EVALUATION OF HUMAN PLATELET LYSATE AS AN ALTERNATIVE GROWTH FACTOR FOR EXPANSION AND HEPATOCYTE DIFFERENTIATION OF DENTAL PULP STEM CELLS

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ABSTRACT

The advancement of stem cells research has pushed the fronteir of regenerative medicine to a different level where range of diseases including liver diseases could be potentially treated using cell therapy. The usage of stem cells offers alternative treatment to organ transplantation, wherein, organ shortage issue and possibility of rejection are major limitations. Dental pulp stem cells from deciduous teeth (SCD) have been identified as a source of adult stem cells with high proliferation activity and multilineage differentiation. Current studies showed that SCD have potential to differentiate into hepatic lineage and they usually cultured in fetal bovine serum (FBS) supplemented media. Thus, this may expose SCD to the possibility of contamination to virus, prions, mycoplasma and transmission of disease. It is necessary to identify animal-free serum media if cell therapy is to play a major role in regenerative medicine. Human platelet lysate (HPL) appears to be a suitable substitution to FBS for stem cells culture as it is a non-animal origin and has a lot of natural growth factors to support stem cells proliferation and differentiation. The aim of this study was firstly to evaluate HPL as an alternative culture media to FBS in the culture, expansion and differentiation of SCD. Secondly, the ability of SCD to differentiate into hepatocyte differentiation when cultured in FBS and HPL will also be assesed. The properties of SCD cultured in both media were examined through growth kinetics, trilineages differentiation and expression of specific surface markers. The differentiation potential of SCD in FBS and HPL into hepatocyte-like cells was investigated from the morphological formation, periodic acid Schiff (PAS) staining and the expression of hepatocyte specific marker with urea secretion assay at day 0, 7, 14 and 21. Results showed SCD cultured in HPL has higher proliferation rate compared to FBS and displayed similar potential of trilineage differentiation and phenotypic expressions. SCD cultured in both media also displayed potential to form hepatocyte-like cells based on the morphology changes from

fibroblast to hexagonal-shaped cells and positive staining for PAS. The expression of hepatocyte specific markers and the urea secretion increased proportionally with the day of hepatocyte induction. Within the limitations in this study, it could be conclude that HPL could be an alternative to FBS as the proliferation of SCD cultured in HPL is statistically higher and exhibited similar characteristic to FBS. SCD in HPL also showed potential to differentiate into hepatocyte cells and thus may become a hope for alternative treatment of liver diseases.

ABSTRAK

Kemajuan penyelidikan sel stem telah membawa perubatan regeneratif ke satu tahap lain di mana pelbagai penyakit termasuk penyakit hati berpotensi untuk dirawat menggunakan terapi sel. Penggunaan sel stem menawarkan rawatan alternatif bagi pemindahan organ di mana isu kekurangan organ dan kemungkinan penolakan merupakan halangan utama bagi kaedah tersebut. Sel stem pulpa gigi daripada gigi susu (SCD) telah dikenalpasti sebagai sumber sel stem dewasa yang mempunyai aktiviti pembiakkan yang tinggi dan pembezaan kepada pelbagai sel lain. Kajian terkini menunjukkan bahawa SCD mempunyai potensi untuk membezakan kepada sel hepatik dan mereka biasanya dikultur di dalam media yang dibekalkan dengan janin lembu serum (FBS) media. Oleh itu, ini menyebabkan SCD terdedah kepada kemungkinan pencemaran virus, prions, mycoplasma dan penyebaran penyakit. Mengenalpasti media bebas serum haiwan adalah penting jika terapi sel akan dijadikan peranan utama dalam perubatan regeneratif. Platelet lysate manusia (HPL) muncul sebagai gantian yang sesuai bagi FBS untuk kultur sel stem kerana ia adalah berasal dari bukan-haiwan dan mempunyai banyak faktor-faktor pertumbuhan semulajadi untuk menyokong proliferasi sel stem dan pembezaannya. Tujuan kajian ini adalah pertamanya untuk menilai HPL sebagai media kultur alternatif kepada FBS bagi kultur, pembiakkan dan pembezaan SCD. Kedua, keupayaan SCD untuk membezakan ke pembezaan hepatosit apabila dikultur di dalam FBS dan HPL juga akan dinilai. Sifat-sifat SCD yang dikultur di dalam kedua-dua media telah diperiksa melalui kinetik pertumbuhan, pembezaan kepada tiga sel lain, dan ekspresi penanda permukaan yang spesifik. Potensi pembezaan SCD dalam FBS dan HPL kepada sel hepatosit telah disiasat melalui pembentukan morfologi, pewarnaan asid berkala Schiff (PAS) dan ekspresi penanda hepatosit yang spesifik serta ujian perembesan urea pada hari 0, 7, 14 dan 21. Hasil kajian menunjukkan SCD yang dikultur di dalam HPL mempunyai kadar proliferasi yang lebih

