses of MoETE (100, 10, 1, 0.1 µg/mL) and controls were added and incubated for 48 hours. The proliferation was measured through fluorimetric assay using a fluorescence microplate reader with excitation of ~485 nm and emission detection at ~530 nm.

RESULTS AND DISCUSSION

Bonferroni pairwise comparison for the dependent variable of white blood cells (WBC) provide details on the group significant value. Group 1 is significant (P=0.05) towards group 3 (0.02), group 2 is significant towards group 5 (0.00), group 3 is significant toward group 5 (0.00). Meanwhile group 4 is significant towards group 5 (0.00). Post hoc test for group had two subsets of homogeneous with groups and 3 subset for days. The administration of MoETE did not significantly affect the level white blood cells in the healthy rats; however, upon induction of immunosuppression, the supplementation of MoETE normalised the white blood cells (Figure 1 and 2).

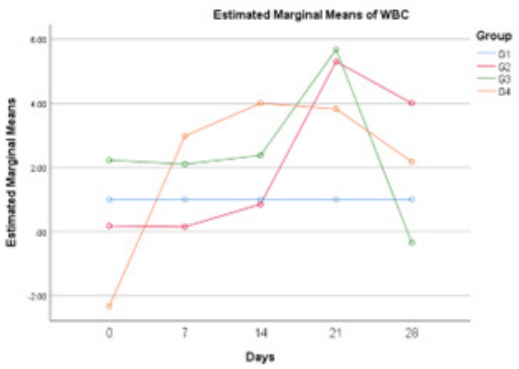

Figure 1: Normalised estimated marginal means of white blood cells vs days for normal animal group 2-5 with G1(negative control)

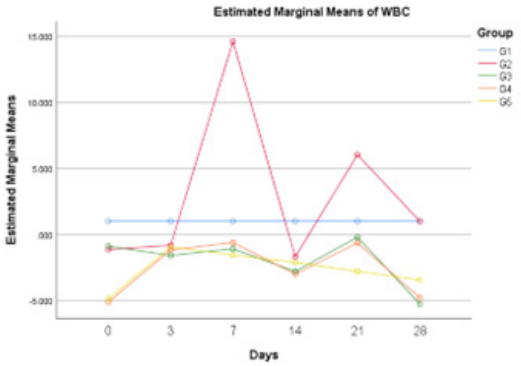

Figure 2: Profile plot normalised estimated marginal means of white blood cells vs days for immunosuppressed animal group 2-5 with G1 (negative control)

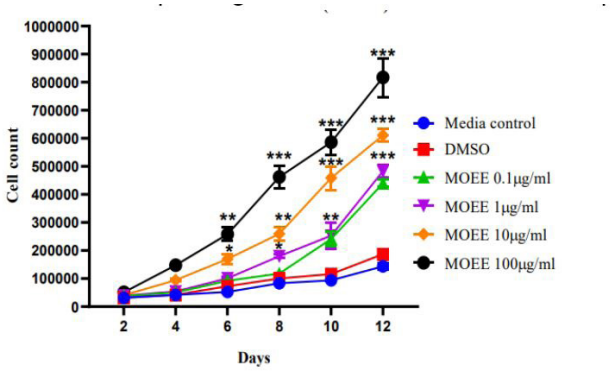

Figure 3: MoETE promotes the proliferation of late passage of UC-MSCs

The IC_{50} for MoETE was determined at 840 µg/ml in the UC-MSCs. Supplementation with a graded concentration of MoETE (100, 10, 1, 0.1 µg/mL) for 48 hours profoundly improved the proliferation and viability of culture-expanded late passaged UC-MSCs (P7-P10) compared to early passage MSCs (P3-P6) (Figure 3). These findings were corroborated with a decrease in culture-induced early apoptotic cells in the late passage UC-MSCs and increased Sphase cells of the cell cycle.

CONCLUSION

The administration of MoETE did not significantly affect the level of white blood cells in the healthy rats; however, upon induction of immunosuppression, the supplementation of MoETE normalised the immune cell levels. This study reports that MoETE enhances the proliferation of late passage UC-MSCs culture through inhibition of culture induce apoptosis and re-entry into the cell cycle.

