

شبكة المعلومات الجامعية التوثيق الإلكتروني والميكروفيلو

بسم الله الرحمن الرحيم





HANAA ALY



شبكة المعلومات الجامعية التوثيق الإلكتروني والميكرونيله



شبكة المعلومات الجامعية التوثيق الالكتروني والميكروفيلم



HANAA ALY



شبكة المعلومات الجامعية التوثيق الإلكترونى والميكروفيلم

جامعة عين شمس التوثيق الإلكتروني والميكروفيلم قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها على هذه الأقراص المدمجة قد أعدت دون أية تغيرات



يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار



HANAA ALY



Adipose - Derived Stromal Vascular Fraction in Treatment of Osteoarthritis: Experimental Study

Thesis

Submitted for Partial Fulfillment of the Requirement of the Master Degree in Physical Medicine, Rheumatology and Rehabilitation

By

Sherine Alaa El Din Mohamed Moussa

M.B, B.CH, Faculty of Medicine, Ain Shams University

Under supervision of

Prof. Dr. Mohamed Gamal El Din Zaki

Professor of Physical Medicine, Rheumatology & Rehabilitation Faculty of Medicine, Ain Shams University

Prof. Dr. Manal Osman Mohamed

Professor of Physical Medicine, Rheumatology & Rehabilitation Faculty of Medicine, Ain Shams University

Assist. Prof. Dina Abou Bakr Farrag

Assistant Professor of Physical Medicine, Rheumatology & Rehabilitation Faculty of Medicine, Ain Shams University

> Faculty of Medicine Ain Shams University 2021



سورة البقرة الآية: ٣٢

Acknowledgment

First and foremost, I feel always indebted to ALLAH, the Most Kind and Most Merciful.

I'd like to express my respectful thanks and profound gratitude to **Prof. Dr. Mohamed Gamal & Din Zaki,** Professor of Physical Medicine, Rheumatology & Rehabilitation, Faculty of Medicine, Ain Shams University for his keen guidance, kind supervision, valuable advice and continuous encouragement, which made possible the completion of this work.

I am also delighted to express my deepest gratitude and thanks to **Prof. Dr. Manal Osman Mohamed,** Professor of Physical Medicine, Rheumatology & Rehabilitation, Faculty of Medicine, Ain Shams University, for her kind care, continuous supervision, valuable instructions, constant help and great assistance throughout this work.

I am deeply thankful to Assist. Prof. Dina Abou Bake Farrag, Assistant Professor of Physical Medicine, Rheumatology & Rehabilitation, Faculty of Medicine, Ain Shams University, for her great help, active participation and guidance.

I wish to express my deepest thanks and respect to **Dr. Asmaa Abdelmonem AboZeid**, Assistant professor of Histology and cell biology. Faculty of Medicine, Ain Shams University, for her valuable supervision, guidance and kind advice throughout this work.

Sherine Alaa

List of Contents

Title	Page No.
List of Abbreviations	i
List of Tables	iv
List of Figures	v
Introduction	1
Aim of the Work	3
Review of Literature	
Osteoarthritis (OA)	4
Stromal Vascular Fraction (SVF)	37
Materials and Methods	62
Results	77
Discussion	103
Summary	116
Conclusion	119
Recommendations	120
References	121
Arabic Summary	

List of Abbreviations

Abbreviation	Full term
	Articular Cartilage A disintegrin and metalloproteases with thromboplastin motifs
ADSCs	Adipose derived stem/stromal cells
Angpt-1	Angiopoietin-1
AP	Anteroposterior
ASCs	Adipose tissue derived stromal cells
ATC	Adipose tissue complex
BADSCs	Brown adipose derived stem cells
BAT	Brown adipose tissue
BBB	Blood brain barrier
bFGF	Basic fibroblast growth factor
BMI	Body Mass Index
BMP	Bone morphogenic protein
CD	Cluster of differentiation
COMP	Cartilage oligomeric matrix protein
COVID-19	Corona virus disease-19
CRP	C-reactive protein
CZ	Cartilage zone
DAMPs	Damage associated molecular patterns.
DMEM	Dulbecco's Modified Eagle Medium
DZ	Deep zone
ECM	Extracellular matrix
ECs	Endothelial Cells
EDTA	Ethylenediaminetetraacetic Acid
EPCs	Endothelial Precursor/Progenitor Cells
EV	Extracellular vesicle
FBS	Fetal bovine serum
FCM	Flow cytometry
FGF	Fibroblast growth factor
FITC	Fluorescein isothiocyanate

List of Abbreviations Cont...

