Laboratorio 4.

Part 1. Collection of Mesenchymal Stem Cells (MSCs)

Part 2. Cells Counting

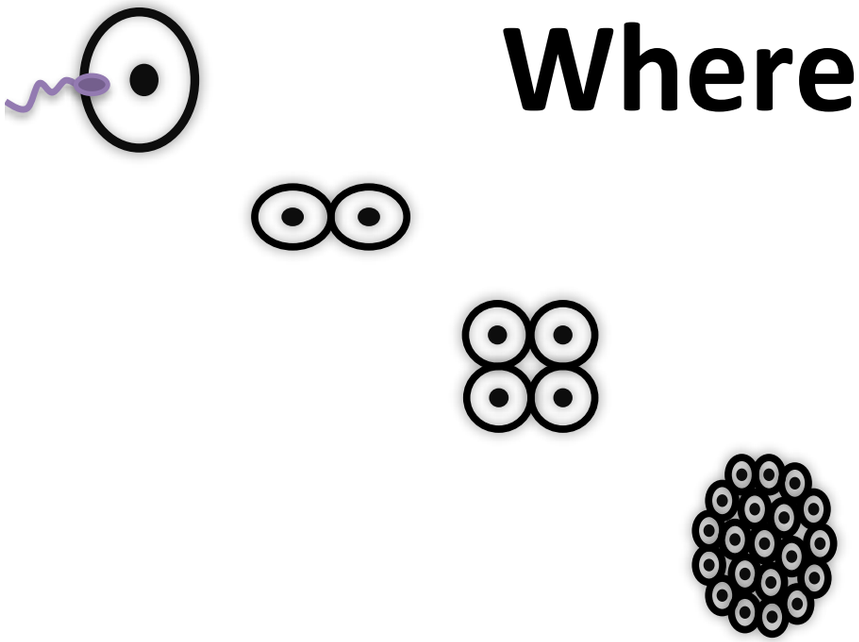
Part 3. Cells Freezing

Stem cells are one of the most fascinating areas of biology today

Stem cells are a special kind of cell that have ability to divide indefinitely and have the potential to give rise to specialized cells (that is, any cell of the body).

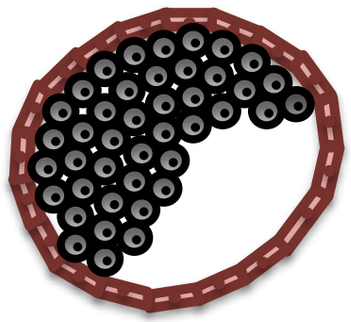
Stem cell Characteristics

- "blank cells" - unspecialized
- Capable of dividing and renewingthemselves for long periods of time
- Have the potential to give rise to specialized cell types (differentiation)
- Plasticity



Where are stem cells found?