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EXTENDED ABSTRACT

Autologous Platelet Rich Plasma (PRP) In Alveolar Bone Graft: A Case Report

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SUMMARY

Platelet rich plasma (PRP) is known to contain growth factors with its capability to accelerate wound healing and tissue regeneration. This case report was intended to demonstrate the use of autologous PRP as a topical adjunct in alveolar bone graft on the patient with post-operative structural socket defect and persistent post-operative infection. The findings from this case indicate that wound healing is good with evidence of tissue regeneration. Therefore, topical autologous PRP has a potential as an adjunct in alveolar bone graft.

Keywords: PRP, autologous, Wound healing, Tissue regeneration, Growth factors

INTRODUCTION

Platelet Rich Plasma (PRP) is obtained from whole blood after the density centrifugation process (1-5). Autologous PRP is one prepared from the patient's own blood and it is enriched with platelets. Besides its procoagulant effect, platelet is known to contain multiple growth factors. Thus, topical PRP application in surgical procedure is expected to accelerate tissue regeneration and healing process (2-5).

MATERIALS AND METHODS

18 years old lady, no known medical illness with five months past surgical history of removal of the impacted teeth (13 and 23) prior to orthodontic treatment. Since then, she had a persistent post-operative infection, associated with swelling, pus discharge and deep periodontal pocket and mobility of 12, 21 & 22 teeth. Despite undergoing another surgical procedure for wound debridement, incisional biopsy and flexible splinting of the mobile teeth, infection still persists.
She was referred to an oral and maxillofacial surgeon for management of persistent post-operative site infected granuloma of maxilla with bony defect and planned for alveolar bone graft with adjunct PRP.

PRP preparation:
Patient's own blood was sampled into the citrated tube. The sample was centrifuged at 800rpm for 8 minutes. PRP was then separated after centrifugation (1).

Surgical Procedure:
Mixed bone graft (Autograft from iliac crest + xenograft) with PRP. Surgical repair was done and followed by bone graft application at areas 11,12 and 21,22. Additional topical PRP was applied to the surgical area.

Clinical & radiographic findings are compared for pre-procedure and post-procedure.

RESULTS AND DISCUSSION

Wound healing is good with successful closure of deep periodontal pocket. There is no more significant mobility of 12,21 and 22 teeth. No more evidence of infection and no graft rejection. Follow-up occlusal x-ray 4 months post operation shows good evidence of bone regeneration.

After removal of impacted teeth, this patient developed structural socket defect and persistent postoperative infection. These conditions had been affecting the healing process. Other than surgical repair, adjunct autologous PRP application provides added benefit to her.

Due to richness in growth factors, autologous PRP is used to promote wound healing in this patient. By direct application of growth factors to the affected area, it is expected to accelerate the regeneration of bone graft and soft tissue healing (2–5) . Clinical & radiographic follow-up shows good improvement & patient recovers

well.

Since autologous PRP is obtained from patient's own plasma, it is relatively safe and free from transfusion-transmitted infection and immunological complications (2). In view of the limited data, further study is required to have case-control comparison and to explore more characteristics of autologous PRP.

CONCLUSION

Autologous PRP application is able to promote local tissue regeneration and accelerate wound healing in alveolar bone graft. It has a great potential as an adjunct in many procedures in oral and maxillofacial surgery.

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EXTENDED ABSTRACT

CAR T vs CAR NK Cells: Big Hunt for the Right Tool

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SUMMARY

Recently CAR NK therapy has emerged as a novel and promising method of adoptive immunotherapy. Only one clinical trial employing CAR NK therapy has been completed to date and has published data, but the unique features of NK cells make them a very attractive carrier of CAR. NK cells could be used as an allogeneic product without additional modifications and are able to recognize tumour "escaper" cells. With the help of available databases, we performed comparative transcriptomic and proteomic analysis of T and NK cells to reveal their potential strengths and disadvantages for cancer immunotherapy in a CAR context.