tinggi berbanding FBS dan mempamerkan potensi pembezaan kepada tiga jenis sel lain serta eksperasi phenotip yang sama. SCD yang dikultur di dalam kedua-dua media juga telah menunjukkan potensi untuk membentuk sel-sel hepatosit berdasarkan perubahan morfologi dari fibroblast kepada sel-sel berbentuk heksagon dan pewarnaan yang positif bagi pewarnaan PAS. Ekspresi penanda hepatosit yang spesifik dan perembesan urea juga telah meningkat secara berkadaran dengan hari penginduksian hepatosit. Dengan had-had di dalam kajian ini, kesimpulan yang boleh dibuat ialah HPL boleh menjadi satu alternatif kepada FBS memandangkan SCD yang dikultur di dalam HPL mempunyai kadar proliferasi yang lebih tinggi secara statistiknya dan mempamerkan ciri-ciri yang serupa dengan FBS. SCD di dalam HPL juga memiliki potensi untuk membentuk sel hepatosit dan seterusnya akan menjadi harapan untuk rawatan alternatif penyakit hati.

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Ag	Antigen surface
ASCs	Adipose stem cells
bFGF	Basic fibroblast growth factor
β-gal	Beta-galactosidase
BMSCs	Bone marrow stem cells
BSA	Bovine serum albumin
CD	Cellular differentiation
cDNA	Complementary DNA
CFU	Colony forming units
CO_2	Carbon dioxide
DAPI	4′,6′-diamidino-2-phenylindole dihydrochloride
ddH_2O	Double distilled water
DEPC	Diethylpyrocarbonate
DFPC	Dental follicle progenitor cells
DMEM-KO	Dulbecco's modified Eagle's medium-knock-out
DPBS	Dulbecco's Phosphate-Buffered Saline
DPSC	Extracted adult dental pulp stem cells
DSCs	Dental stem cells
EDTA-trypsin	Ethylenediaminetetraacetic acid-trypsin
EGF	Epidermal growth factor
EMT	Epithelial to mesenchymal transition
ESCs	Embryonic stem cells

FBS	Fetal bovine serum
FGF	Fibroblast growth factor
FITC	Fluorescein isothiocyanate
HGF	Hepatocyte growth factor
HPL	Human platelet lysate
ICM	Inner cell mass
Ig	Immunoglobulin
IGF-1	Insulin-like growth factor-1
ISSCR	International Society of Stem Cell Research
ITS	Insulin-Transferrin- Selenium-X
MAP 2	Microtubule associated protein 2
MEM	Minimal Essential Medium
MET	Mesenchymal to epithelial transition
mg/ml	Milligram per milliliter
ml	Milliliter
Mm	Millimeter
MSCs	Mesenchymal stem cells
OsM	Oncostatin M
PAS	Periodic acid Schiff
PD	Population doubling
PDEGF	Platelet-derived epidermal growth factor
PDGF	Platelet-derived growth factor
PDLSC	Periodontal ligament stem cells
PDT	Population doubling Time

PF-4	Platelet factor-4
PFA	Paraformaldehyde
PVP-I	Povidone-iodine
rpm	Revolutions per minute
RT-PCR	Reverse transcriptase polymerase reaction
SA-β-gal	Senescence associated β-galactosidase
SCAP	Stem cells from apical papilla
SCD	Dental pulp stem cells from deciduous teeth
SDS	Sequence detection system software
SHED	Stem cells of human exfoliated teeth
SSEA-4	Stage-specific embyonic antigen 4
TGF-β	transforming growth factor-β
UCSCs	Umbilical cord stem cells
µg/ml	Micrograms per milliliter
µl	Microliter
VEGF	vascular endothelial growth factor