embryonic stem cells
blastocyst - a very early embryo

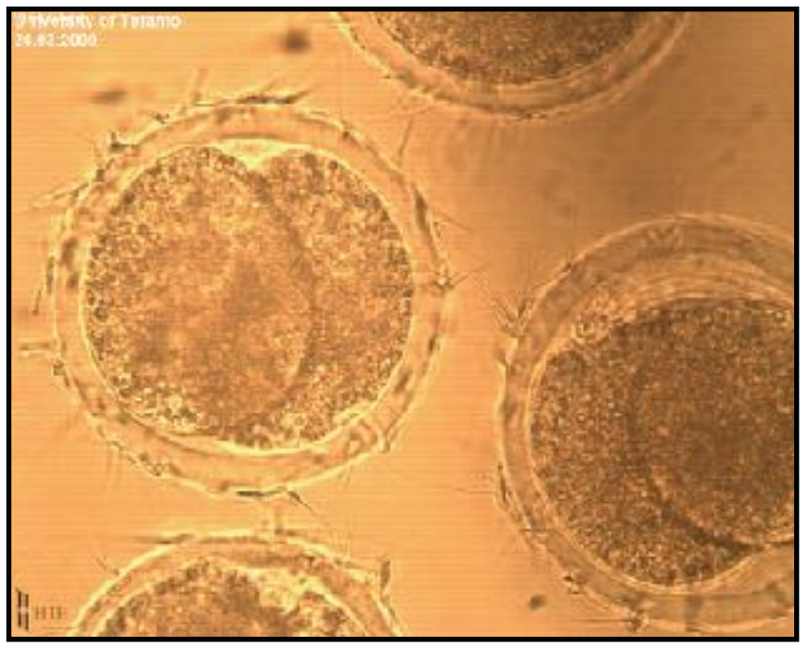


tissue stem cells
fetus, baby and throughout life

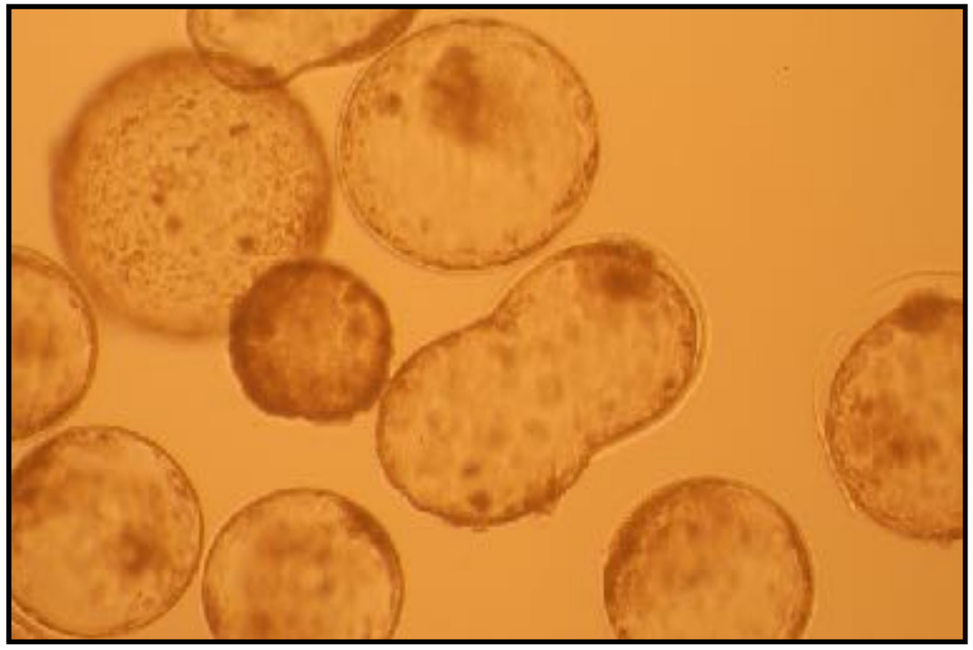
There are different types of stem cells:

1) **Embryonic stem cells**: found in the blastocyst, a very early stage embryo that has about 50 to 100 cells;
2) **Tissue stem cells:** found in the tissues of the body (in a fetus, baby, child or adult).

Stem Cell Type	Description	Examples
Totipotent	Each cell can develop into a new individuals	Cells from early (1-3 days) embryos
Pluripotent	Cells can form any (over 200) cell types	Some cells of blastocysts (5-14 days)
Multipotent	Cells differentiated, but can form a number of other tissues	Fatal tissue, cord blood and adult stem cells.