Keywords: CAR T, CAR NK, receptors, cytotoxicity, cancer immunotherapy

INTRODUCTION

CAR T cell therapy is rapidly moving from the scientists' bench to the patients' bedside. With more than 300 completed clinical trials and 6 FDA-approved products we have a lot of data on CAR T efficacy, safety and limitations. CAR NK cells, on the other hand, still exist as a cutting-edge experimental approach with only a handful of completed clinical trials. In contrast with T cells, NK cells do not express TCR, therefore they could be used without additional modifications in allogeneic studies. Another unique feature of NK cells is the unique palette of pattern recognition receptors on their surface. In this work we analysed data from clinical trials, transcriptome and proteome datasets of T and NK cells, to figure out which effector is more prospective for cancer therapy.

MATERIALS AND METHODS

Data regarding clinical trials was extracted from publicly available databases clinicaltrials.gov and PubMed. Proteomics data for NK and T cells subpopulations was used from Immprot (1). To retrieve single-cell transcriptome data, a single cell portal of Broad Institute was used (singlecell.broadinstitute.org).

RESULTS AND DISCUSSION

Compared with T lymphocytes NK cells express a much wider variety of activation receptors (Fig.1). In clinical practice this phenomenon could translate into better eradication of "escaper" tumour cells that become invisible for CAR recognition due to the antigen loss. Another potential benefit of NK cells is lower expression of PD-1 checkpoint receptor (PDCD1 gene), although CTLA-4, TIM-3 (HAVCR2 gene) and KIRs are up-regulated.

To avoid excessive KIR signalling NK cells could be used in an allogeneic setting. NK cells seem to be better equipped for direct target cell lysis. NK cells express more granzymes, perforin and synapse-forming integrins than T cells, and therefore could lysis tumour cells in a more efficient way (Fig.1). A potential flaw of NK cells is the low expression of several cytokine receptors, so the trafficking of NK cells to solid tumours needs to be further modified by ectopic expression of these receptors.

From the industrial point of view, NK cell expansion, transduction and storage pose additional challenges compared with T cells (2). There is a lack of feeder free solutions for efficient expansion of NK cells; transduction of cells requires higher MOIs, and cryopreservation of NK cells could significantly compromise their cytotoxicity. These disadvantages do not outweigh the key benefit of NK cells – compatibility with allogeneic use. Therefore,

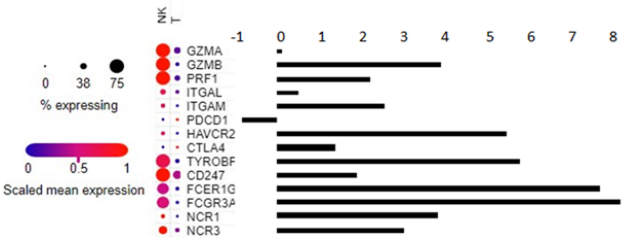

Figure 1: Single-cell transcriptome (left side of figure) and pooled-cell proteome (right side of figure) analysis of NK and T cells.

CAR NK cells have all chances to become affordable off-the-shelf therapy.

Only one CAR NK trial is completed to date and has published results (3). Therapy with allogeneic CD19 specific CAR NK cells was rather safe and comparable in efficacy with autologous CAR T therapy. Additional data from ongoing clinical trials will provide more information about benefits of CAR NK therapy over CAR T therapy and potential disadvantages of this approach.

CONCLUSION

NK cells possess several unique features that could make them an ideal carrier for CAR. NK cells express more pattern recognition receptors and potentially could more effectively lyse tumour cells. Some improvements still have to be done in CAR NK cells manufacturing process to reduce costs and increase affordability of such therapy.

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EXTENDED ABSTRACT

Novel Strategies to Detect Ovarian Cancer Using a Precision Medicine Approach Capturing Cancer Stem Cell Markers

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SUMMARY

One of the major drivers of tumor initiating cancer stem cells (CSCs) is the upregulated self-renewal mechanism, the Wnt - catenin pathway. Herein, we elaborate on the Wnt mechanism and its status in ovarian cancer. The hyperregulated Wnt catenin can be exploited in a two-pronged strategy that we propose: one, to use it as a diagnostic tool indicative of the presence of CSCs and other as a therapeutic target to inhibit and suppress rapid proliferation and sustenance of CSCs. This exhaustive diagnostic panel that is elucidated is directed towards a precision approach to enable an early and accurate detection of ovarian cancer.

