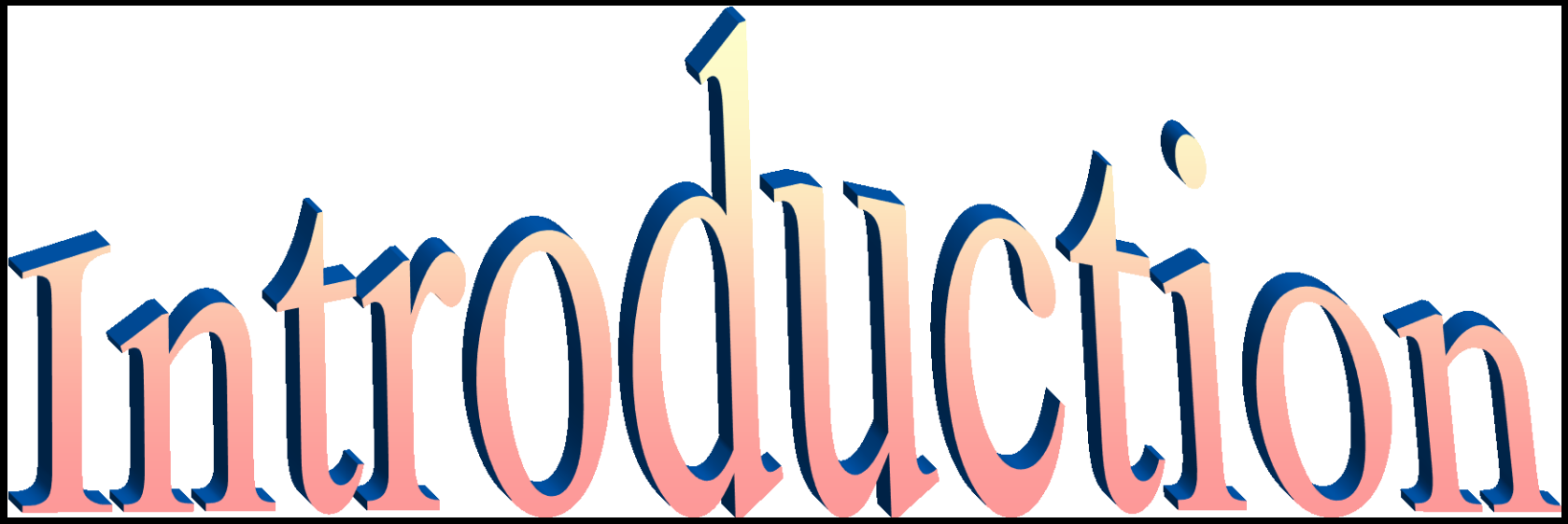
Stem Cells in Hereditary neuropathies

Professor Assistant: Dr. Maeda H. Mohammad
Specialization : Molecular Genetic, Animal Tissue Culture
Vice Director General of the Iraqi Center for Cancer & Medical Genetic Research

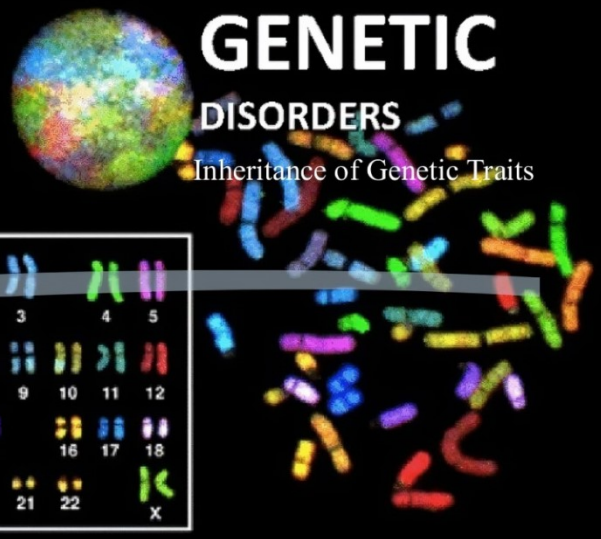
outlines:

- Introduction
- What is Genetic disorders?
- What is Stem cells?
- Stem cell therapy
- Types of stem cells used in treated Genetic Disorders
- Methodology
- Results


Introduction

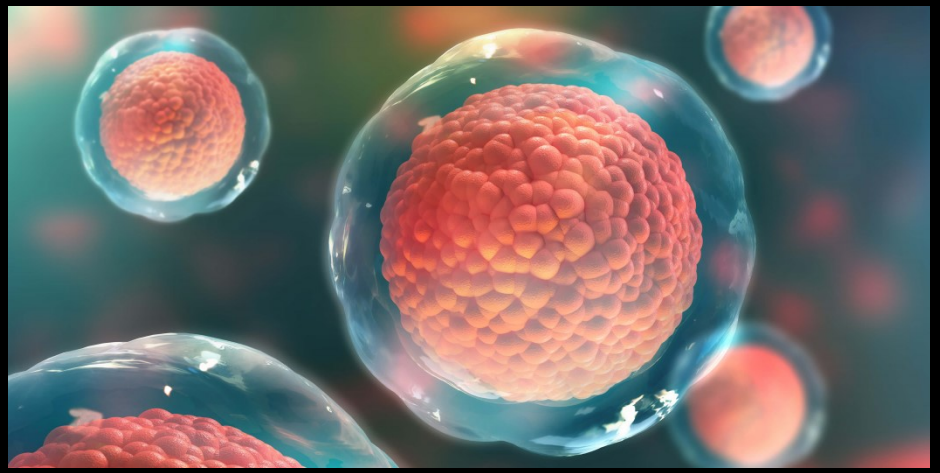
Genetic Disorders

- A disorders resulting from abnormality in the genome that is either inherited or acquired.
- **Symptoms :** Symptoms depend on the type of disorder and are highly variable.
- **Causes :** May be caused by inherited genetic abnormalities such as single gene defects, acquired mutations, or chromosomal abnormalities.
- **Treatment :** Treatment or management may include medication, therapy, surgery, transplantation and use of other aids such as orthotic or technological devices.



What is stem cell therapy ?

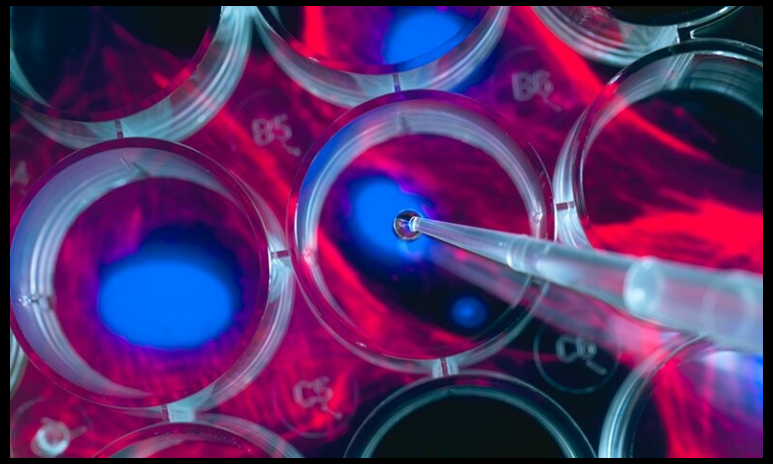
The process of isolating stem cells (hematopoietic and non hematopoietic) from patients with genetic disorders, genetically correcting the stem cells, possibly expanding them ex vivo, and transplanting them back into patients with the goal of producing genetically corrected cells in vivo.



Types of stem cells in used in treated Genetic Disorders

Adult stem cells used for treated genetic disorders

Adult stem cells are already being used in treating genetic diseases. **Bone marrow transplants and the hematopoietic stem cells** within are used to treat genetic and acquired disorders of the blood and immune system. New developments include genetic engineering of hematopoietic stem cells to cure some genetic disorders.



HSCs to treat inherited disorders

The use of allogeneic hematopoietic stem cells (HSCs) to treat genetic blood cell diseases has become a clinical standard but is limited by availability of suitable matched donors and potential immunologic complications. Gene therapy using autologous HSCs should avoid these limitations and thus may be safer.

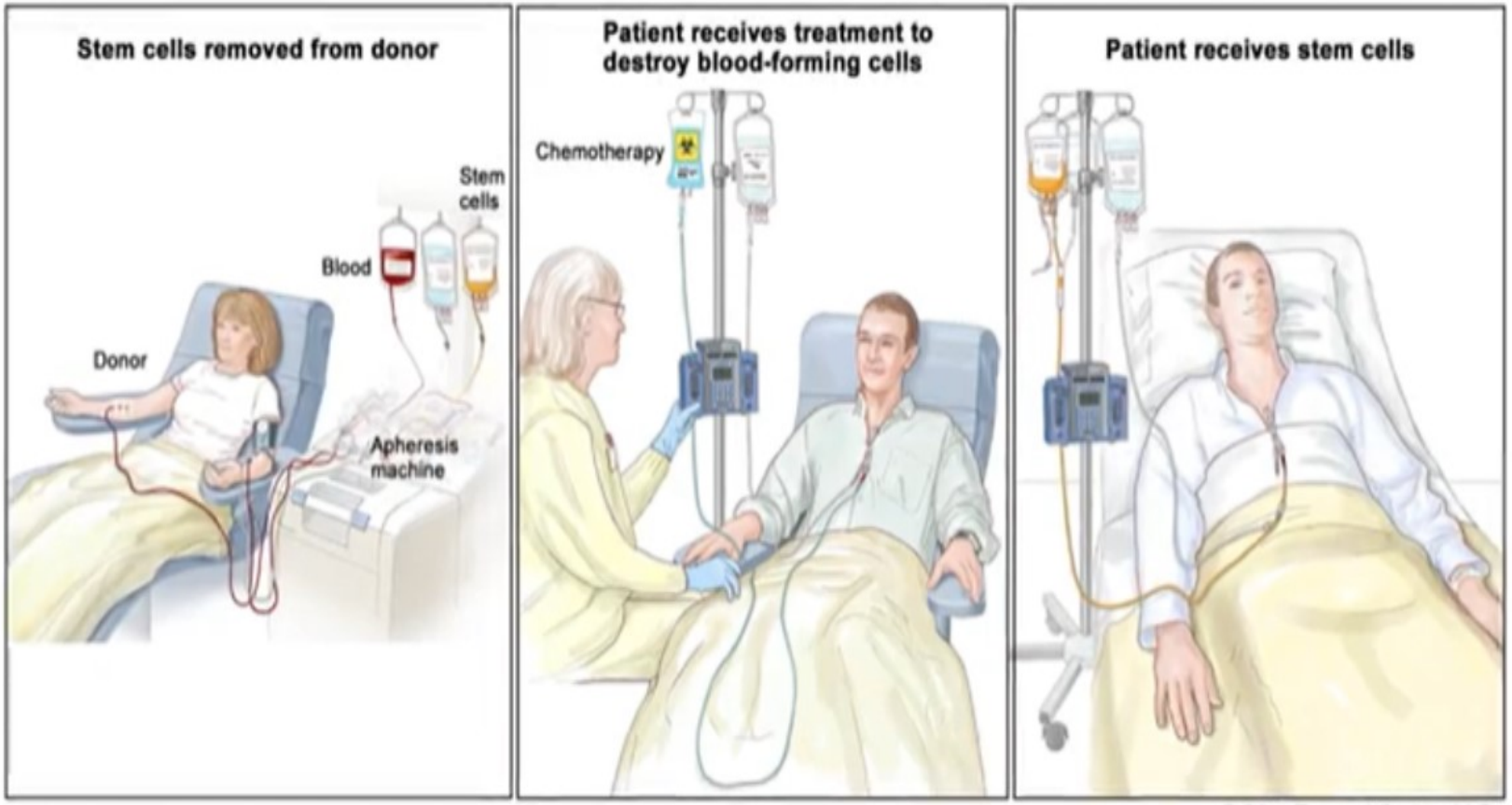
Disease	Approach	Gene
SCID-X1	LV	IL2RG
ADA-SCID	LV	ADA
Wiskott–Aldrich Syndrome	LV	WAS
X-linked chronic granulomatous disease (CGD)	LV	Gp91phox
Leucocyte adhesion deficiency (LAD)	LV	CD18
SCID due ARTEMIS defect	LV	DCLRE1C
Transfusion dependent β-thalassemia	LV	HBB
Transfusion-dependent β-thalassemia	GE	HBB
Sickle cell disease	LV	HBB
Fanconi anemia	LV	FANCA
Metachromatic leukodystrophy (MLD)	LV	ARSA
X-Adrenoleukodystrophy (ALD)	LV	ABCD1
Mucopolysaccharosidosis type I	LV	IDUA