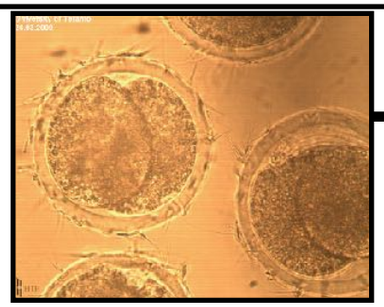


2-cells - totipotent



Blastocyst - pluripotent

This cell
Can form the
Embryo and placenta



Totipotent

fertilised egg

totipotent stem cells

This cell
Can just form the
embryo

Pluripotent

blastocyst containing
pluripotent stem cells

isolated pluripotent SCs
from inner cell mass

cultured pluripotent SCs

hematopoeitic SCs

neural SCs

mesenchymal SCs

tissue-specific SCs

Fully mature

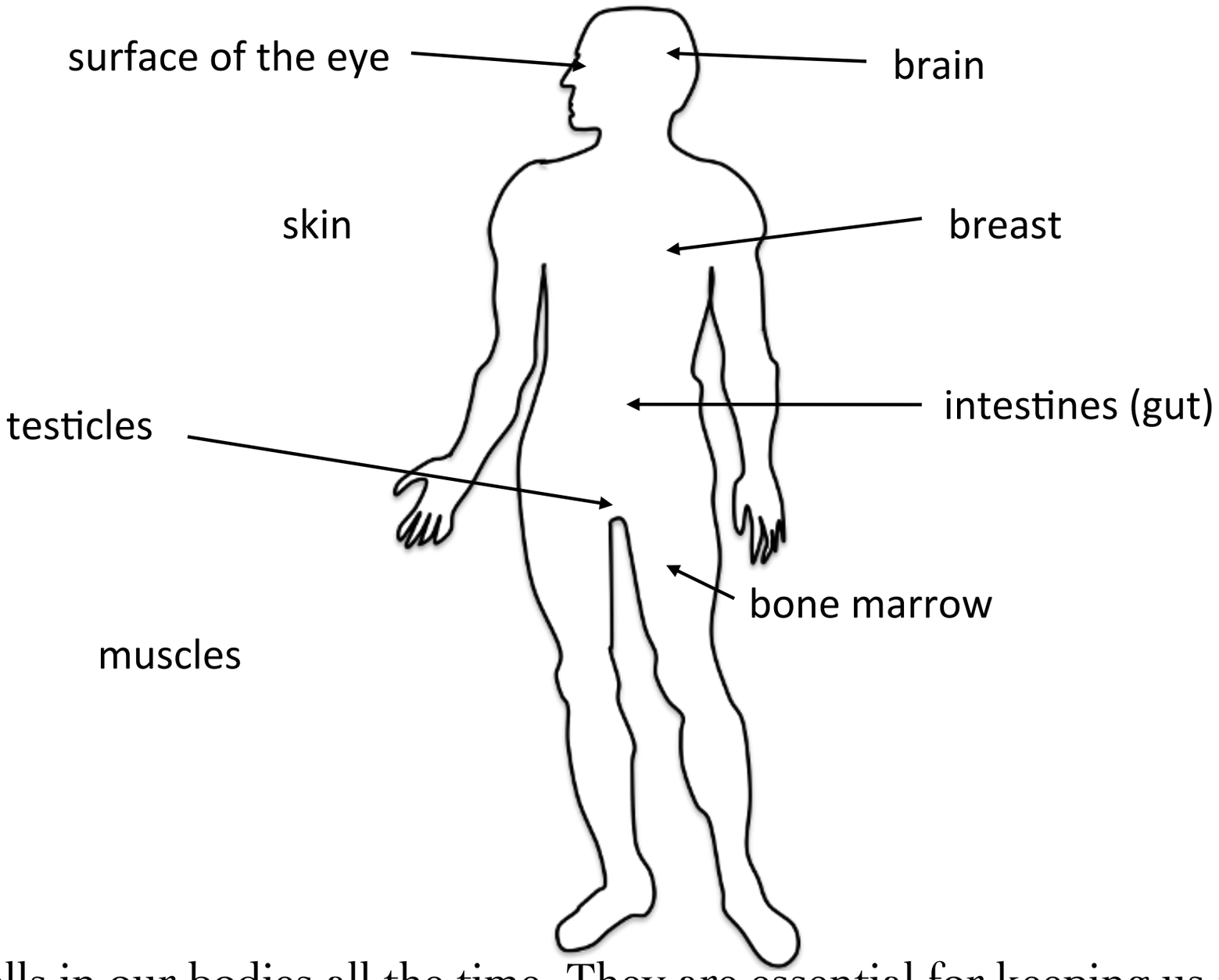
blood cells

cells of nervous system

connective tissue,
bones, cartilage, etc.