Keywords: Ovarian cancer, cancer stem cells, Wnt signaling, sFRP4, CSC diagnostics

INTRODUCTION

Among various cancers of the genital tract, ovarian cancer (OC) remains the deadliest due to its indolent course and the absence of specific symptoms. Patients report with vague abdominal discomfort and distension more commonly in the advanced stages. The most widely used biomarker to detect ovarian cancer is CA125 but the definitive diagnosis is established only through histopathology of the resected ovarian mass. Current treatment modalities involve debulking surgery followed by adjuvant platinum based chemotherapy and/or immunotherapy depending on the disease stage (1). However, these strategies have failed to yield a favorable outcome in patients with metastasis. Tumor relapse and metastasis is primarily driven by an elusive chemoresistant population of stem cells. These are the cancer stem cells (CSCs), which are a minority population within the tumor and which are also referred to as tumor initiating cells (2). The low sensitivity and specificity of the existing ovarian cancer biomarkers reflects on the lack of precise and early diagnosis.

MATERIALS AND METHODS

We prepared a complete and exhaustive list of reported markers indicative of cancer stemness in ovarian cancers. Furthermore, we also reviewed the available literature on circulating tumor cell markers specific for ovarian cancers. In addition, we elaborate on the Wnt mechanism and its status in ovarian cancer to utilize the upregulation of this pathway as a diagnostic tool and to target it using the Wnt antagonist secreted frizzled related protein 4 (sFRP4).

RESULTS AND DISCUSSION

We propose a novel panel of biomarkers which are emerging as early identifiers of CSCs. These include cell surface markers which are unique to OC stem cells such as CD44 and CD24. In addition, the proteins which regulate chemo-resistance are also of substantial relevance in a personalized medicine approach to OC, because markers of drug resistance have a differential pattern between naive and drug-treated tumors. Another emerging diagnostic approach which is non-invasive is the detection of circulating tumor cells (CTCs). CTCs originate from the primary tumor as cells that disseminate into blood/lymphatic circulation and has been shown to have increased expression of CSCs and EMT markers, TIMP1 and CXCR4. This points to an interesting possibility of the use of circulatory CSC markers in OC for diagnosis. The use of Wnt mediators such as β catenin, axin, APC, which are upregulated in ovarian CSCs can also be used as a diagnostic indicator of OC. We also describe how a well reported natural antagonist of Wnt, the secreted frizzled related protein

4 (3) and its domain peptides can be used to effectively target the CSC population of ovarian cancer.

CONCLUSION

Using cancer stemness related markers and circulating tumor cell markers, we propose an exhaustive diagnostic panel that is elucidated and directed towards a precision approach to enable an early and accurate detection of ovarian cancer. The role of Wnt pathway upregulation in promoting cancer stemness is also highlighted. To exploit this upregulation as a therapeutic target, we proposed the use of unique peptides derived from the Wnt antagonist, sFRP4, to specifically destroy ovarian CSCs. This could be the latest strategy in peptide based drugs for ovarian cancer.

ACKNOWLEDGEMENT

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EXTENDED ABSTRACT

Viral Reprogramming of Primary Human Dermal Fibroblasts for Generation of Induced Pluripotent Stem Cells

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SUMMARY

Induced pluripotent stem cells (iPSCs) mimic embryonic stem (ES) cells in nature, possessing self-renewal abilities and pluripotent differentiation potential with the added benefit of bypassing any ethical issues faced by the derivation of ES cells. However, the choice of reprogramming method for iPSC generation may vary based on the efficiency, characterization of resulting clones formed and potential downstream applications. Here, two viral methods were used for generation of iPSCs, and the resulting clones were characterized to allow for comparison of both methods.

Keywords: Induced pluripotent stem cells, lentivirus, Sendai Virus, reprogramming.