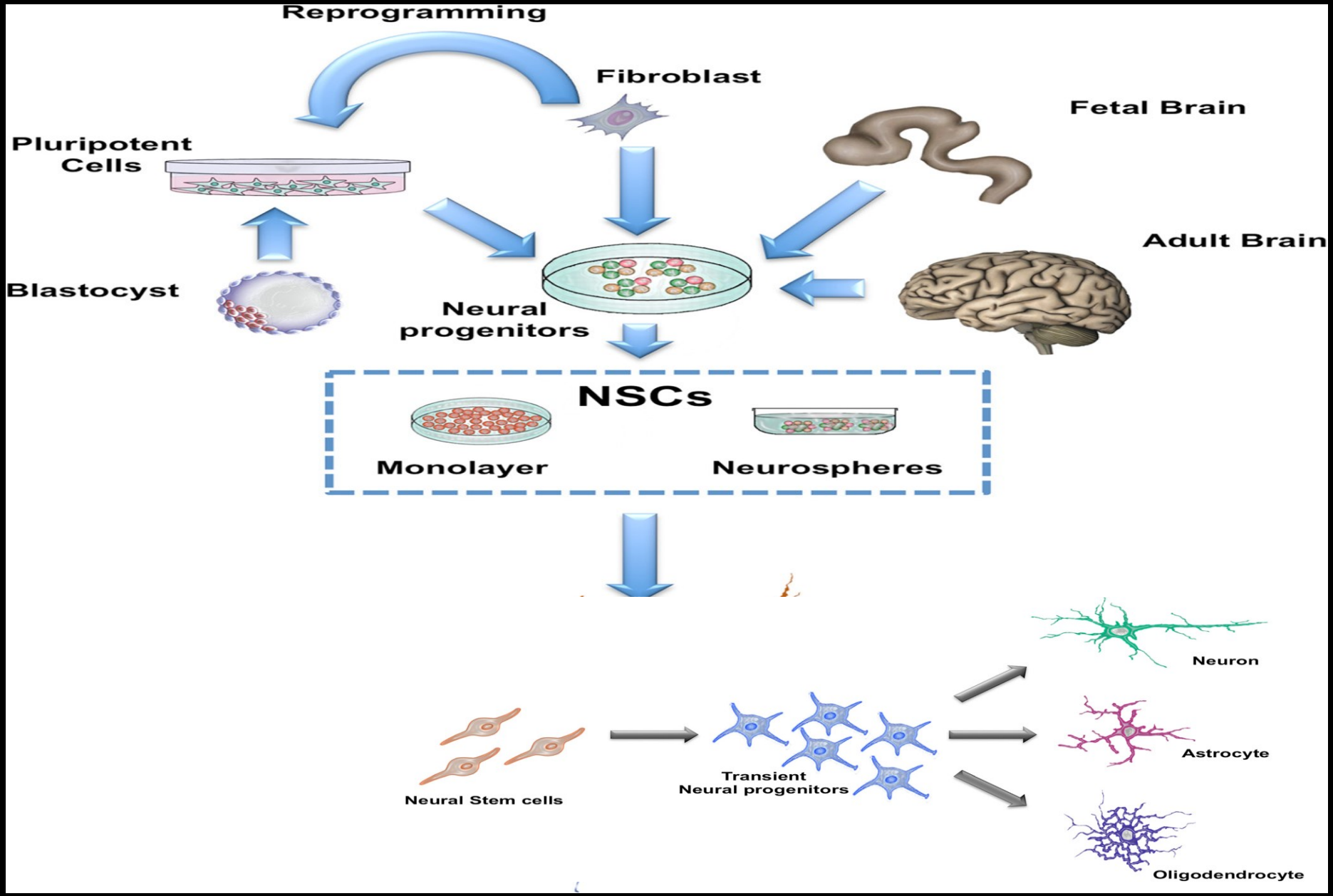
LV, lentiviral vector; GE, gene editing.

Cord blood stem cells in genetic disorders

Cord blood stem cells can be harvested from the umbilical cord of a baby after birth. The cells can be frozen (‘cryopreserved’) in cell banks and are currently used to treat children with cancerous blood disorders such as leukemia, as well as genetic blood diseases like A rare Fanconi anemia.

Bone Marrow Transplantation




Reprogramming
Fibroblast
Fetal Brain
Pluripotent Cells
Adult Brain
Blastocyst
Neural progenitors
NSCs
Monolayer
Neurospheres
Neuron
Astrocyte
Neural Stem cells
Transient Neural progenitors
Oligodendrocyte


objective

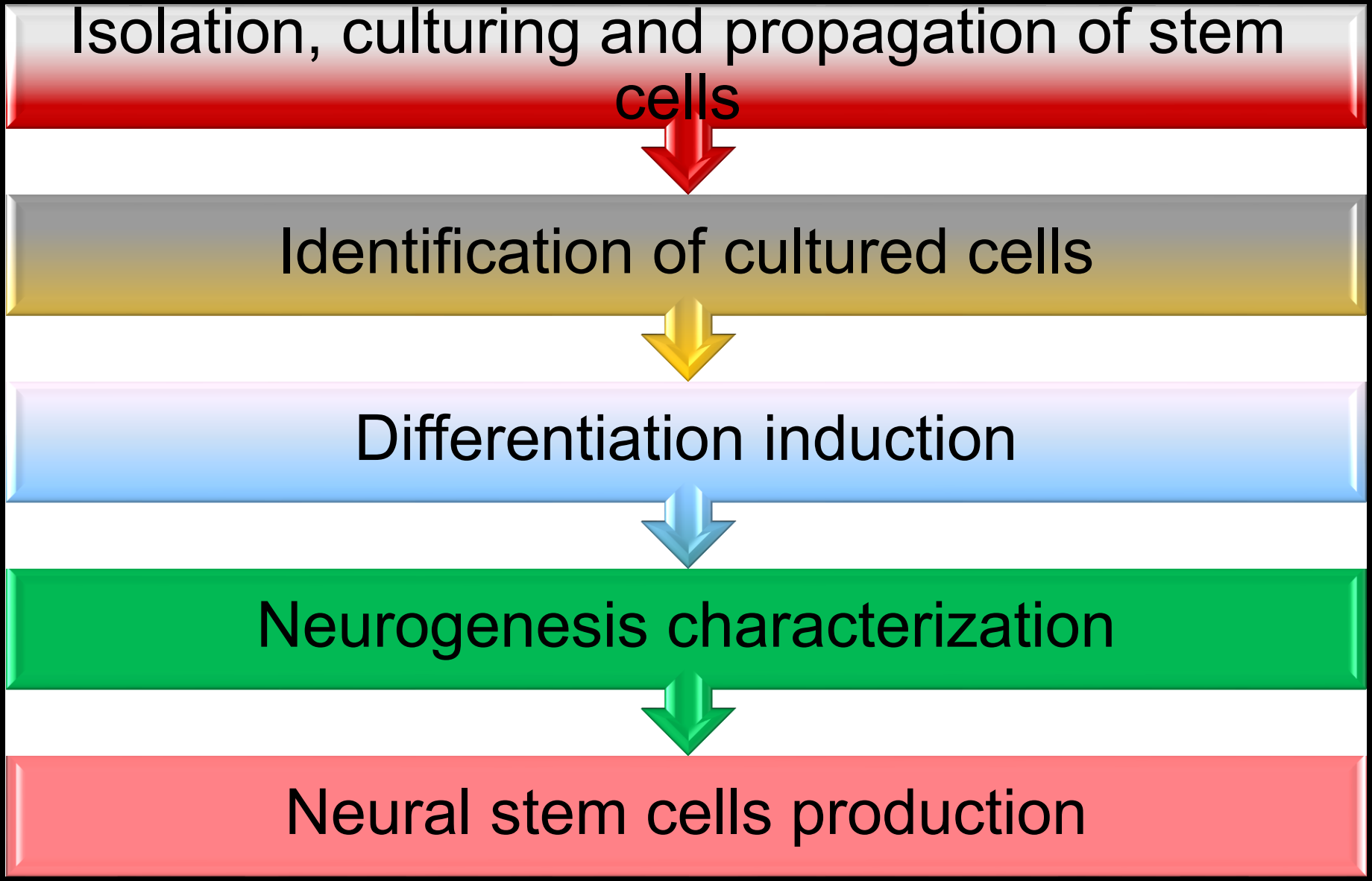
The Aims:

The aims of this project are to:

- Produce neural and neural stem cells in culture for use it as a source in cell therapy later.
- Study their morphological, protein profile, and molecular characteristics to do a full characterization of these differentiated cells.

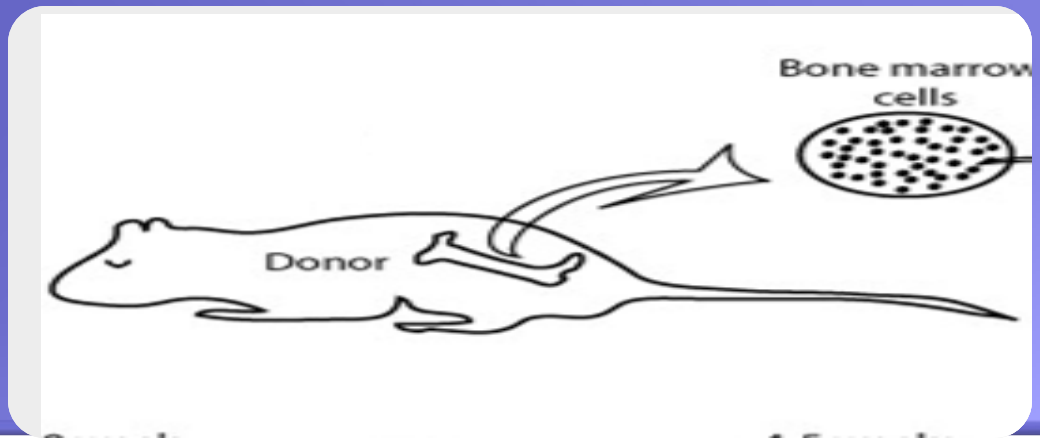
Methodology

Methodology:



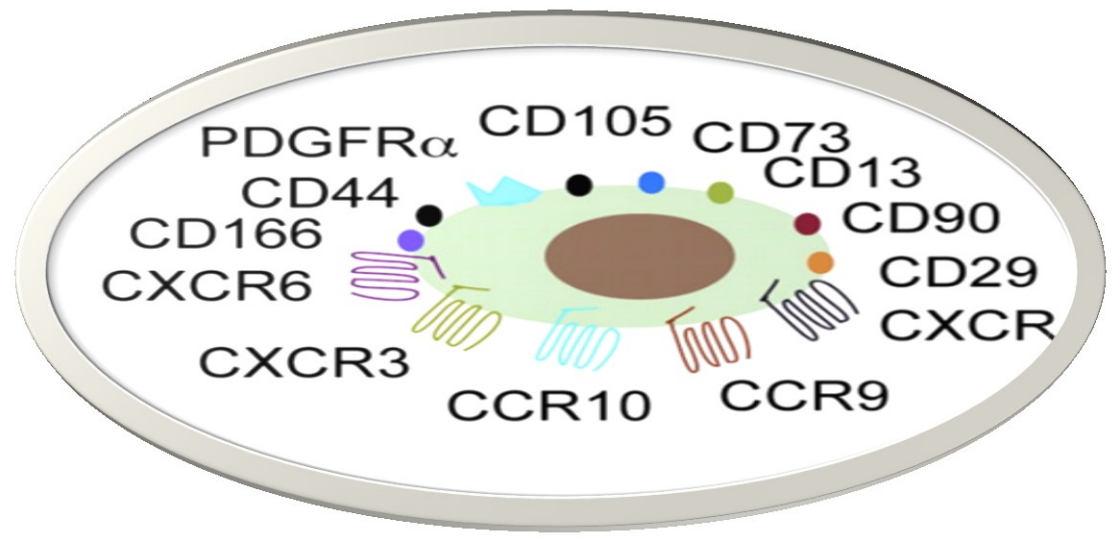
Step 1

ISOLATION



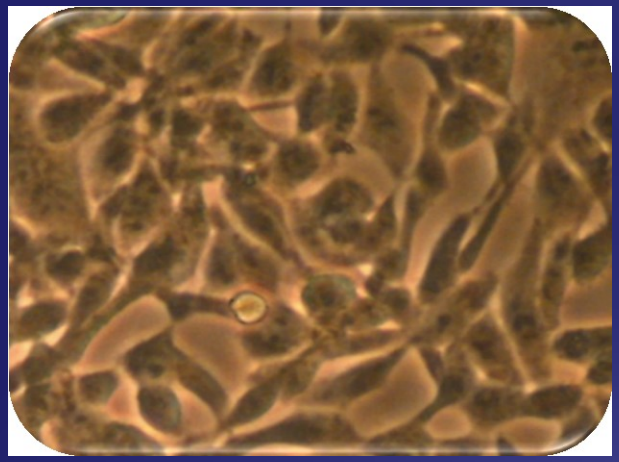
Step 2

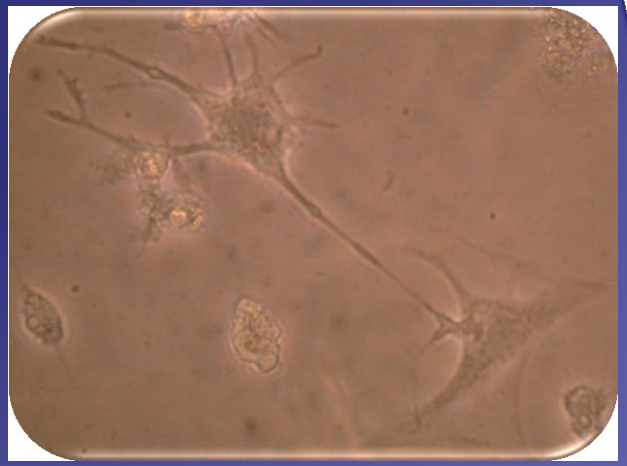
IDENTIFICATION



Step 3

DIFFERENTIATION





Step 4

Study the neurogenesis characterization through differentiation process from MSCs towards neural cells differentiation by using:

- Cytological study by H.& E. stains (morphological study).

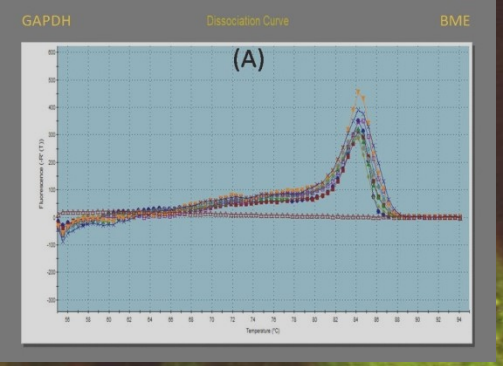
- ICC assays to determined of differentiated cells by using NES, NF-L, MAP-2.

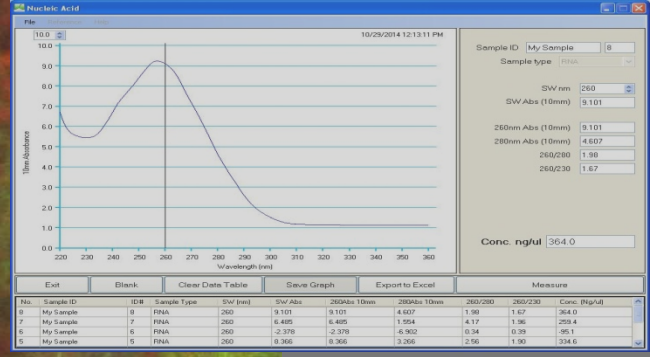
- *Study Gene expression analysis for differentiated markers(NES, NF-L ,MAP-2).*

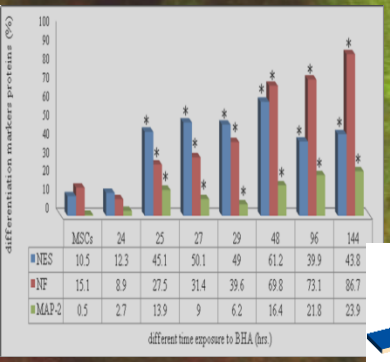
Step 5

NSCs Production

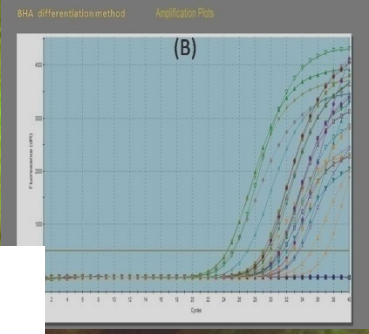
- *NSCs Induction*
- *Proof of Stemness state*



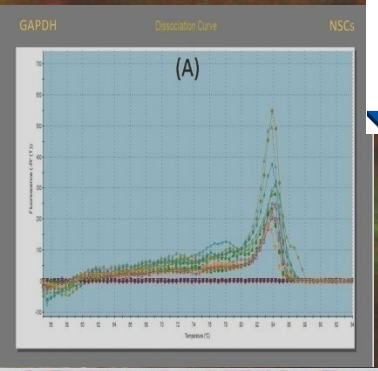


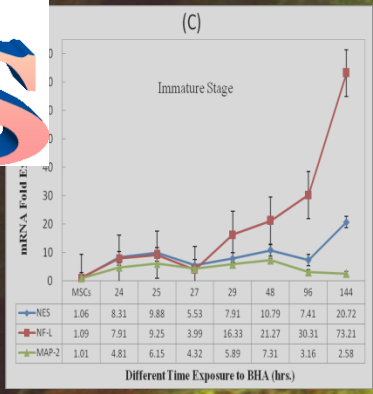


	MSCs	24	25	27	29	48	96	144
NES	10.5	12.3	45.1	50.1	49	61.2	39.9	43.8
NF	15.1	8.9	27.5	31.4	39.6	69.8	73.1	86.7
MAP-2	0.5	2.7	13.9	9	6.2	16.4	21.8	23.9

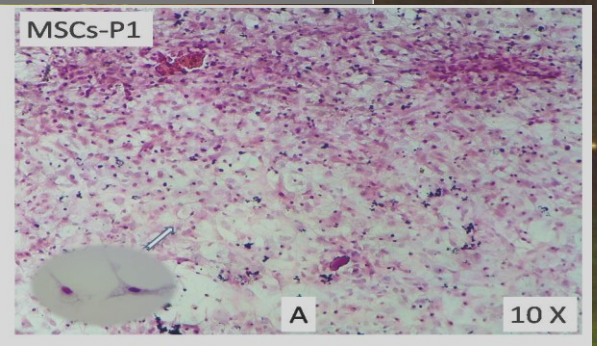


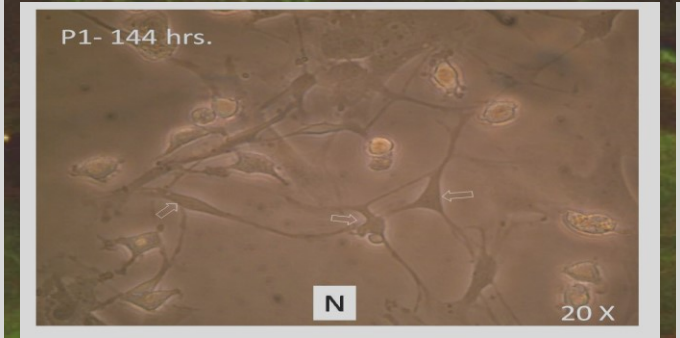
Results

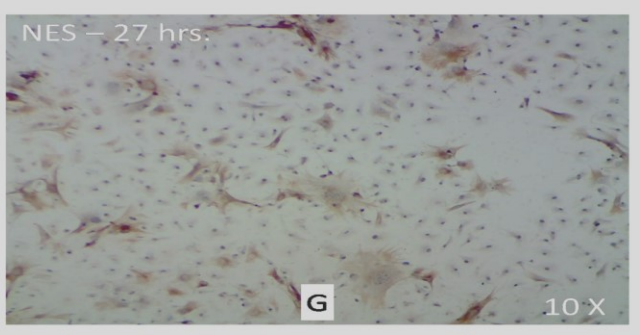




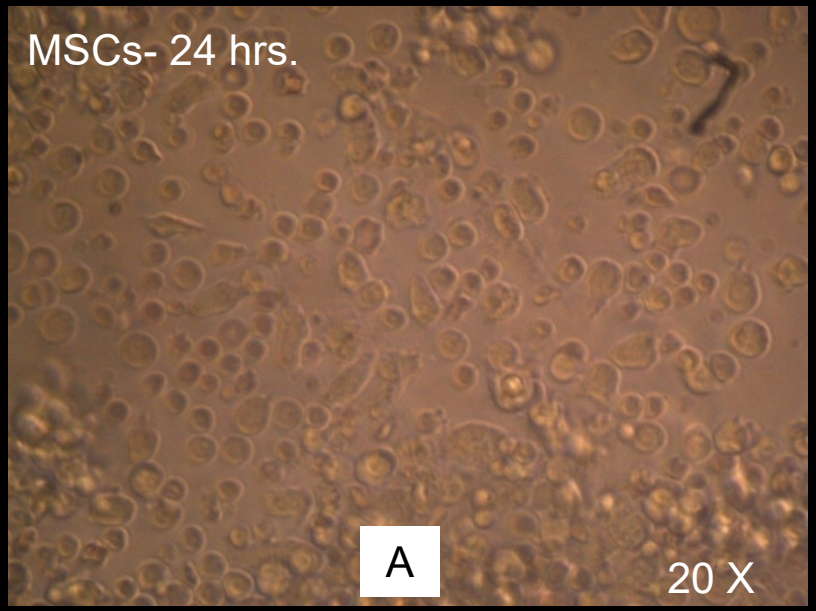
	MSCs	24	25	27	29	48	96	144
NES	1.06	8.31	9.88	5.53	7.91	10.79	7.41	20.72
NF-L	1.09	7.91	9.25	3.99	16.33	21.27	30.31	73.21
MAP-2	1.01	4.81	6.15	4.32	5.89	7.31	3.16	2.58