Multipotent

Tissue stem cells:
Where we find them



We all have stem cells in our bodies all the time. They are essential for keeping us fit and healthy. They replace cells that are damaged or used up.
Scientists are still learning about all the different kinds of tissue stem cells found in our bodies and how they work.

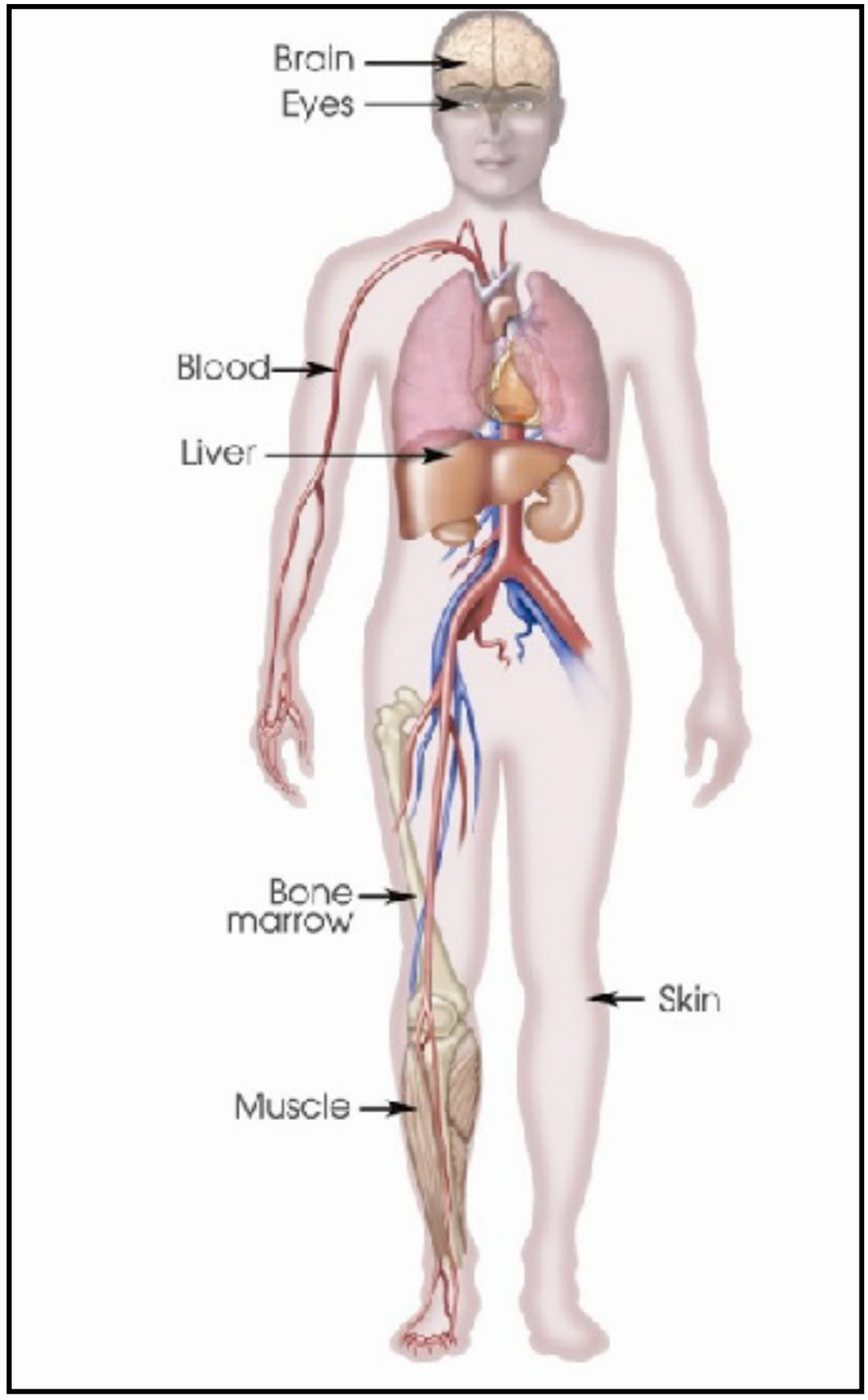
Adult Stem cells

- An adult stem cell is an undifferentiated cell, found among differentiated cells in a adult tissue or organ

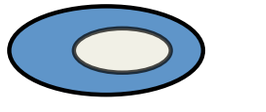
- Adult stem cell **a self renewal** and can differentiate to some or all of the major specialised cell types of the tissue or organ.