INTRODUCTION

Induced pluripotent stem cell (iPSC)-based models using human-derived samples are useful resources for studying disease mechanisms *in-vitro,* particularly in the field of personalized medicine. iPSCs can be differentiated into any cell type in the body and be utilized for disease modelling and therapeutic drug screening. The successful reprogramming of iPSCs from primary adult somatic cells are dependent on multiple factors, including the choice of a robust reprogramming method. Viral reprogramming methods are well established and include both integrative methods such as lentiviruses and non-integrative methods such as Sendai Virus. Reprogramming involves forced expression of using a combination of key transcription factors most commonly OCT4, SOX2, KLF4 and C-MYC. In this study, we examined the efficiency of generating iPSCs using two different viral reprogramming methods: Lentivirus and Sendai Virus and characterize the resulting iPSC clones generated. .

MATERIALS AND METHODS

Primary human dermal fibroblasts were transduced with either lentiviruses or Sendai Viruses encoding transcription factors OSKM. Transduced cells were grown on inactivated fibroblast feeder layer in embryonic stem (ES) cell medium for 20-27 days were subjected to daily morphological analysis. The resulting number of ES-like colonies were calculated, and reprogramming efficiency was calculated based on the initial number of transduced cells. ES-like cell colonies were picked and expanded by clonal isolation in feeder-free culture system and further characterized by morphology and immunophenotyping for pluripotency and tri-lineage differential potential by spontaneous differentiation of embryoid body. Genetic stability of reprogrammed iPSCs were analyzed by qPCR analysis of 8 common chromosomal mutations seen in reprogrammed iPSCs. Sendai Virus reprogrammed iPSC clones were investigated for evidence of viral integration by rt-PCR analysis.

RESULTS AND DISCUSSION

Transduction with both lentiviruses and Sendai virus produced ES-like cell colonies. Morphological changes in transduced cells were seen as early as 9 days in lentiviral reprogrammed cells compared to day 12 in Sendai virus reprogrammed cells. Both viral reprogramming systems formed similar ES-like colonies by day 28 (Figure 1(A)). All lentivirus reprogrammed fibroblasts (n=3) were able to form ES-like colonies, whereas only 2 of Sendai Virus reprogrammed fibroblasts (n=3) successfully formed ES-like colonies. Reprogramming efficiency of lentivirus was 0.001%-0.002% whereas reprogramming using Sendai Virus was 0.001% in reprogrammed cells (Figure 1(B)), correlating with previous reports (1,2). All

expanded ES-like colonies were able to expand and maintain ES-like cell morphology on both feeder-layer and feeder free systems (Figure 1(C)). ES-like colonies generated by both viral systems expressed pluripotent nuclear markers OCT4, SOX2 and NANOG, and surface markers TRA-1-81, TRA-1-61 and SSEA4 (Figure 1(D)). Expression of >90% of pluripotent markers SSEA4 and TRA-1-81 and <5% differentiation marker SSEA1 was identified by flowcytometry (Figure 1(E)). No difference in pluripotency expression was seen between both viral reprogramming systems. IPSCs generated were able to form embryoid bodies and spontaneously differentiate into cells expressing markers of ectoderm (AFP), mesoderm (SMA) and endoderm (BIII-tubulin) (Figure 1(F)). Expanded iPSC clones showed similar karyotype as parent cells (Figure 1(G)). Sendai Virus reprogrammed iPSC clones showed evidence of viral clearance by rtPCR (Figure 1(H)). The higher reprogramming efficiency of integrative methods such as lentivirus may provide a more reliable choice compared to non-integrative Sendai Virus as it provides more stable transgene expression (3).

CONCLUSION

Viral reprogramming of primary human dermal fibroblasts is a robust reprogramming strategy for the generation of iPSCs. The choice of integrative or non-integrative viral reprogramming methods may further require assessment on the downstream use of the generated iPSC clones and cost of each reprogramming method.