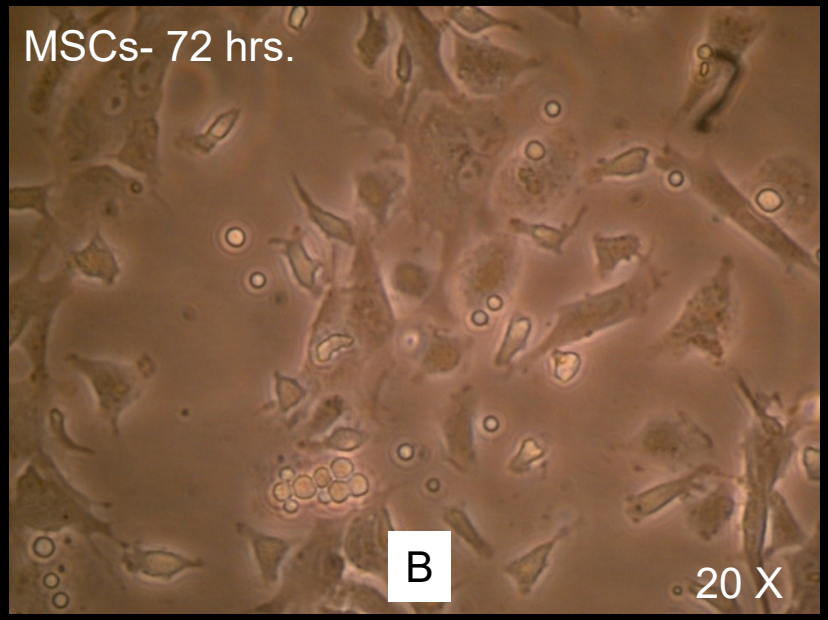




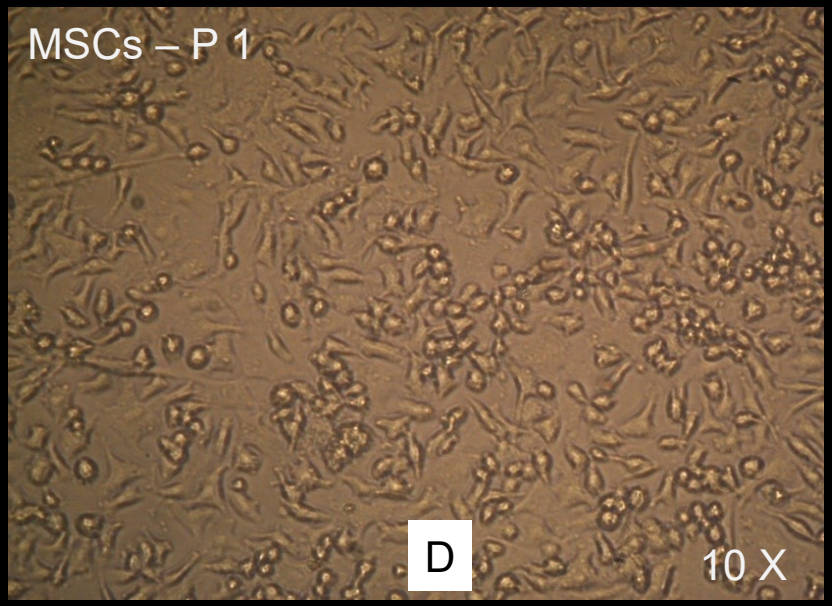


Isolation

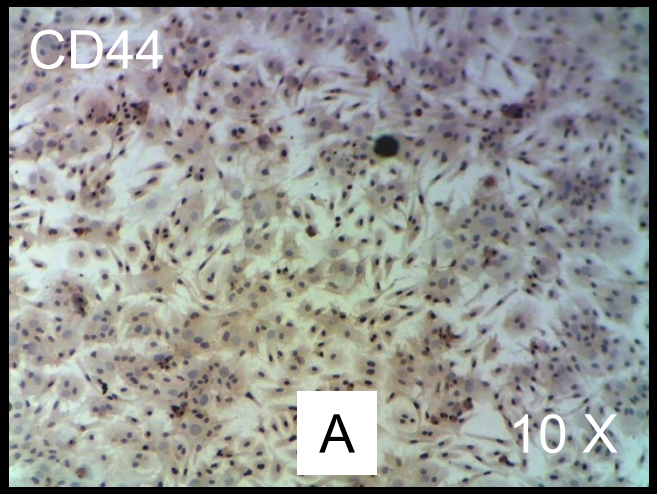

MSCs- 24 hrs.
A
20 X

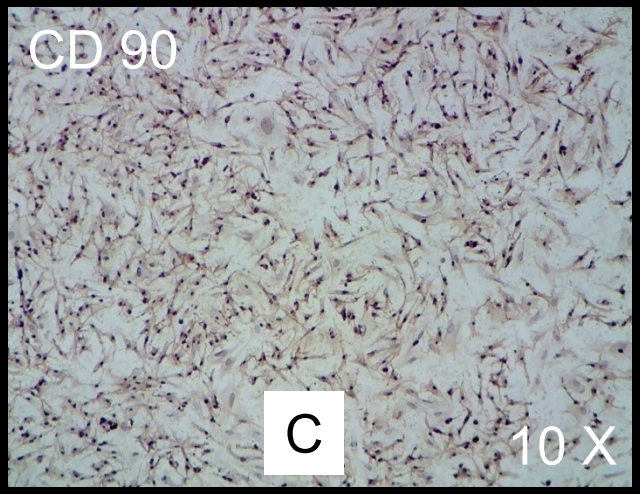

MSCs- 72 hrs.
B
20 X

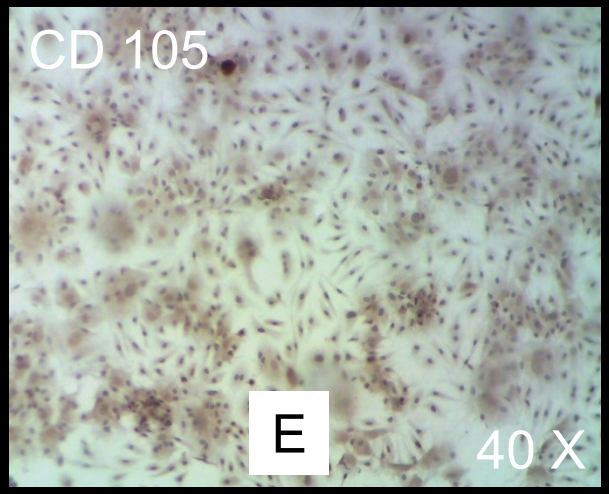

MSCs- monolayer
C
10 X

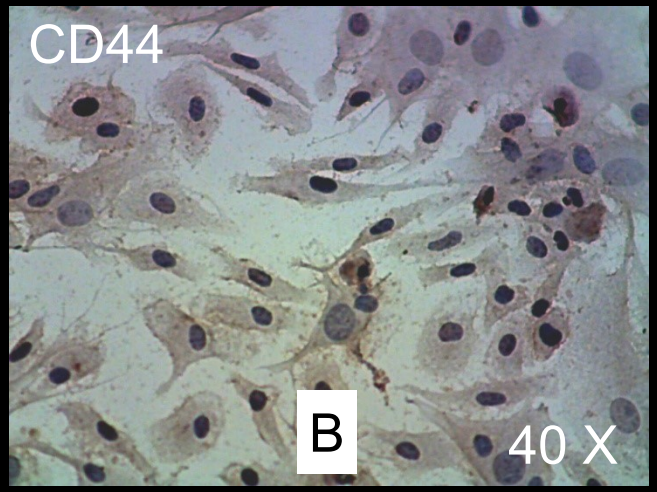

MSCs – P 1
D
10 X

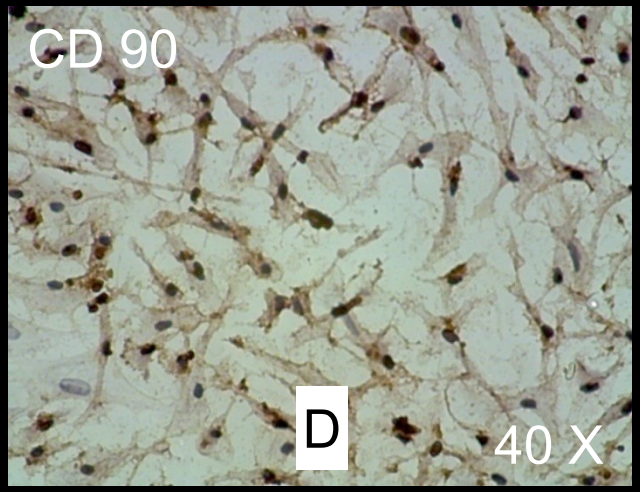
Identification

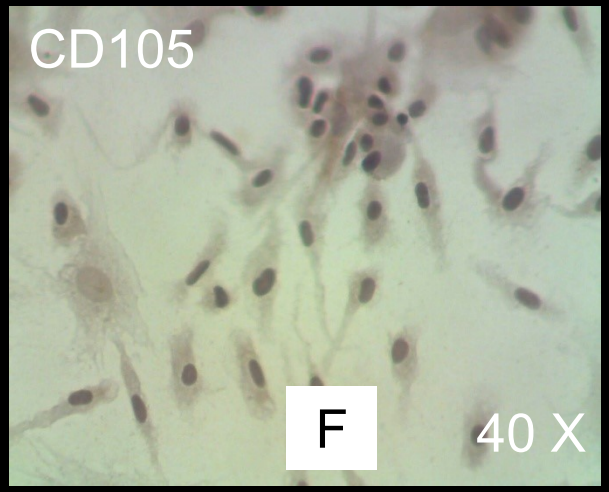




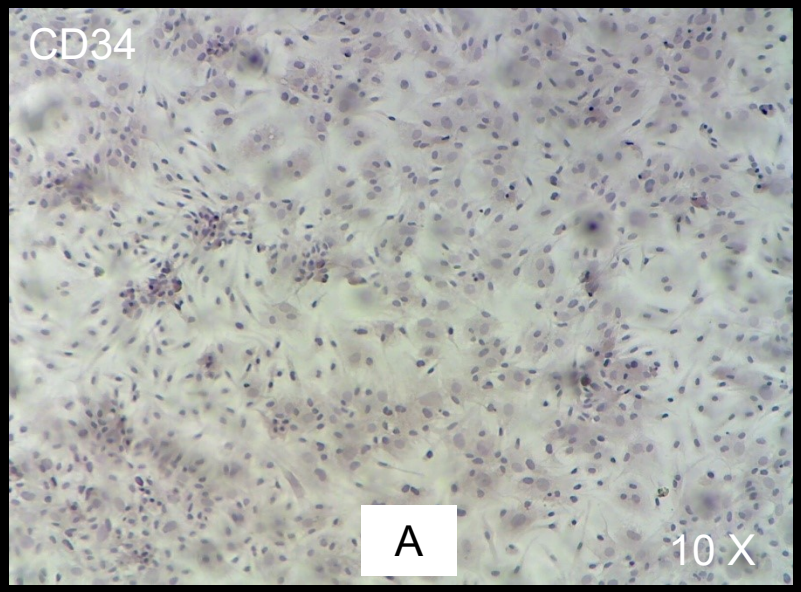


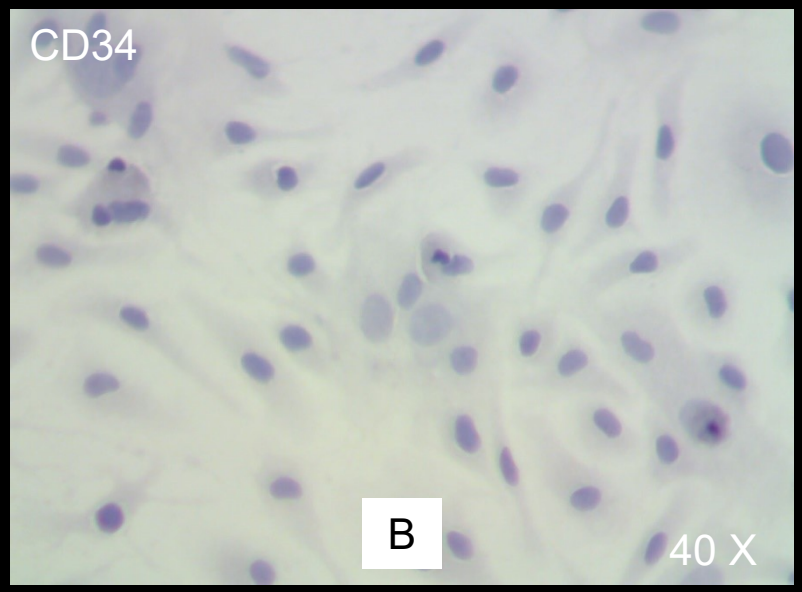