- The roles of adult stem cells are: to **maintain and repair the tissue in which they are found.**

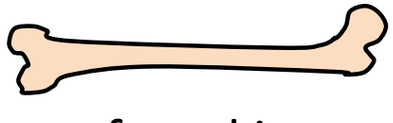
- Adult stem cells have been identified in many organs and tissues, including brain, bone marrow, peripheral blood, blood vessels, skeletal muscle, skin, teeth, heart, gut, liver, ovarian epithelium, and testis.



Tissue stem cells:
What they can do

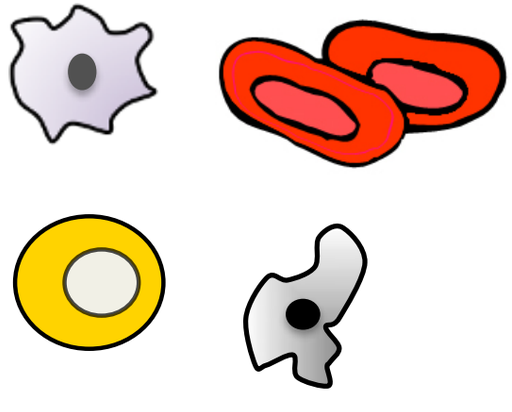


blood stem cell



found in
bone marrow

differentiation



only specialized types of blood cell:
red blood cells, white blood cells,
platelets

MULTIPOTENT

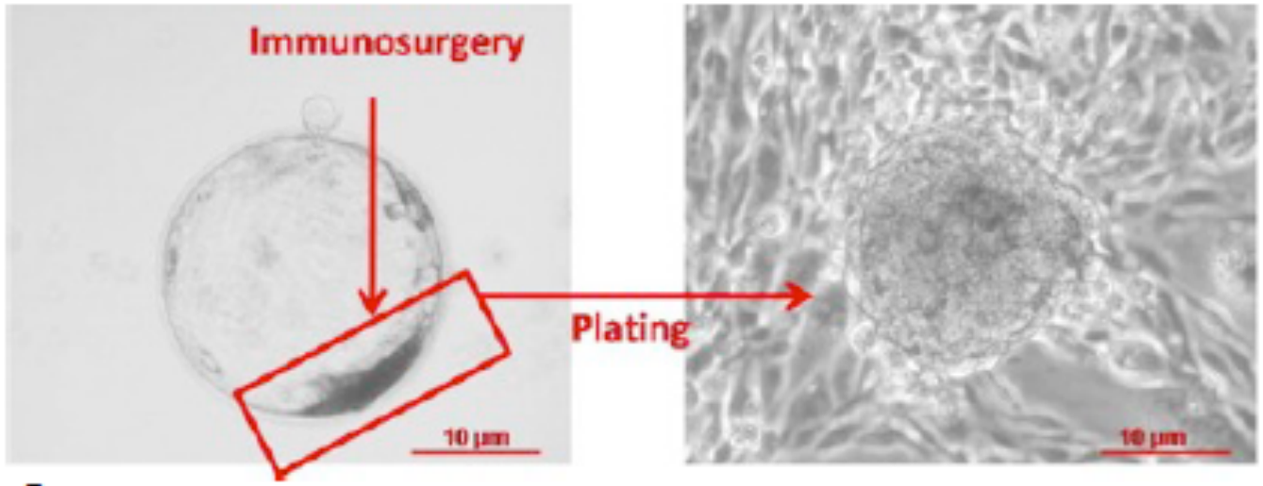
Tissue stem cells can often make several kinds of specialised cell, but they are more limited than embryonic stem cells. Tissue stem cells can ONLY make the kinds of cell found in the tissue they belong to. So, blood stem cells can only make the different kinds of cell found in the blood. Brain stem cells can only make different types of brain cell. Muscle stem cells can only make muscle cells. And so forth.