ACKNOWLEDGEMENT

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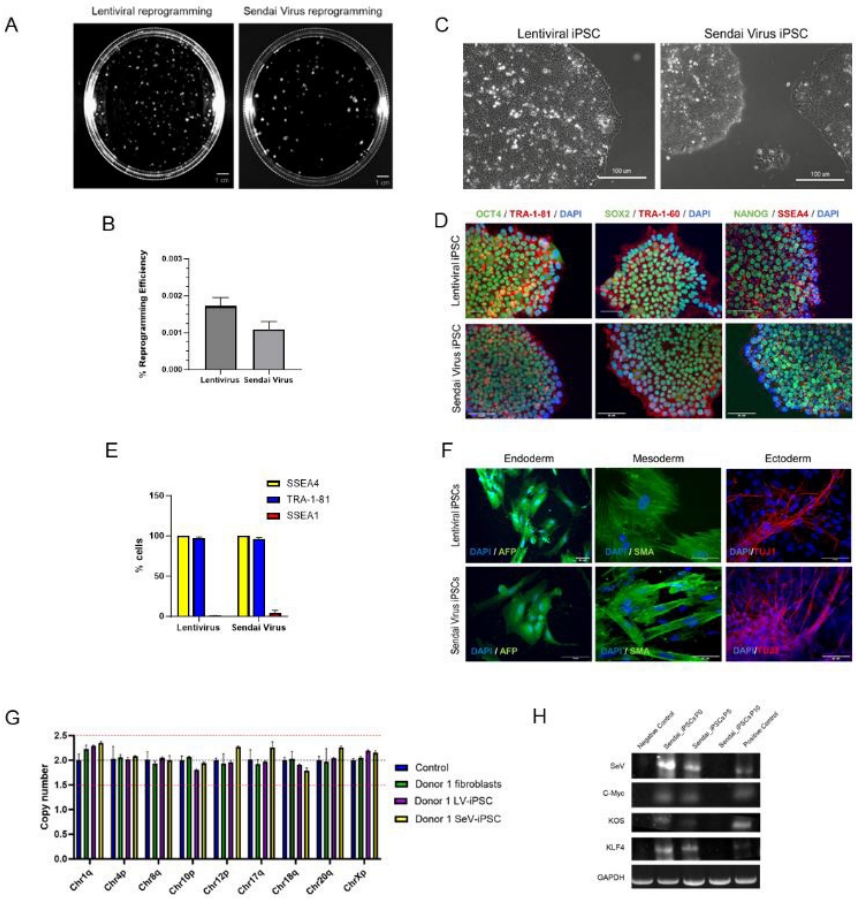

Figure 1: Generation and characterization of Lentivirus and Sendai Virus reprogrammed iPSCs. (A) Visible ES-like colonies 28-days post-transduction with Lentivirus or Sendai Virus reprogramming factors. (B) Reprogramming efficiency of Lentivirus and Sendai Virus reprogramming methods on dermal fibroblasts. (C) Morphology of generated iPSCs on Vitronectin-coated wells following clonal expansion. (D) Immunocytochemical analysis of pluripotent marker proteins OCT4A/ TRA-1-81, SOX2/TRA-1-61, and NANOG/SSEA4 in iPSC clones. (E) Flow cytometry analysis of expanded iPSC clones. (F) Immunocytochemical analysis of AFP (Endoderm), SMA (Mesoderm), and TUJ1 (Ectoderm) expression in 28-day spontaneously differentiated embryoid bodies produced from lentivirus and Sendai virus iPSC clones. (G) qPCR analysis of 8 common karyotypic mutations occurring in prolonged iPSC culture. (H) rtPCR analysis of Sendai Virus presence in P0, P5 and P10 Sendai virus reprogrammed iPSCs.

EXTENDED ABSTRACT

A Case of Granulocyte Transfusion in a Patient with Severe Neutropenia

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SUMMARY

Granulocyte transfusions have been used to treat infections in neutropenic patients, despite persistent controversy over their effectiveness. This poster illustrates a case study of a 14-year-old boy who was recently diagnosed with acute myeloid leukaemia (AML) complicated with neutropenic fever which did not improve despite optimal antimicrobial treatment. Subsequently, the medical team decided to administer granulocyte transfusions. From this case report, it was observed that granulocyte transfusion did not yield improvements due to a few factors which are the patient's advanced condition and the granulocyte content.

Keywords: granulocyte transfusion, severe neutropenia

INTRODUCTION

Granulocyte transfusions have been used to treat infections in neutropenic patients for over four decades, despite persistent controversy over their effectiveness. Most evidence suggests that granulocyte transfusions may be useful in a few cases as a temporary measure to control infection which is resistant to optimal antimicrobial treatments (1). This poster illustrates a case study of a 14-year-old boy who was recently diagnosed with acute myeloid leukaemia (AML) with neutropenic fever. He continued to have recurrent fevers and persistently low absolute neutrophil counts (ANC) despite receiving multiple antibiotics and antifungal treatment. Thus, the medical team decided to administer granulocyte transfusions in an effort to increase his ANC and improve his symptom.