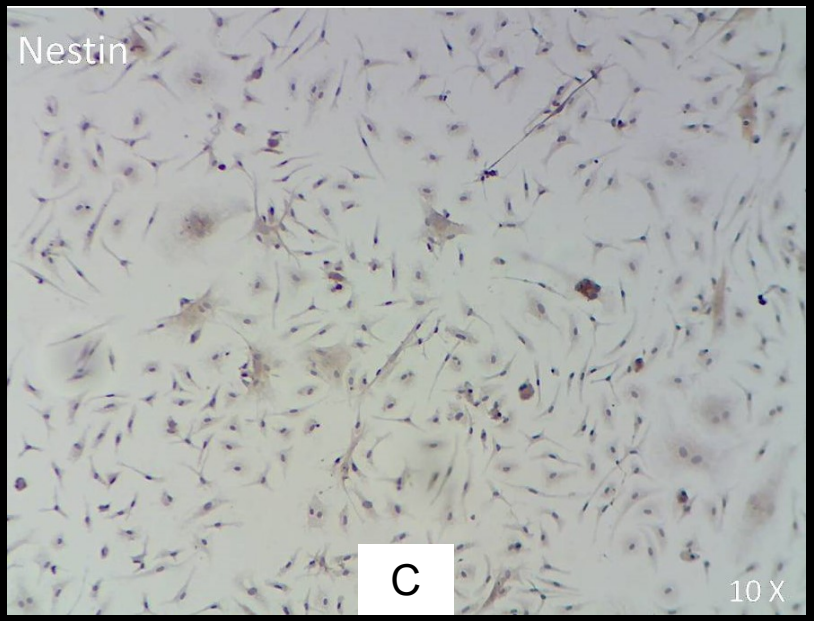


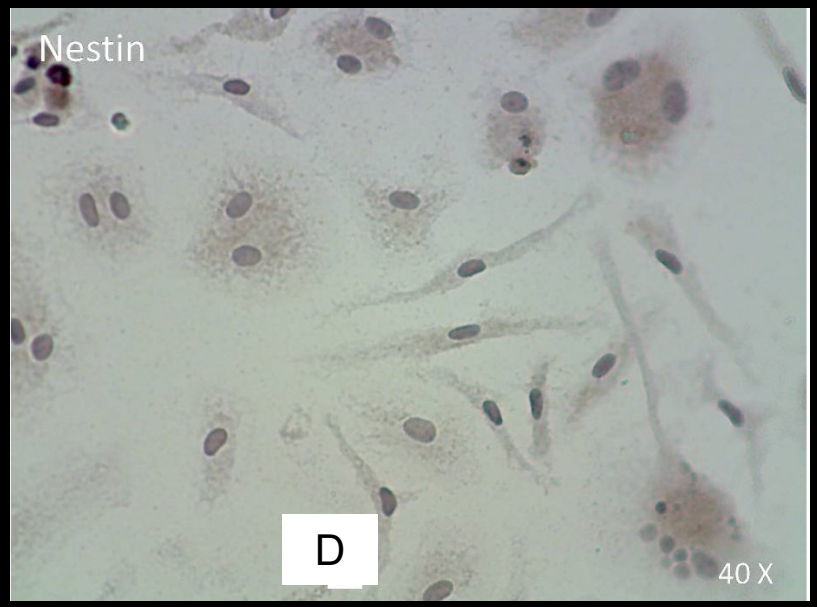


Identification

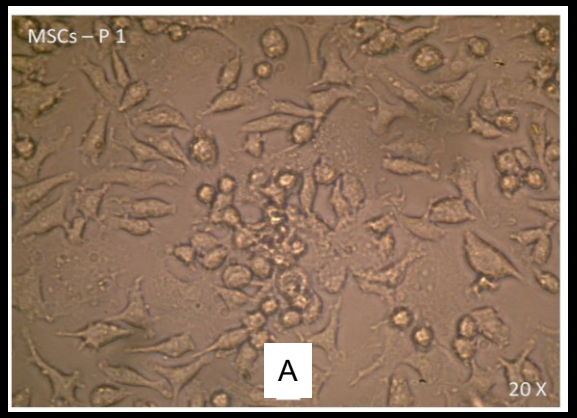


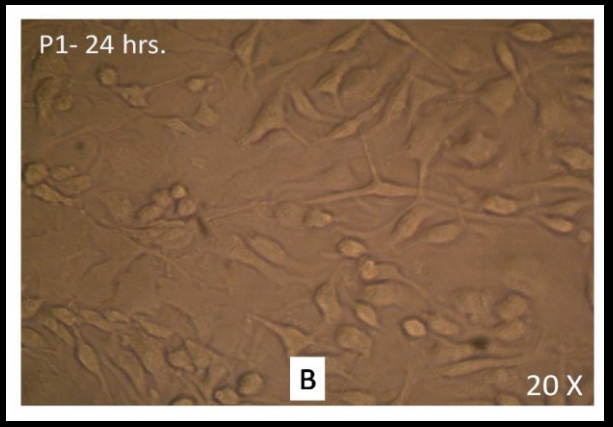


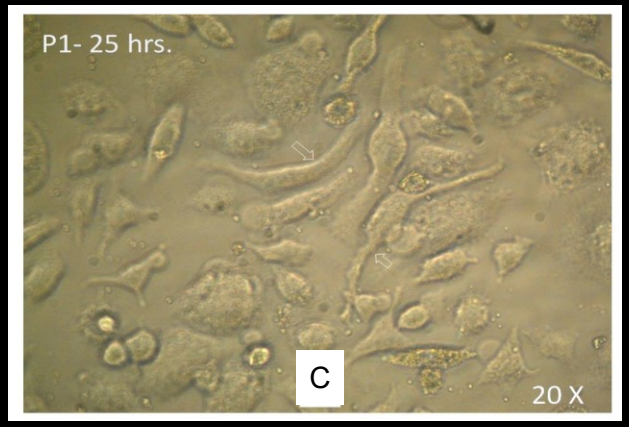


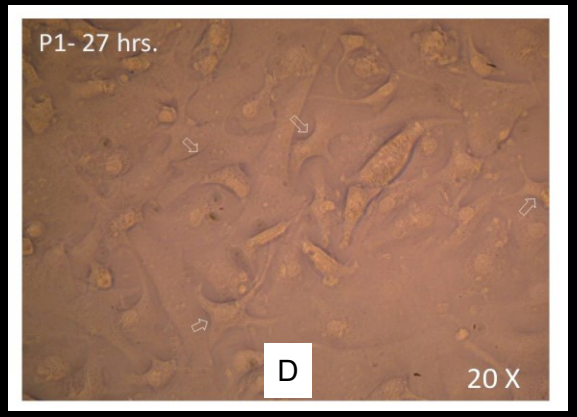


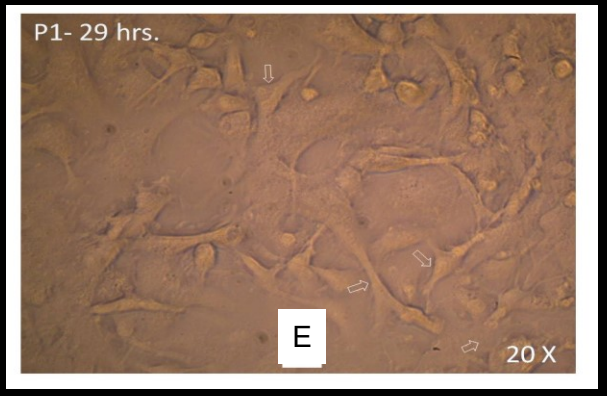
Differentiation

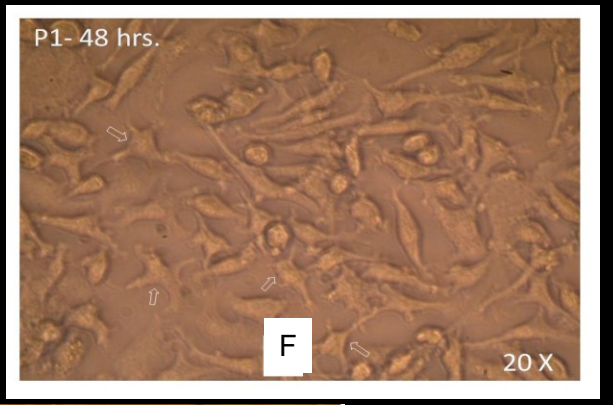


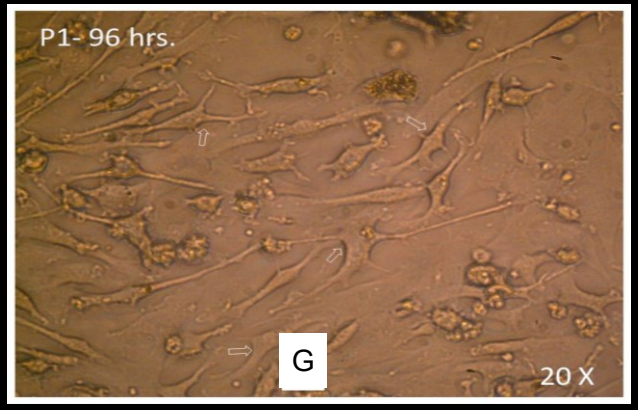






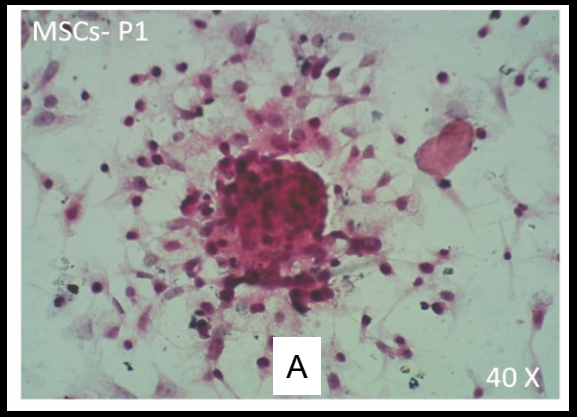


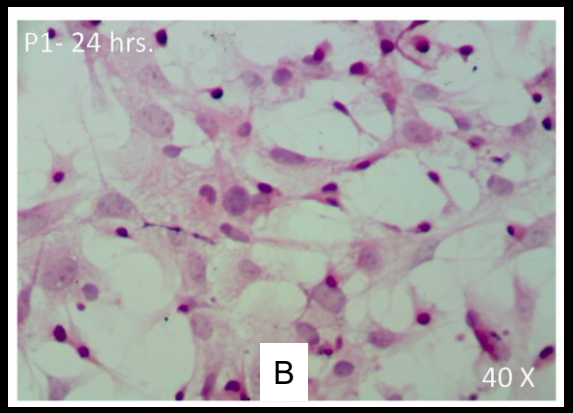


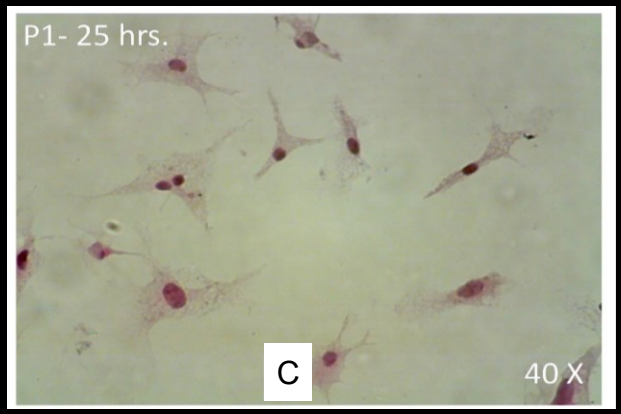