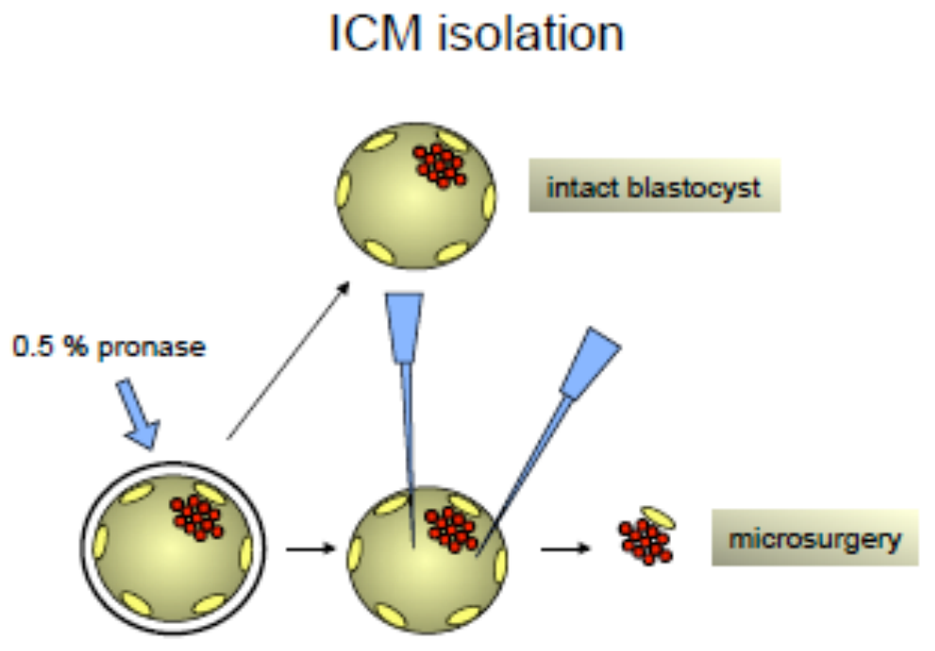
Scientists say that tissue stem cells are **multipotent** because they can make **multiple** types of specialised cell, but NOT all the kinds of cell in our body.

