"Human Dental Pulp Stem Cell Proliferation and Maintenance in Xeno Free/Serum Free Defined Culture Media (in Vitro Study)"

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By

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Abstract

Background: Dental pulp stem cells (DPSCs) are considered an easily accessible source of mesenchymal stem cells which hold great promise for use in tissue repair and regenerative medicine. Routinely, culture media used for culturing stem cells are supplemented by animal serum for promoting growth and successful maintenance of stem cells. Although, fetal bovine serum (FBS) is now considered a universal growth supplement effective for almost all types of human and animal cells, it still bears a number of disadvantages. Thus, there is a growing demand for optimizing a well-defined culture media that could safely increase the efficacy and reproducibility of the cultured cells especially when clinical applications are intended. **Objective:** In this research, we aimed at optimizing a defined culture medium enriched by a various supplements that is capable of enhancing DPSCs proliferation and maintenance. **Methods:** various supplements intended to enrich DPSCs proliferation in defined concentrations were designed such as recombinant human Basic Fibroblast Growth Factor (hBFGF), Insulin Transferrin Selenium (ITS), Ascorbic acid, Beta mercaptoethanol and Cholesterol. The effect of this optimized media on the proliferation of DPSCs was assessed by MTT assay and expression of stemness-related genes *(OCT4, SOX2* and *NANOG)* was analyzed by qRt-PCR. **Results:** Proliferation results by MTT illustrated a significant increase in the proliferation rate of DPSCs cultured in the proposed culture media. Expression of *OCT4, SOX2* and *NANOG* genes was also up-regulated, confirming that the proposed combination of supplements not only increased the proliferation potential of DPSCs but also enhanced its stemness properties. **Conclusions**: Taken together, our results indicate that proposed optimized media is safe and efficient and can readily substitute traditional animal derived supplements like FBS.

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Dedication

To my Family

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Abbreviations and Symbols

Abbreviation	Full name
Ala	Alanine
Arg	Arginine
Asn	Asparagine
Asp	Aspartic acid
bFGF	basic fibroblast growth factor
BSA	Bovine serum albumin
CD	Chemically defined
CFU	Colony forming unit
Ct	Cycle threshold
Cu	Copper
Cys	Cysteine
DFSCs	Dental Follicle Stem Cells
DMEM	Dulbecco modified Eagle medium
DPSCs	Dental pulp stem cells
EGF	epidermal growth factor
FACS	Fluorescent activated cell sorting
FBS	Fetal Bovine Serum
Fe	Fe
FGF	Fibroblast growth factor
GAPDH	Glyceraldehyde 3-phosphate dehydrogenase
Gln	Glutamine
Glu	Glutamic acid
Gly	Glycine
hES	human embryonic stem cells
HGF	hepatic growth factor
His	Histidine
hMSCs	human mesenchymal stem cells
hPL	human platelet lysate
hs	human serum
hUCBS	umbilical cord blood serum
IGF-1	insulin growth factor-1
IGF-2	insulin growth factor-2
Ile	Isoleucine
iPS cells	induced pluripotent stem cells
ITS	Insulin Transferrin Selenium
Leu	Leucine

Lys	Lysine
Met	Methionine
MSCs	Mesenchymal stem cells
MTT	3(-4,5-dimethylthiazol-2-yl)2,5 diphenyltetrazolium bromide).
P0	passage zero
PBS	phosphate buffer solution
PCR	polymerase chain reaction
PDGF	platelet-derived growth factor
PDLSCs	Periodontal Ligament Stem Cells
Phe	Phenylalanine
PI	propidium iodide
Pro	Proline
qRt-PCR	Quantitative real time PCR
Res	relative expressions
REST	Relative expression software tool
SCAPs	Stem Cells from the dental Apical Papilla
SCC	supplement for cell culture
Se	Selenium
SEM	standard error of the mean
Ser	Serine
SFM	Serum Free Medium
SHEDs	Human Exfoliated Deciduous teeth
SSM	Serum supplemented media
T3	triiodothyronine
TGF-*β*1	transforming growth factor-*βeta*1
Thr	Threonine
Trp	Tryptophan
Tyr	Tyrosine
Val	Valine
VEGF	vascular endothelial growth factor
Zn	Zinc
ΔΔCT	Delta cycle threshold

Introduction and Review of Literature