Isolation and Characterization of Stem Cells from the Submandibular and Parotid Salivary Glands of Albino Rats

Thesis Submitted For Partial Fulfillment of the Requirements of Doctorate Degree in Oral Biology

By

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ABSTRACT

Background: Salivary gland stem cell therapy is an attractive putative option to treat various salivary gland disorders and cases causing salivary hypofunction.

Aim: The aim of this work is to isolate stem cells from the Submandibular and Parotid salivary glands of albino rats for identification and characterization. Also, to assess the proliferation rate, the cryopreservation ability, and to culture isolated stem cells in cell culture inserts.

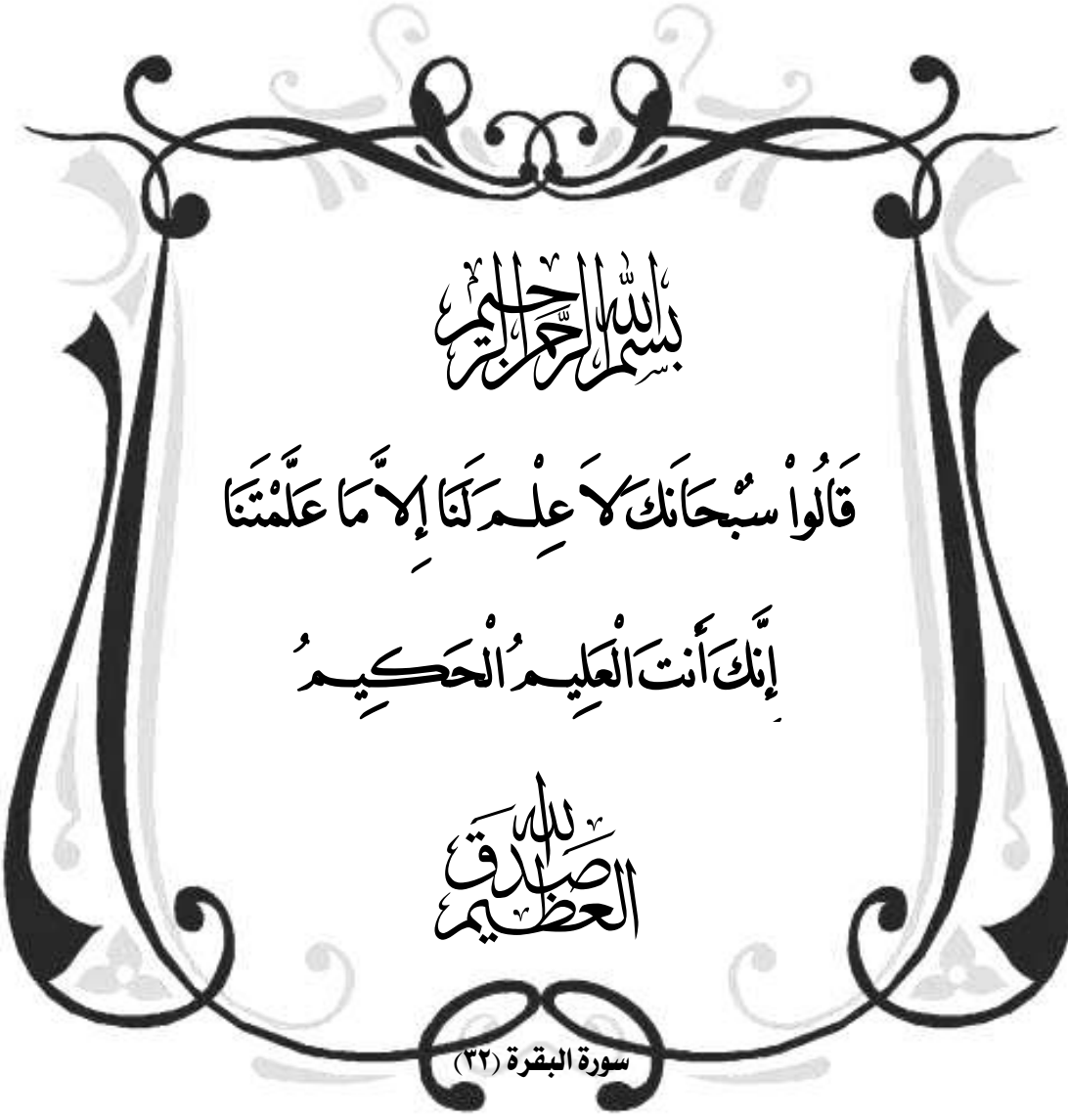
Methodology: Fifteen adult male albino rats were used in this study. The Submandibular and Parotid salivary glands were dissected and prepared for tissue culture. Identification and characterization were carried out through assessment of proliferation rate, performing flow cytometry analysis and colony forming unit-fibroblast assay. Cryopreservation, Culturing of stem cells in cell culture inserts and ultrastructural study of the SG stem cells using Scanning electron microscopy were also carried out.