MATERIALS AND METHODS

This is a retrospective case study wherein patient particulars and clinical records were obtained from the hospital's respective information system and Blood Bank Information System Version 2.0 (BBISV2). This study features a 14-year-old boy recently diagnosed with AML. As part of his curative treatment plan, he underwent intense chemotherapy, which resulted in marrow suppression and a decrease in neutrophil count—an anticipated effect of the regimen. Given the risk of bacterial infections due to compromised immune function resulting from very low neutrophil levels, the patient received antibiotics and antifungal treatments. Nevertheless, the patient continued to have a persistent fever and his ANC remained critically low. Consequently, the medical team opted for multiple granulocyte transfusions aimed to effectively augment the ANC and alleviate the patient's symptoms.

RESULTS AND DISCUSSION

The patient's blood group is O Rh(D) positive and his body weight is 55 kilograms. The patient's treatment plan involved three cycles of granulocyte transfusions. Granulocyte requests were submitted to Pusat Darah Negara, where granulocyte preparation was conducted utilizing whole blood collection from donors with a similar blood group as the patient. The platelet-rich plasma (PRP) method was employed in the preparation process. Prior to the transfusions, the patient's ANC was zero. Subsequently, granulocyte transfusions were administered at a dose of 1×10^{10} granulocytes (approximately 10-12 units of granulocytes) on three separate days (refer Table 1). Following the multiple transfusions, the patient's ANC remained persistently at zero, and he continued to exhibit clinical fever. From this study, it was observed that granulocyte transfusion did not yield improvements in the patient's condition (clinical and laboratory parameters). This outcome could be attributed to several factors. Firstly, the patient's critical state and advanced infection might have limited

the potential benefits of the intervention (1). Additionally, the granulocyte content in the transfusions may have been below the required dosage level (2). To enhance the effectiveness of granulocyte transfusions, employing apheresis granulocyte collection with stimulants and sedimentation agents could be a more favourable approach. This method is likely to yield a higher concentration of granulocytes, potentially increasing the therapeutic efficacy of the transfusions (1).

CONCLUSION

The findings of this case study align with the conclusion drawn from the Cochrane review (3). However, it is essential to acknowledge the existence of other case series that have demonstrated the benefits of granulocyte transfusion (2). Therefore, the significance and potential advantages of granulocyte transfusion cannot be disregarded. Furthermore, the emergence of resistant bacteria and the rising number of immunosuppressed patients, susceptible to a diverse spectrum of uncommon pathogens, may sustain the relevance and necessity of this specialized intervention.

ACKNOWLEDGEMENTS

The authors would like to thank the National Blood Centre and Hospital Tuanku Azizah in Malaysia for providing the patient's details. The authors would also like to acknowledge the support of the Ministry of Health Malaysia.

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Table I: Patient's ANC trend before and after granulocyte transfusions.

Date	21/7/2023	22/7/2023	23/7/2023	24/7/2023	25/7/2023
Granulocyte transfusion	No	1st cycle	No	2nd cycle	3rd cycle
ANC	0	0 (post-transfusion)	0	0 (post-transfusion)	0 (post-transfusion)

EXTENDED ABSTRACT

Allogeneic Human Umbilical Cord-Derived Mesenchymal Stem Cells Ameliorates LPS-Induced Cardiac Injury and Inflammation

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SUMMARY

Myocardial infarction produces systemic inflammation which in turn leads to a larger infarct size and cardiomyopathy. Human umbilical cord-mesenchymal stem cells (hUC-MSCs) possess anti-inflammatory effects and may potentially protect the heart from worsening myocardial damage. In this study, systemic inflammation was induced in BALB/c mice by lipopolysaccharide (LPS). Cardiac injury or myocardial damage was represented by plasma level of cardiac troponin-T. The study successfully demonstrated attenuation in myocardial damage associated with systemic inflammation reduction in mice treated with hUC-MSCs compared to sham injection.