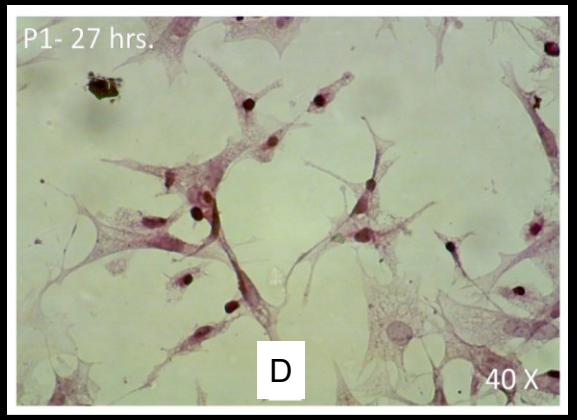
Neurogenesis Characterization Results

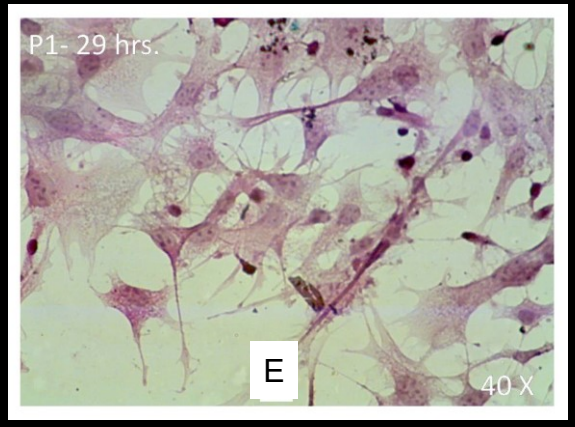
Morphological Study

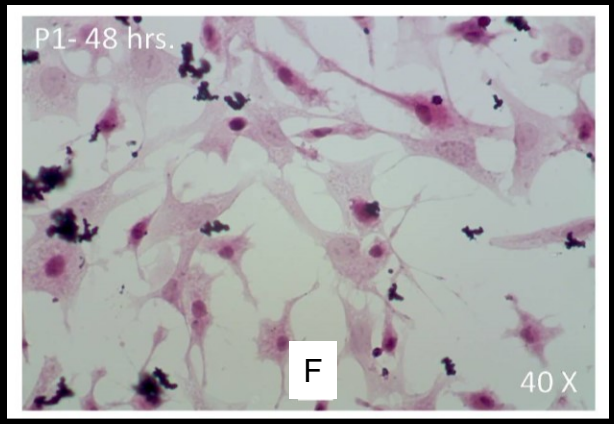


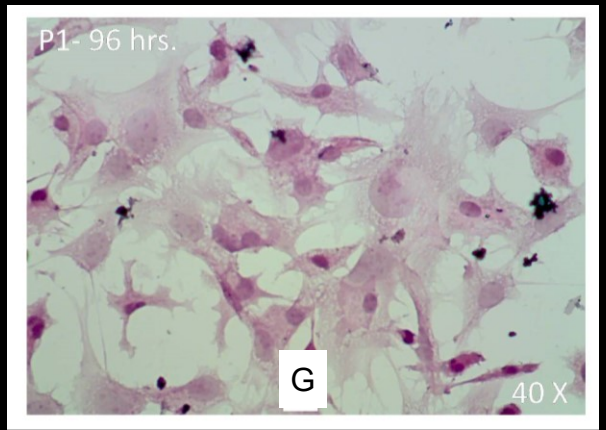


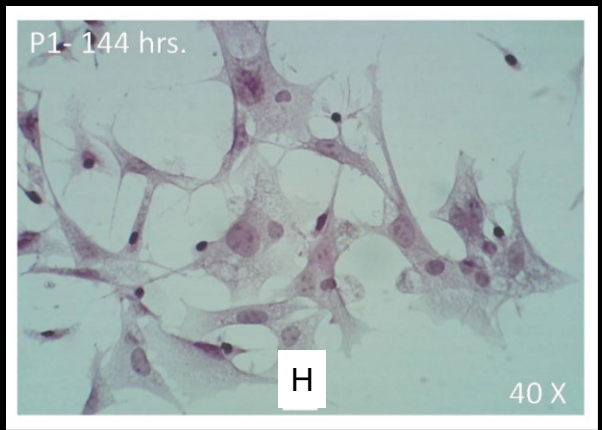




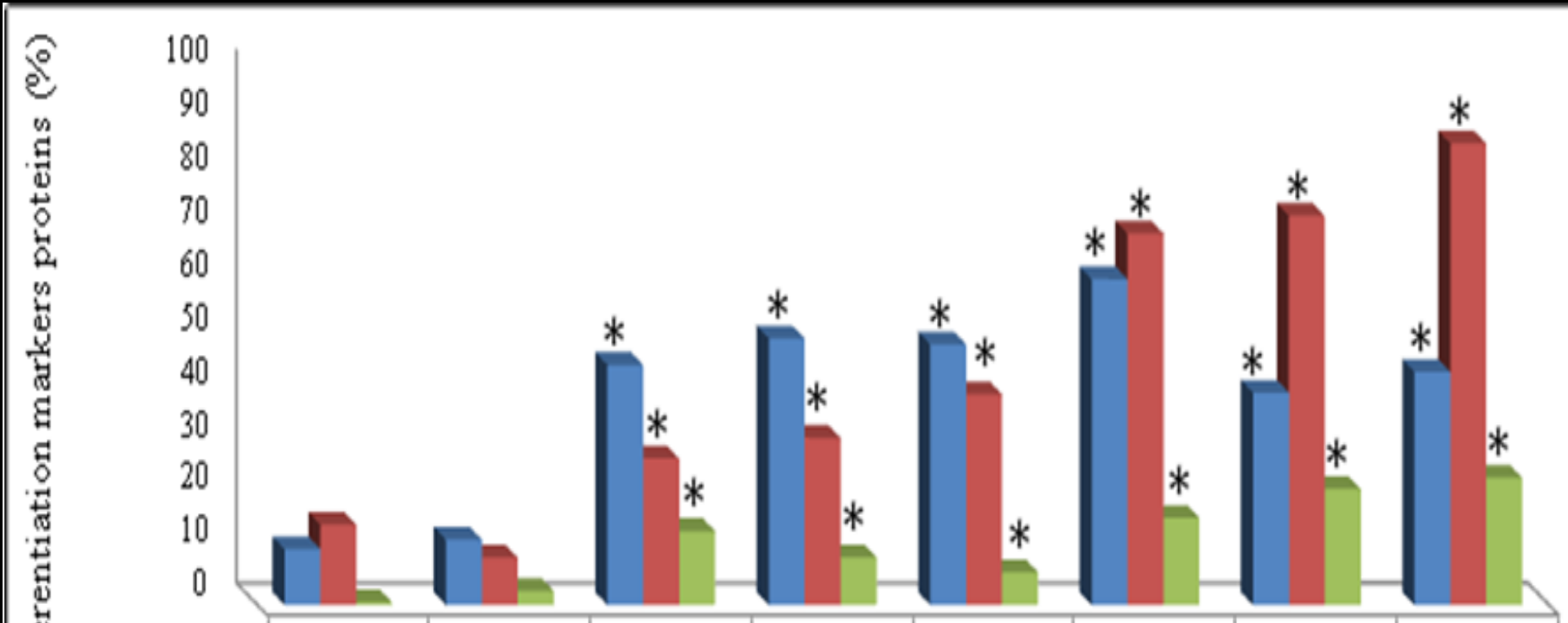






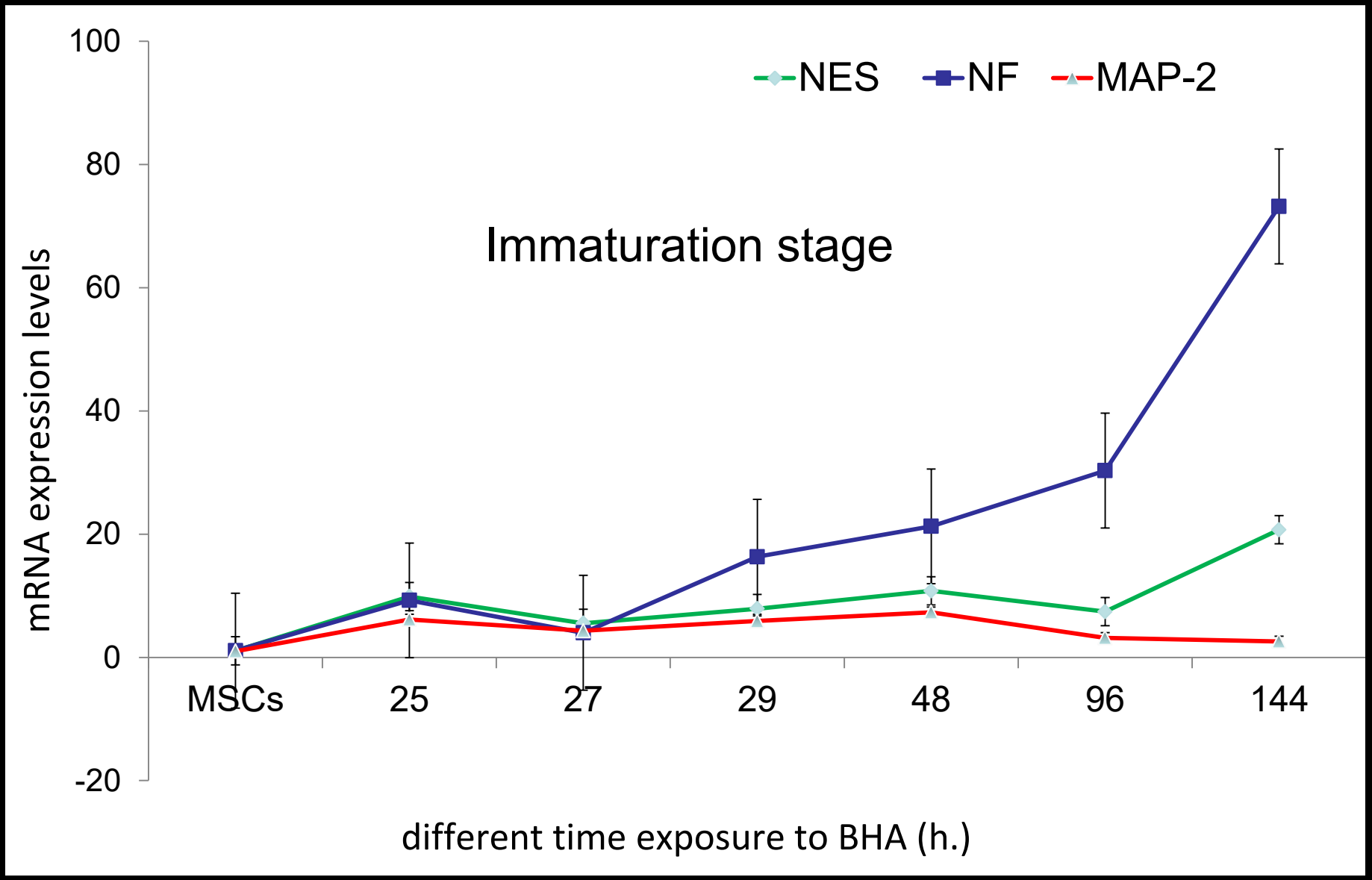


ICC Study

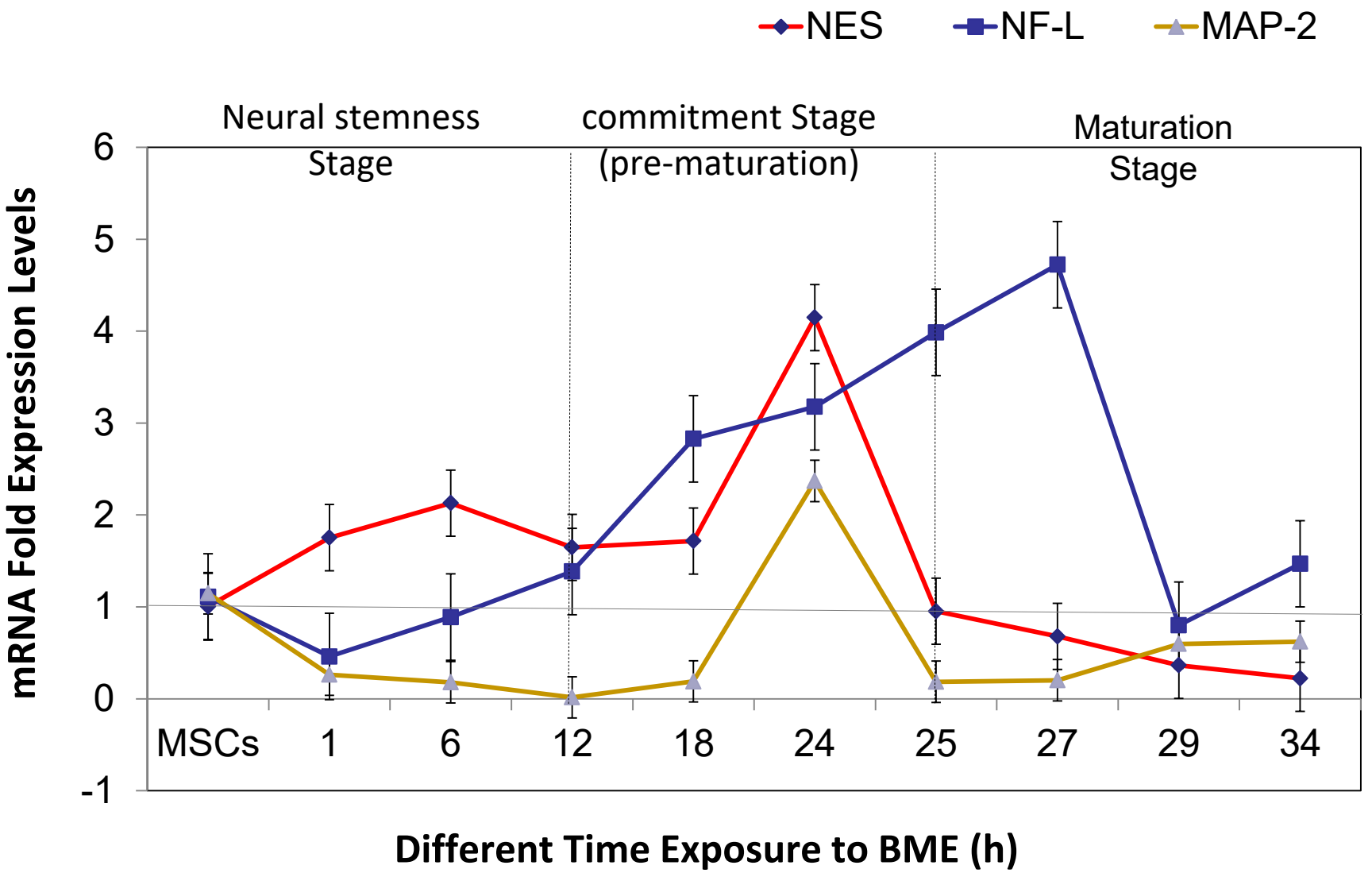


	MSCs	24	25	27	29	48	96	144
NES	10.5	12.3	45.1	50.1	49	61.2	39.9	43.8
NF	15.1	8.9	27.5	31.4	39.6	69.8	73.1	86.7
MAP-2	0.5	2.7	13.9	9	6.2	16.4	21.8	23.9