Results: Stem cells from both glands were successfully isolated and were positive for salivary gland stem cell markers CD133 and CD117. The Submandibular salivary gland stem cells showed faster and higher proliferation rate and also formed more colonies than the parotid gland stem cells. Stem cells from both glands formed multicellular layers when cultured on the

transmembrane culture inserts yet the submandibular multicellular layer was apparently thicker than that of the Parotid gland. Cryopreservation was performed for both groups and thawed cells of the Submandibular group showed a higher percentage of viability than the parotid group.

Conclusions/Significance: Rats salivary glands contain a ‘putative’ stem cell population, expressing salivary gland stem cell markers that are capable of forming multicellular layers when cultured on transmembrane cell culture inserts. Also, stem cells isolated from salivary glands can be successfully cryopreserved showing sufficiently high number of viable cells after thawing.

To My Family


بسم الله الرحمن الرحيم
قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا
إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ
صدق الله العظيم
سورة البقرة (٣٢)

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LIST OF ABBREVIATIONS

A/M	**:**	Acetone/ Methanol
AFM	**:**	Atomic Force Microscope
BMC	**:**	Bone Marrow derived Cells
BrM	**:**	Branching morphogenesis
CAC	**:**	Cacodylate
CD117	**:**	Cluster of Differentiation 117
CFU-F	**:**	Colony Forming Unit Fibroblast assay
CK7	**:**	Cytokeratin 7
CPA	**:**	Cryoprotectant Agent
dH_2O	**:**	Distilled water
DMEM	**:**	Dulbeco Modified Eagle's Medium
DMSO	**:**	Dimethyl Sulfoxide
ECM	**:**	Extracellular Matrix
EDTA	**:**	Ethylene Diamine Tetra-acetic Acid
EGF	**:**	Epidermal Growth Factor
ESCs	**:**	Embryonic Stem Cells
ETOH	**:**	Ethanol
FITC	**:**	Fluorescin isothiocyanate
GA	**:**	Gluteraldehyde
G-CSF	**:**	Granulocyte Colony Stimulating Factor
GCTs	**:**	Granular Convoluted Tubules
GFR	**:**	Growth Factor Reduced
GVHD	**:**	Graft Versus Host Disease
GY	**:**	Gray
H&E	**:**	Haematoxylin and Eosin
HBSS	**:**	Hank's Buffered Saline Solution
HMDS	**:**	Hexamethyl disilasane

hMSGMSCs	**:**	Human Minor Salivary Gland Mesenchymal Stem Cells
HS	**:**	Human Serum.
hSGSC's	**:**	Human Submandibular Salivary Gland Stem Cells
ID	**:**	Intercalated Duct
iPSC's	**:**	Induced Pluripotent Stem Cells
LI	**:**	Labeling Index
MSCs	**:**	Mesenchymal Stem Cells
NGF	**:**	Nerve Growth Factor
OsO_4	**:**	Osmium Tetraoxide
PAS	**:**	Periodic Acid Schiff
PBS	**:**	Phosphate Buffered Saline
PFA	**:**	Paraformaldehyde
PL	**:**	Platelet Lysate
PLGA	**:**	Polylactic-co-Glycolic Acid
pSGEC's	**:**	Primary Salivary Gland Epithelial Cells
RT–PCR	**:**	Reverse Transcription–Polymerase Chain Reaction
SEM	**:**	Scanning Electron Microscope
SFS	**:**	Silk Fibroin Scaffold
SG	**:**	Salivary Gland
SGP	**:**	Salivary Gland Progenitor Cells
SGSCs	**:**	Salivary Gland Stem Cells
SM	**:**	Submandibular
SMG	**:**	Submandibular Salivary Gland
SS	**:**	Sjogren's Syndrome