



# ***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

**Dina Hazem H. Gomaa**

B.D.S-(2002) Faculty of Dentistry - Ain shams University

Master Degree Oral Biology (2011)

Faculty of Dentistry - Ain Shams University

## **Supervision**

**Prof. Dr. Souzi M. Farid Shinaishin**

Head of Oral Biology Department

Faculty of dentistry- Ain shams University

**Dr. Rania Mossad Hassan**

Assistant professor of Oral Biology

Faculty of Dentistry - Ain shams University

**Dr. Sayed Bakry Ahmed**

Assistant professor of Experimental Biology

Faculty of Science – Al Azhar University

**Faculty of Dentistry**

**Ain Shams University**

**2015**



# عزل وتوصيف الخلايا الجزعية من الغدد اللعابية تحت الفكية والنكفية فى الفئران البيضاء

رسالة مقدمة من

الطبيبة / دينا حازم حسن جمعة

المدرس المساعد بقسم بيولوجيا الفم  
كلية طب الأسنان - جامعة عين شمس

كجزء من مقومات الحصول على درجة الدكتوراه فى بيولوجيا الفم

تحت إشراف

أ.د/ سوزى محمد فريد شنیشن

أستاذ ورئيس قسم بيولوجيا الفم  
كلية طب الأسنان - جامعة عين شمس

أ.م.د/ رانيا مسعد حسن

أستاذ مساعد بقسم بيولوجيا الفم  
كلية طب الأسنان - جامعة عين شمس

أ.م.د/ سيد بكرى أحمد

أستاذ مساعد بقسم البيولوجيا التجريبية  
بكلية العلوم - جامعة الأزهر

كلية طب الأسنان

جامعة عين شمس

٢٠١٥

## ***Acknowledgment***

*All thanks and praise to **ALLAH** who guided and enabled me to fulfill this work.*

*I would like to express my deepest gratitude and appreciation to **Prof. Dr. Souzi Farid Shinaishan**, Head of Oral Biology department, Faculty of dentistry , Ain shams university. Dr. Souzi have always helped, advised and guided me throughout the years since I joined the department.*

*I would also like to thank **Dr Rania Mosaad Hassan**, Assisstant Professor of Oral Biology, Faculty of Dentistry-Ain shams University, who provided me with all the help, knowledge and guidance and supported me in every way.*

*I deeply appreciate and would like to thank **Prof Dr. Medhat A. El-Zainy** professor of Oral Biology and former vice dean of Society and Environmental affairs Faculty of Dentistry, Ain shams university, for his constant encouragement, sound advice and fatherly guidance.*

*My gratitude is extended to **Dr Sayed Bakry** Assisstant Professor of Experimental Biology, Faculty of Science- Al Azhar university, for helping me accomplish this work.*

*Finally, I would like to thank the entire staff of Oral Biology department Faculty of dentistry, Ain Shams university for their cooperation and valuable support.*

# 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***

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قَالُوا سُبْحَانَكَ لَا عِلْمَ كُنَّا إِلَّا مَا عَلَّمْتَنَا

إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ

اللَّهُ  
صَدِّقُ  
الْعَظِيمِ

سورة البقرة (٣٢)

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# LIST OF ABBREVIATIONS

<b>A/M</b>	: Acetone/ Methanol
<b>AFM</b>	: Atomic Force Microscope
<b>BMC</b>	: Bone Marrow derived Cells
<b>BrM</b>	: Branching morphogenesis
<b>CAC</b>	: Cacodylate
<b>CD117</b>	: Cluster of Differentiation 117
<b>CFU-F</b>	: Colony Forming Unit Fibroblast assay
<b>CK7</b>	: Cytokeratin 7
<b>CPA</b>	: Cryoprotectant Agent
<b>dH<sub>2</sub>O</b>	: Distilled water
<b>DMEM</b>	: Dulbeco Modified Eagle's Medium
<b>DMSO</b>	: Dimethyl Sulfoxide
<b>ECM</b>	: Extracellular Matrix
<b>EDTA</b>	: Ethylene Diamine Tetra-acetic Acid
<b>EGF</b>	: Epidermal Growth Factor
<b>ESCs</b>	: Embryonic Stem Cells
<b>ETOH</b>	: Ethanol
<b>FITC</b>	: Fluorescein isothiocyanate
<b>GA</b>	: Gluteraldehyde
<b>G-CSF</b>	: Granulocyte Colony Stimulating Factor
<b>GCTs</b>	: Granular Convolute Tubules
<b>GFR</b>	: Growth Factor Reduced
<b>GVHD</b>	: Graft Versus Host Disease
<b>GY</b>	: Gray
<b>H&amp;E</b>	: Haematoxylin and Eosin
<b>HBSS</b>	: Hank's Buffered Saline Solution
<b>HMDS</b>	: Hexamethyl disilane

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<b>hMSGMSCs</b>	: Human Minor Salivary Gland Mesenchymal Stem Cells
<b>HS</b>	: Human Serum.
<b>hSGSC's</b>	: Human Submandibular Salivary Gland Stem Cells
<b>ID</b>	: Intercalated Duct
<b>iPSC's</b>	: Induced Pluripotent Stem Cells
<b>LI</b>	: Labeling Index
<b>MSCs</b>	: Mesenchymal Stem Cells
<b>NGF</b>	: Nerve Growth Factor
<b>OsO<sub>4</sub></b>	: Osmium Tetraoxide
<b>PAS</b>	: Periodic Acid Schiff
<b>PBS</b>	: Phosphate Buffered Saline
<b>PFA</b>	: Paraformaldehyde
<b>PL</b>	: Platelet Lysate
<b>PLGA</b>	: Polylactic-co-Glycolic Acid
<b>pSGEC's</b>	: Primary Salivary Gland Epithelial Cells
<b>RT-PCR</b>	: Reverse Transcription-Polymerase Chain Reaction
<b>SEM</b>	: Scanning Electron Microscope
<b>SFS</b>	: Silk Fibroin Scaffold
<b>SG</b>	: Salivary Gland
<b>SGP</b>	: Salivary Gland Progenitor Cells
<b>SGSCs</b>	: Salivary Gland Stem Cells
<b>SM</b>	: Submandibular
<b>SMG</b>	: Submandibular Salivary Gland
<b>SS</b>	: Sjogren's Syndrome