Keywords: Human Umbilical Cord Mesenchymal Stem Cell, Cardiac Injury, Inflammation.

INTRODUCTION

Cardiovascular disease is closely linked to inflammation (1). Systemic inflammation results in atherosclerosis and thrombosis (2). In myocardial infarction, the accompanying acute inflammation could lead to a larger myocardial damage. hUC-MSCs have been reported to ameliorate the systemic inflammation by regulating the homeostasis of cytokines level (3). The present study aimed to investigate the anti-inflammatory effects of Cytopeutics® hUC-MSCs in attenuating systemic inflammation and reducing myocardial damage.

MATERIALS AND METHODS

Cardiac injury and systemic inflammation were induced by intraperitoneal (i.p) injection of 5 mg/kg body weight (BW) of LPS at 0.1 mL/mouse. Eighteen BALB/c mice were randomly grouped into 3 groups: 1) healthy group; 2) LPS only group; and 3) LPS + Cytopeutics® hUC-MSCs group. Healthy group and LPS only group were administered with normal saline by slow intravenous (i.v) bolus injection at 0.2 mL/mouse (sham injection). LPS + Cytopeutics® hUC-MSCs group was treated with 0.2 mL of 18.5 x 10^6 cells/kg BW. This dosage translated to a human equivalent dose (HED) of 1.5 x 10^6 cells/kg BW. At 24 h and day 7 post-treatment, blood plasma was collected for enzyme-linked immunosorbent assay (ELISA) assay. Tumor Necrosis Factor–alpha (TNF-α), Interleukins-6, -1beta (IL-6, IL-1β) and cardiac troponin-T were measured as highly specific marker of myocardial damage.

RESULTS AND DISCUSSION

LPS injection induced both systemic inflammation and cardiac injury as evidenced by elevated levels of pro-inflammatory cytokines and cardiac troponin-T. At 24 h post-treatment, the TNF-α level in Cytopeutics® hUC-MSCs treatment group (3.7 ± 1.8 pg/mL) was significantly reduced compared to the LPS only group (7.4 ± 1.0 pg/mL; $p = 0.0221$). IL-1β level also showed significant reduction in Cytopeutics® hUC-MSCs treatment group (0.1 ± 0.1 pg/mL) compared to the LPS only group (0.8 ± 0.4 pg/mL; $p = 0.0419$) at 24 h. These results were consistent with IL-6 level in Cytopeutics® hUC-MSCs treatment group which was reduced to 47.9 ± 15.7 pg/mL compared to LPS only group (63.4 ± 22.0 pg/mL) at 24 h albeit no significant statistical difference was observed ($p > 0.05$). At day 7, TNF-α, IL-1β and IL-6 levels in all groups were undetected and returned to near-normal levels. The result showed that LPS was able to successfully mimic a transient systemic inflammation which is a typical of acute myocardial infarction.

In addition, the Cytopeutics® hUC-MSCs treatment group had lower mean cardiac troponin-T (61.9 ± 13.0 pg/mL) compared to the LPS only group (88.6 ± 42.9 pg/mL) and the healthy group (76.0 ± 12.6 pg/mL). This indicated that Cytopeutics® hUC-MSCs may potentially

reduce cardiac injury. Cytopeutics® hUC-MSC treatment was able to control the acute inflammation quickly (at 24 h) and this anti-inflammatory effect may result in a smaller myocardial damage (at day 7).

CONCLUSION

18.5 x 10^6 cells/kg BW (HED 1.5 x 10^6 cells/kg BW) of Cytopeutics® hUC-MSCs was able to reduce LPS-induced systemic inflammation and LPS-induced cardiac injury at 24 h and 7 days, respectively. This finding is relevant when planning future clinical trials of MSCs for acute myocardial infarction.

ACKNOWLEDGEMENTS

This study (POD0002/PreCP/R) was funded by Cytopeutics Sdn. Bhd. The stem cells used in the study were provided by Cytopeutics Sdn. Bhd. We would like to acknowledge the contributions and support of the Molecular Pathology Unit, IMR and Cytopeutics Sdn. Bhd. in this study.

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