Gene Expression

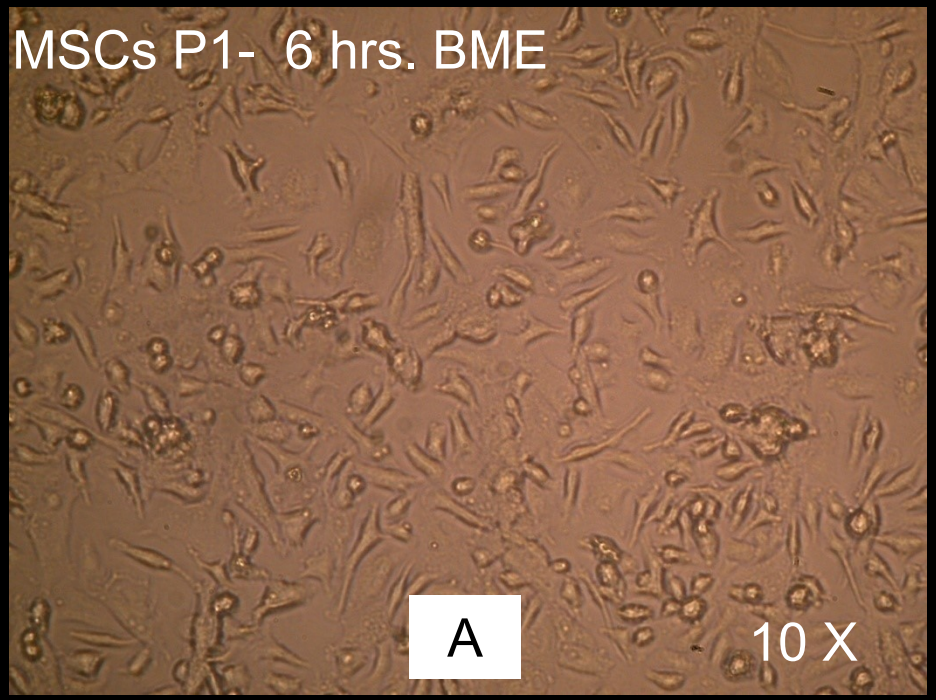


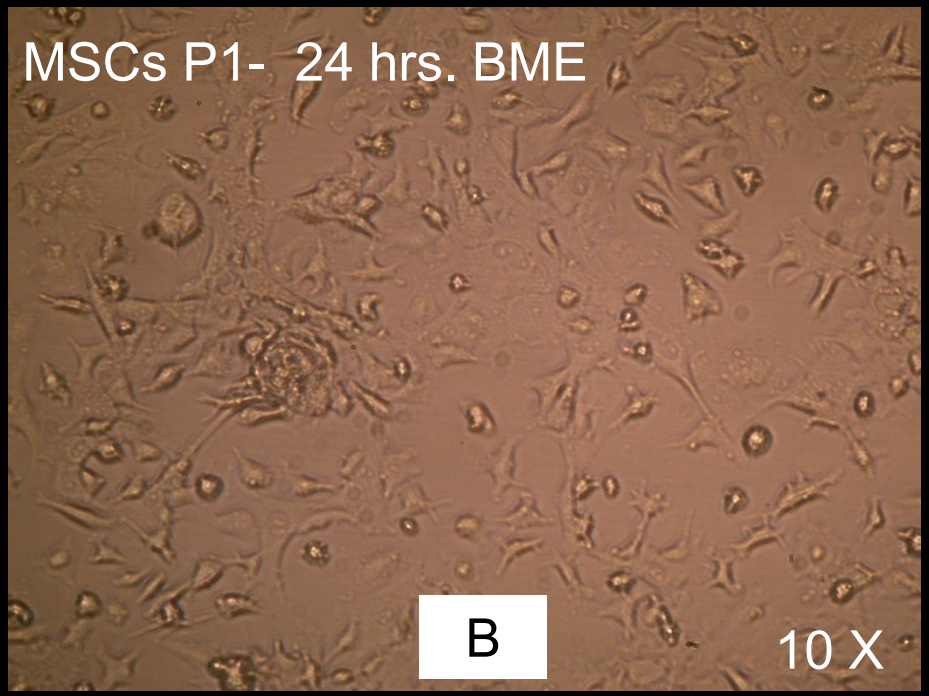
Defining of Neurogenesis stages



Neural Stem Cells Production Results

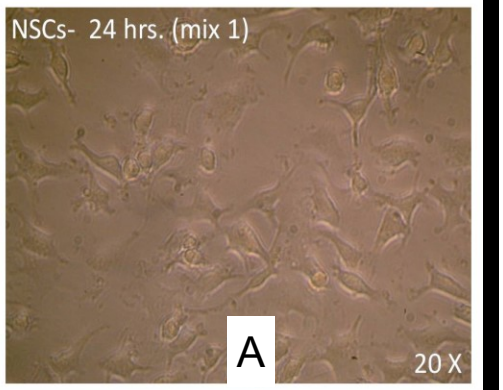
Neural Stem Cells Induction

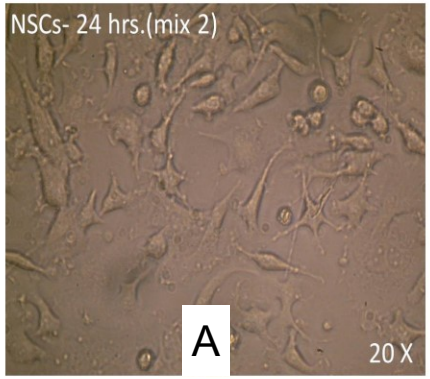


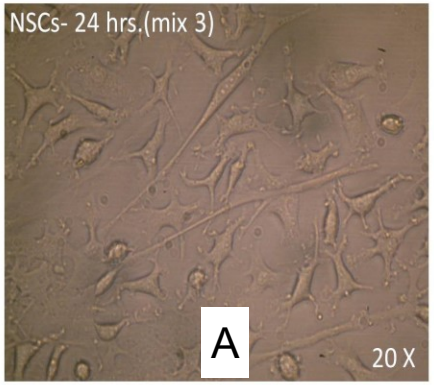


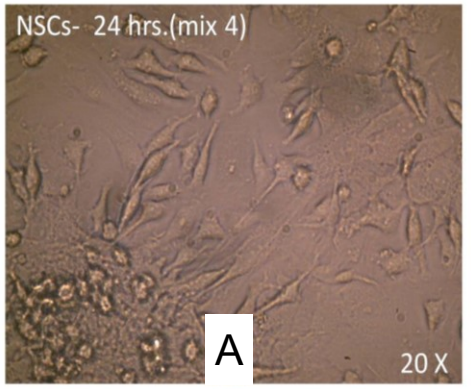
Proof of Neural Stemness

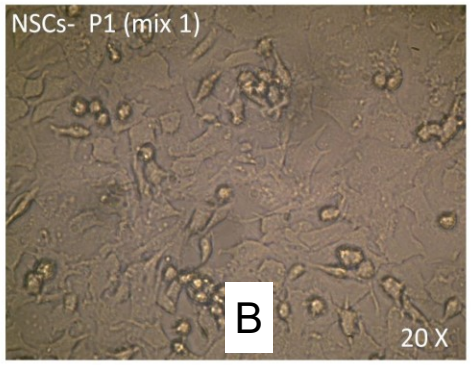
Microscopic Examination

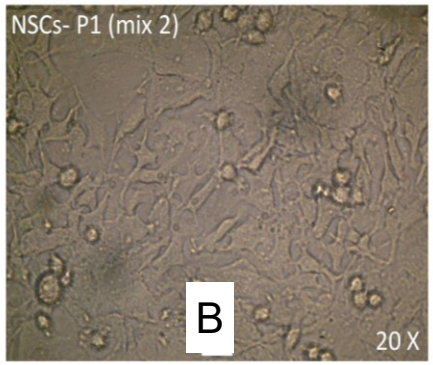


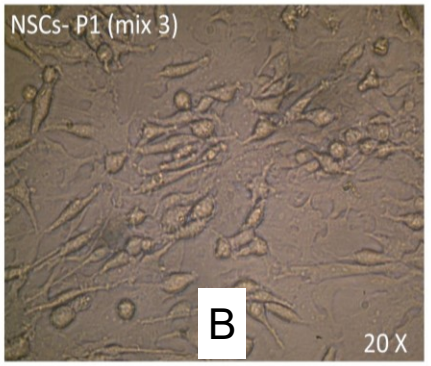


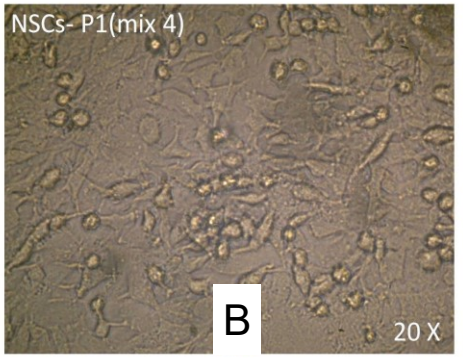


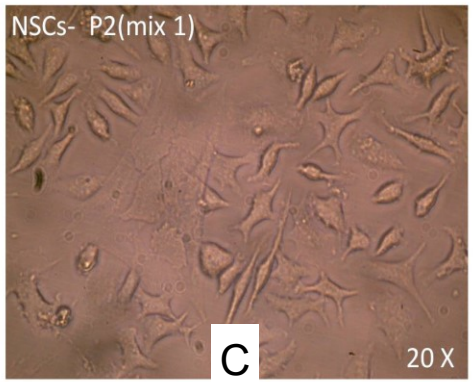