Abb.	Full term
GAG	Glycosaminoglycan
	Good laboratory practices
	Good manufacturing practices
	Hyaluronic acid
	Hepatocyte growth factor
	High power field
	Hematopoietic stem cells
	Hematoxylin and Eosin stain
	Induced arthritis
I-CS	Intra-articular corticosteroids
IFN-γ	Interferon gamma
	Insulin growth factor
IGFBPs	Insulin like growth factor binding proteins
I-HA	Intra- articular hyaluronic acid
IL	Interleukin
IL-1RA	Interleukin 1 receptor antagonist
IZ	Intermediate zone
JAK/STAT	Janus kinase / signal transduction and
	activator of transcription.
	Joint space Narrowing
	Knee Osteoarthrtis
	Monoclonal antibody
	Metalloproteases
	Magnetic resonance imaging
	Mesenchymal stem cells
	Nerve growth factor
	Non- steroidal Anti- inflammatory drugs
OA	
	Posteroanterior
	Phosphate buffer saline
PCR	Polymerase chain reaction

List of Abbreviations Cont...

Abb.	Full term
PDGF-BB	Platelet derived growth factor- BB
	Platelet rich plasma
	Red blood cell
	Radiofrequency
	Range of motion
	Standard deviation
SLRP	Small leucine rich proteoglycan
SRY	=
	Stromal vascular Fraction
Sy-SADOA	Systemic slow acting drugs of OA
·	Superficial zone
	Transcutaneous electrical nerve stimulation
TGF –β	Transforming growth factor β
	Transforming growth factor
TIMPs	Tissue inhibitors of MMPs
TKA	Total knee arthroplasty
	Tumor necrosis factor
Trk	Tropomyosin-receptor-kinase
US	
VAS	Visual analogue scale
	Vascular endothelial growth factor
	Vulvar lichen sclerosis
WAT	White adipose tissue
WBC	White blood cells
WOMAC	Western Ontario and McMaster universities
	osteoarthritis index

List of Tables

Table No.	Title	Page No.
Table (1):	Characterization of SVF	58
Table (2):	Shows the most frequent side effect SVF treatment	
Table (3):	Modified Mankin Score	75
Table (4):	Shows the flow cytometry analysis cells in a sample from SVF using CD45 and CD44 markers	CD34,
Table (5):	Shows the expression of SRY gene in samples by Real time PCR (qPCR) in treated with SVF (Group III) and unsample of induced osteoarthritis (Gro	sample ntreated
Table (6):	Shows the Comparison between the groups regarding MS	
Table (7):	Shows the Comparison re Chondrocyte cell count between the groups:	studied
Table (8):	Comparison between the studied regarding their articular c thickening.	artilage
Table (9):	Showing difference in optical denimage analysis method in sections with Hx &E between studied groups.	stained
Table (10):	Showing difference in optical den image analysis method in sections with Safranin between studied group	stained

List of Figures

Fig. No.	Title Page N	V o.
Figure (1):	Showing schematic representation of cartilage extracellular matrix and its changes in osteoarthritis	
Figure (2):	Showing (A) Normal histology. (B) Early osteoarthritic changes.(C) late osteoarthritis changes	
Figure (3):	Shows anterior-posterior (AP) radiograph of a left knee with mild osteoarthritis (Kellgren and Lawrence Grade 1)	
Figure (4):	Shows AP radiograph of a left knee with moderate OA (Kellgren and Lawrence Grade 2)	
Figure (5):	Shows anterior-posterior (AP) radiograph of a left knee with moderate to severe osteoarthritis (Kellgren and Lawrence Grade 3)	18
Figure (6):	Shows AP radiograph of a left knee with severe OA (Kellgren and Lawrence Grade 4)	
Figure (7):	Showing Wistar albino rat.	
Figure (8):	Showing injection day 0	
Figure (9):	Showing injection day 3	
Figure (10):	Showing evident swollen right knee after 2 weeks after induction of osteoarthritis in one of the female rats.	65
Figure (11):	Showing section of Knee joint showing early degenerative changes in cartilage 2 weeks after induction of arthritis	65
Figure (12):	Showing the processed adipose tissue in a petridish	68

List of Figures Cont...