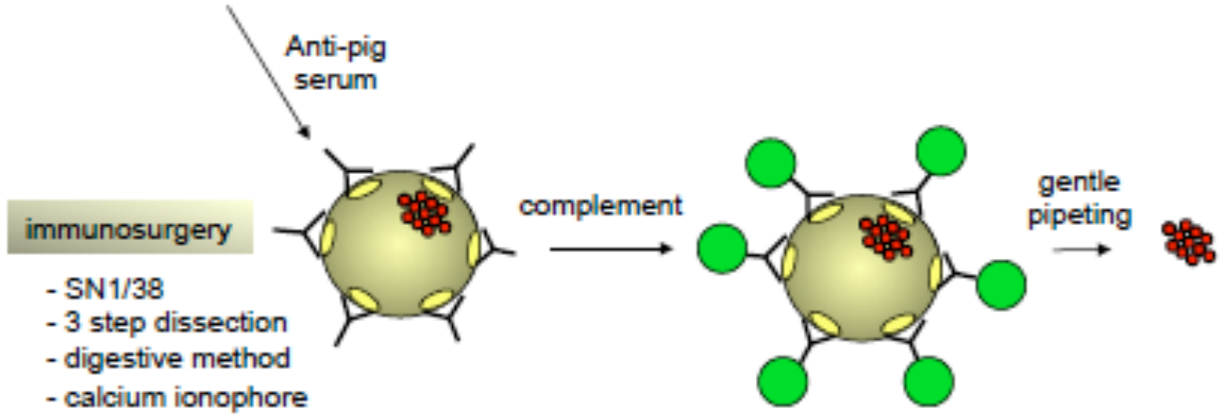
Isolation of Stem Cells

Immunosurgery
Microsurgery
Enzymatic (trypsin + EDTA)
Laser

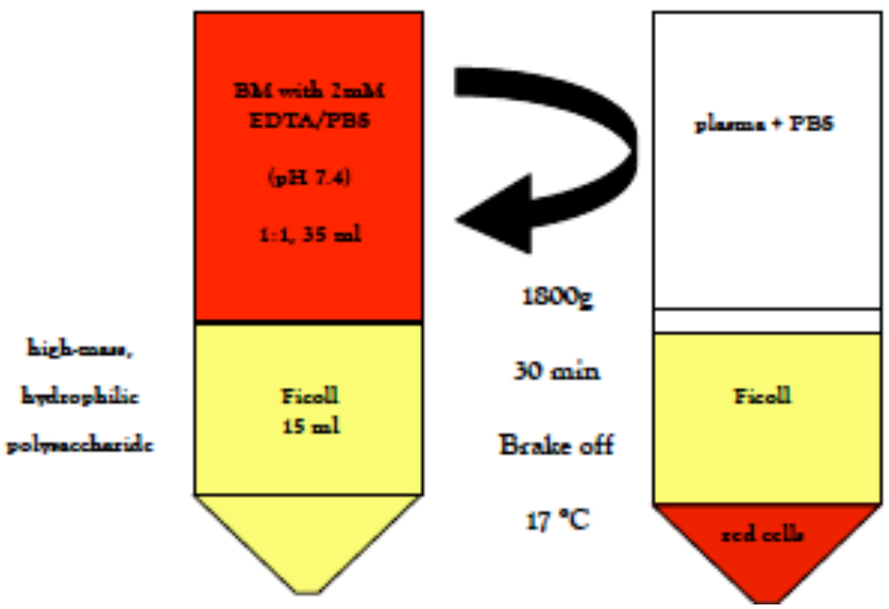
Embryonic Stem Cells - ESCs







Mesenchymal Stem Cells - MSCs



MSCs

Wash cells 1x with 2mM EDTA, 2880g 10min

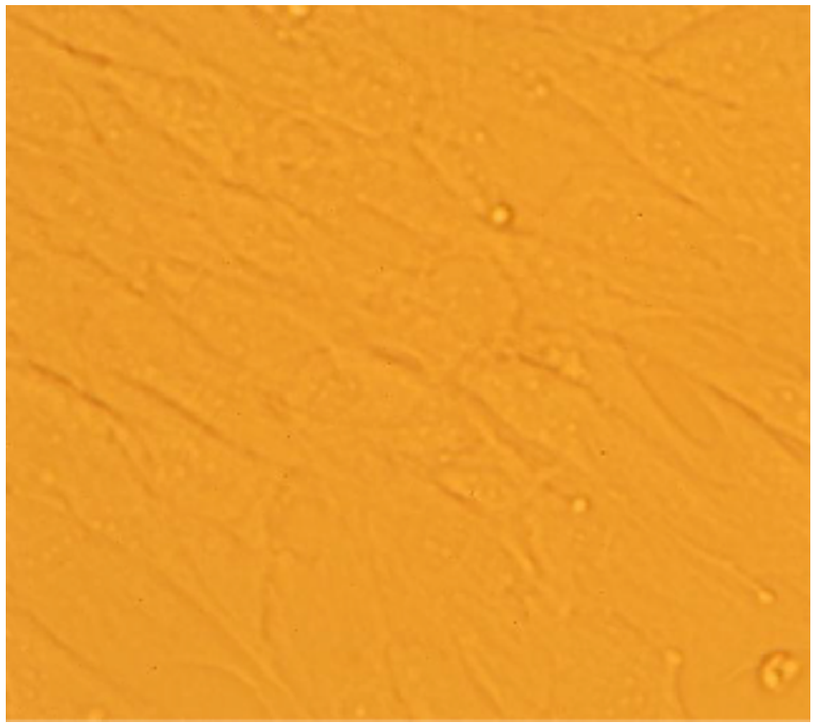
Wash cells 1x in alfa-MEM medium, 2880g, 10min