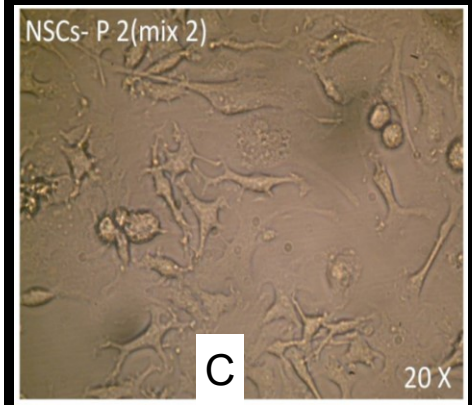


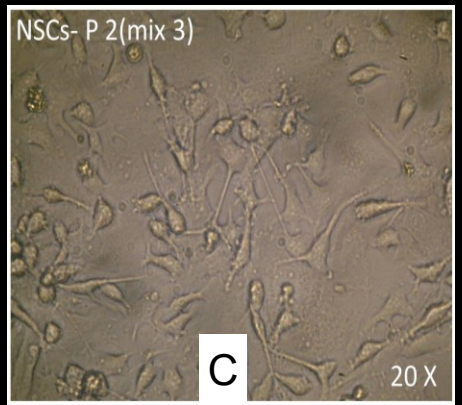


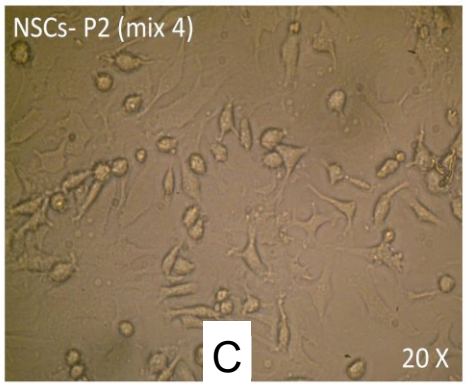




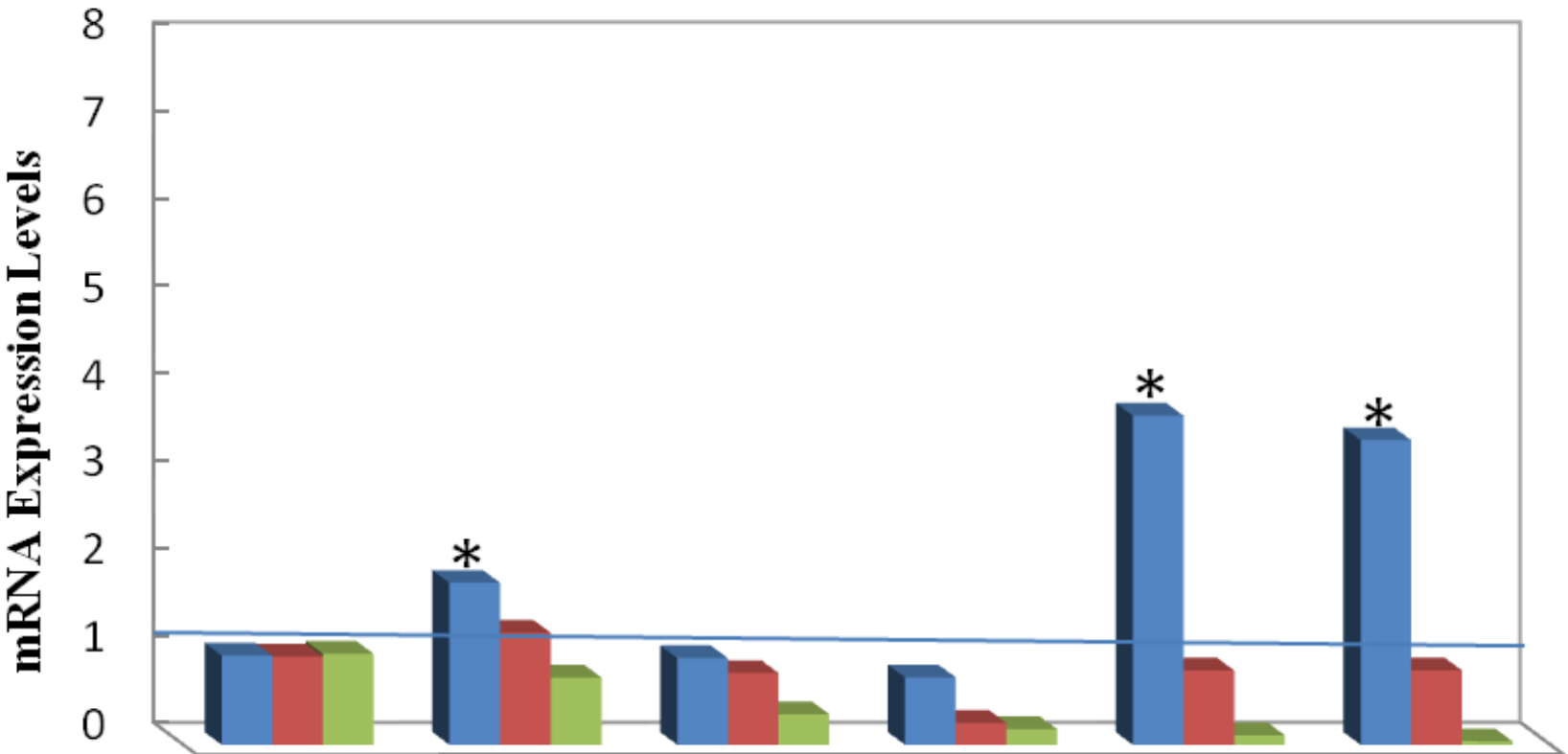








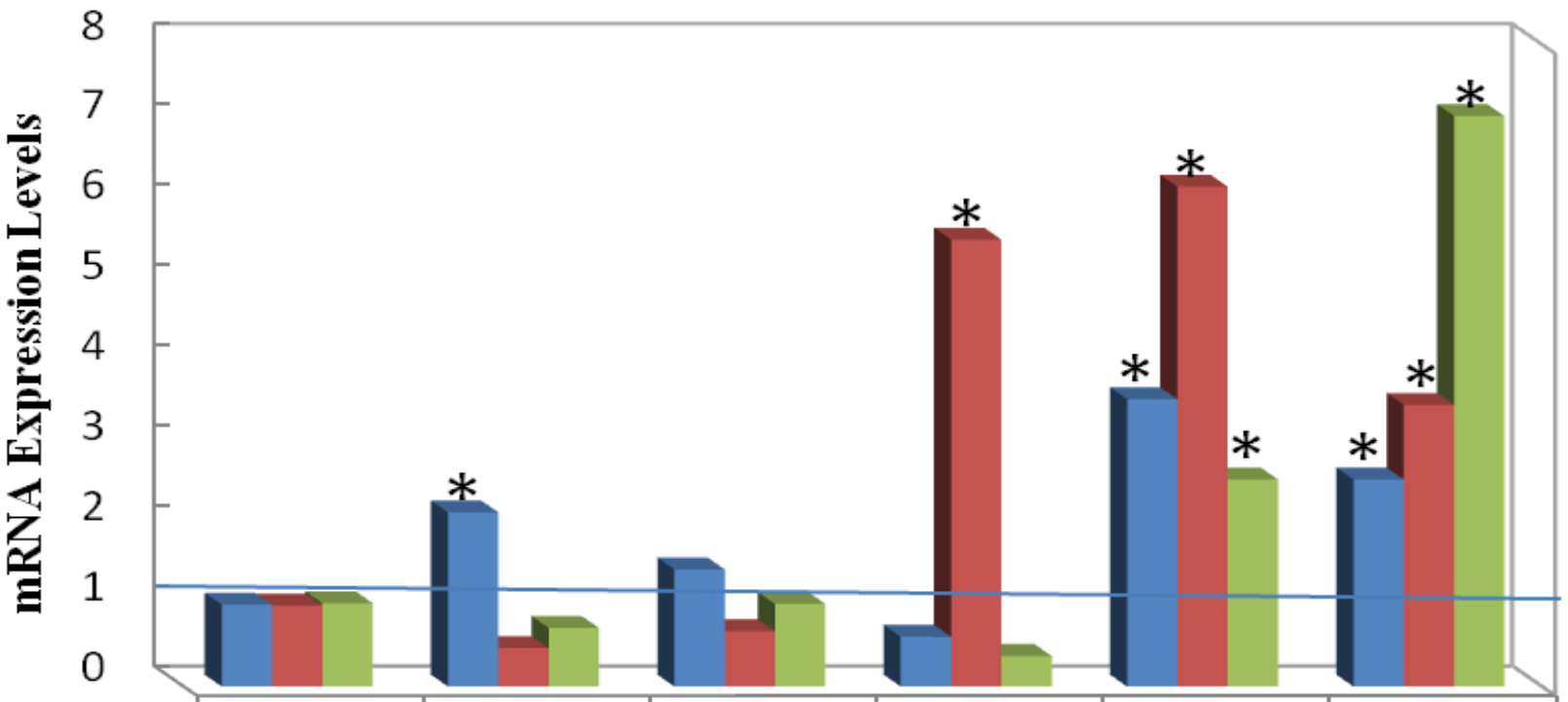
Gene Expression Study



	MSCs	6 h.	5% FBS	EGF	FGF	EGF+FGF
NES	1.02	1.85	0.99	0.77	3.76	3.48
NF	1.01	1.28	0.82	0.25	0.85	0.85
MAP-2	1.04	0.77	0.35	0.18	0.11	0.04

Different Media Mix

Gene Expression



	MSCs	24 h.	5% FBS	EGF	FGF	EGF+FGF
NES	1.02	2.17	1.46	0.62	3.58	2.58
NF	1.01	0.48	0.69	5.57	6.23	3.51
MAP-2	1.04	0.73	1.03	0.38	2.58	7.11

Different Media Mix


Thank you for your kind listening
Elena Pankova-Fotolia.com