

**Isolation and Characterization of Gingival Mesenchymal Stem
Cells from Healthy and Inflamed Gingival Tissue: Migration
and Proliferation Potential Through a Guided Tissue
Membrane. (In vitro study)**

Presented by:

Mohamed Al Bahrawy

Assistant Lecturer Ain Shams University

In the partial fulfillment of the requirements of PhD degree

Supervisors:

Dr Khaled Abdel Ghaffar*

Professor and Dean of Faculty of Dentistry Ain Shams University

Dr Ahmed Yousef Gamal*

Professor and Chair of Oral Medicine and Periodontology department

Dr Vincent J Iacono**

Professor and Chair of Periodontology department

Dr Ahmed Abdel Aziz*

Lecturer of Oral Medicine and Periodontology

Ain Shams University* – Stony Brook University**

2016

Acknowledgment:

Before and after all the thanks to Allah. Because who don't thanks people didn't thank Allah, I would like to show my deepest appreciation and thanks to Professor Dr Khaled Abdel Ghaffar dean of school of dentistry, Ain Shams University, and the primary mentor of this study, for his valuable efforts and close supervision.

I would show my deepest thanks to Professor Dr Ahmed Gamal, Chair of periodontology department, school of dentistry, Ain Shams University, for his great efforts, persistent help, and close supervision of each step in this study at home and at USA, so as this work could proceed to an end. Without his advices and support I don't think this study could proceed to reach its goal.

I would like to show my greatest appreciation for Dr Ahmed Abdel Aziz lecturer in Periodontology department, school of dentistry, Ain Shams University, for his support, close supervision and valuable observations.

Last but not least, I would thank Dr Vincent Iacono, for giving me the opportunity to develop my scientific experience by working in his lab.

Dedication:

TO MY LOVELY MOTHER WHO DEDICATED HER LIFE TO RAISE ME UP, AND SUFFERED A LOT IN HER OLD AGE NOT FINDING ME BESIDE HER FOR WHOLE 2 YEARS TILL MY PROJECT REACHED AN END.

TO THE SOUL OF MY FATHER WHO SUPPORTED ME TO BE A MAN, AND WHO SCULPTURED MY SCIENTIFIC STAMINA.

List of abbreviations:

MSC: Mesenchymal stem cells.

GTR: Guided tissue regeneration.

OM: Occlusive membrane.

MPM: Modified perforated membrane.

BMP: Bone morphogenic proteins.

VEGF: Vascular endothelial growth factors.

PDGF: Platelets derived growth factors.

ICML: Inner cell mass blastocyst.

OCT: Octamer binding protein.

SSEA: Stage specific embryonic antigen.

Sox: Sex determining region box.

ASC: Adult stem cell.

CFU-F: Colony forming unit fibroblast.

MUSE: Multi-lineage enduring stem cells.

BMSC: Bone marrow mesenchymal stem cells.

DPSCL: Dental pulp stem cells.

FCS: Fetal calf serum.

FFPP: Fresh frozen plasma platelets.

PDLSC: periodontal ligament stem cells.

DFC: Dental Follicle cells.

SCAP: Stem cells from root apical papilla.

List of figures

	Page
Figure 1	9
Different types of stem cells and their differentiation potential.	
Figure 2	36
The different sources of stem cells of dental origin.	
Figure 3	61
The design and rationale of perforated GTR membrane	
Figure 4	70
The gingival tissue was sliced then meshed so as to enhance the effect of the digestive enzymes to isolate the GMSCs.	
Figure 5	76
Macro perforation of the trans-well inserts using syringe needles.	
Figure 6	81
Magnetic sorting kit.	
Figure 7	83
Histological hematoxylin and Eosin staining confirmation of healthy vs inflamed gingival tissue.	

Figure 8 **84**

Sox 2 as marker for GMSCs in tissue sections.

Figure 9 **85**

Nucleostamin as a marker for GMSCs in tissue sections.

Figure 10 **86**

OCT 4 and CD 105 as a marker for GMSCs in tissue sections.

Figure 11 **87**

L6 as a marker for GMSCs in tissue sections.

Figure 12 **88**

Gingival Tissue Extract colony forming Unit formation.

Figure 13 **90**

Graphs showing the flow cytometry results of the healthy GMSC line.

Figure 14 **92**

Differentiation abilities of GMSCs.

Figure 15 **93**

MTT assay.

Figure 16 **95**

Image shows Macro-perforated trans-well collagen membrane, using 10% bovine serum as chemo-attractant.

Figure 17 **95**

Images show Macro-perforated trans-well collagen membrane, with no chemo-attractant.

Figure 18 **98**

Representative images of migrated GMSCs through 8 microns perforated membranes.

Figure 19 **100**

Representative images staining and counting of migrated GMSCs through 3 and 0.4 μm perforated collagen membrane.

Figure 20 **102**

A comparison of the migrated GMSCs in the inflamed tissue origin cells versus healthy tissue origin cells through 0.4, 3, 8 microns perforated membrane respectively.

Figure 21 **103**

Scanning electron microscopy of GMSCs migrated through ultra-fine perforated membranes.

Figure 22 **104**

A bar chart comparing the migration of GMSCs through different ultra-fine pore sizes membranes.

Figure 23 **106**

The mean of the migrated GMSCs isolated from healthy and inflamed gingival tissue through different pore sizes.

Figure 24 **107**

Staining GMSCs attached to cover slip using CD 105, Oct 4, and DAPI.

Figure 25 **108**

Staining GMSCs attached to cover slip using L6, Oct 4, and DAPI.

Figure 26 **109**

Staining GMSCs attached to cover slip using CD 105, Oct 4, without DAPI

List of Tables:

	Page
Table 1	18
The major characteristic to define a stem cell.	
Table 2	41
The general properties of stem cells of dental origin.	
Table 3	55
The sensitivity of the BM-MSCs to different growth and chemotactic factors.	
Table 4	88
The mean and SD of colony forming unit experiments.	
Table 5	89
Population Doubling Assay outcomes of 3 experiments of GMSCs, and the total assay outcome of each experiment.	

Table 6

94

The Spectrophotometer reading of MTT staining of gingival tissue extracted mesenchymal stem cells in comparison to Control.

Table 7

96

Average number of attached MSCs on macro-perforated collagen membrane with pore sizes 400, 700 and 1000 microns.

Table of contents:

	Page
Introduction	1
Review of literature	7
Aim of the study	65
Materials and methods	67
Discussion	111
Summary	131
Conclusions	133
Recommendations	137
References	139