Fig. No.	Title	Page No.
Figure (13):	Showing the Polyscience water be shaker device	
Figure (14):	Showing the sample inside the water	erbath 69
Figure (15):	Showing the Centurion Scientific K device	3 Series
Figure (16):	Showing Beamocytometer	70
Figure (17):	Showing Trypan Blue 0.4%,0.85NaCl	stain70
Figure (18):	Showing the Beamocytometer photocells took no stain, dead ones t stain.	o, viable ook the
Figure (19):	Shows the Characterization of SV using Multiparametric analysis	
Figure (20):	Shows the Amplification plot of treated sample (SVF) showing expression "orange" of SRY gene in rats, comparative to ACTB " brown expression	g high n female 'n" gene
Figure (21):	Shows the amplification plot of unsample showing negative expression	pression female n" gene
Figure (22):	Showing the histopathological examples of healthy control demonstrated to surface of the articular cartilar regular and smooth	that the ge was
Figure (23):	Showing a Section of Knee joint early degenerative changes in car weeks after induction of osteoarthri	$\operatorname{tilage}\ \overset{\circ}{2}$

List of Figures Cont...

Fig. No.	Title	Page No.
Figure (24):	Comparison of Synovial membrar in healthy controls and 2 wee induction of ostearthritis	eks after
Figure (25):	Shows the comparison between the groups regarding MS at 1 and 2 me	
Figure (26):	Showing Section in rat knee stair. Hx &E showing comparison treated group and induced osteountreated group after 1 month	between parthritis
Figure (27):	Synovial membrane lining in treat after 1 month (Group IIIa)	· ·
Figure (28):	Section in rat knee stained with showing comparison between group (Group IIIb) and osteoarthritis untreated group months (Group IIb).	treated induced after 2
Figure (29):	Showing Photomicrograph of seadult female rat knee joint stair Safranin O stain	ection in ned with
Figure (30):	Comparison between the studied regarding thickening.	d groups
Figure (31):	Difference in optical density in stained with Hx&E between groups	sections studied
Figure (32):	Difference in optical density in stained with Hx &E between groups	sections studied

Introduction

Osteoarthritis (OA) is one of the leading causes of joint pain and disability (Arden and Nevitt, 2006). It's characterized by degeneration of articular cartilage, subchondral sclerosis and marginal osteophyte formation. OA is associated with chronic pain, stiffness, joint deformity and decreased range of motion (Hannan et al., 2000). Etiology of OA is multifactorial with hereditary, metabolic, hormonal and mechanical factors (Bhatia et al., 2013). Apart from these factors, age has been a primary factor, causing changes in extracellular matrix by increasing inflammatory cytokines and increase in the free radicals that causes the loss of the ability of the cartilage to regenerate in response to mechanical stress (Freitag et al., 2016).

Regenerative cell therapies in treatment of knee OA such as adipose derived stromal vascular fraction (SVF) have been recently investigated (*Bansal et al.*, 2017). SVF is the aqueous fraction derived from enzymatic digestion of lipoaspirate (the product of liposuction). This aqueous fraction is a combination of endothelial precursor cells (EPCs), endothelial cells (ECs), macrophages, smooth muscle cells, lymphocytes, pericytes, and pre-adipocytes among others. It has shown promise in regenerative and reconstructive medicine (*Bony et al.*, 2016). SVF derived mesenchymal progenitor/stem cells can be easily expanded in vitro and has the potential in differentiation into diverse cell lineages (*Han et al.*, 2015).