Transfer isolated cells into the culture

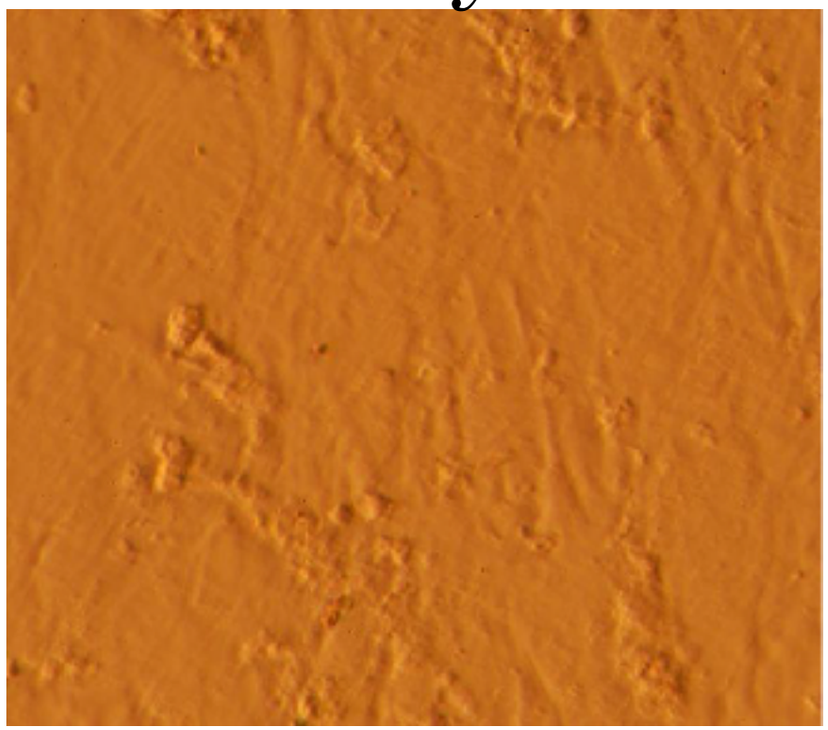
Part 1. Collection of Mesenchymal Stem Cells

https://www.youtube.com/watch?v=8vuoU9_mEWg

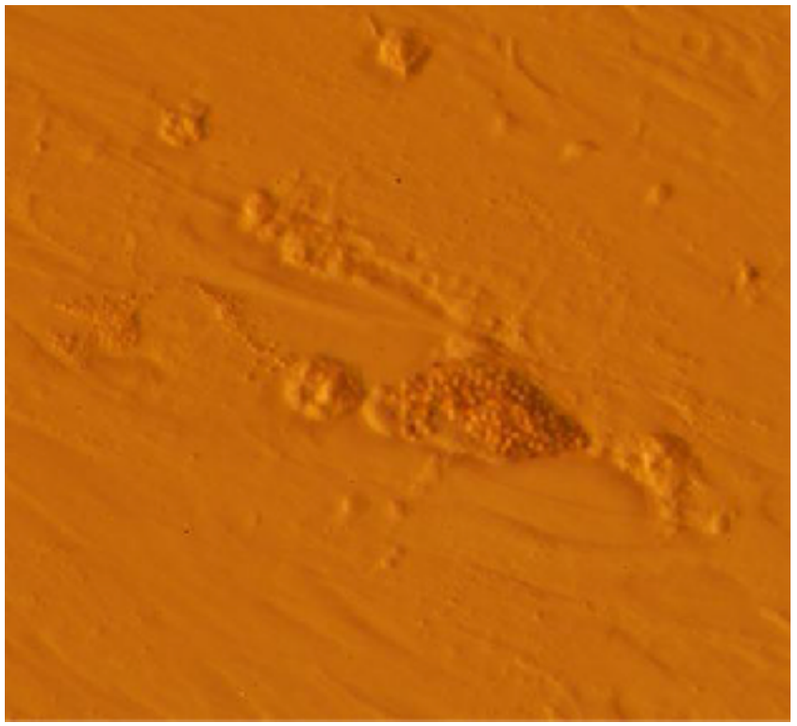
Before

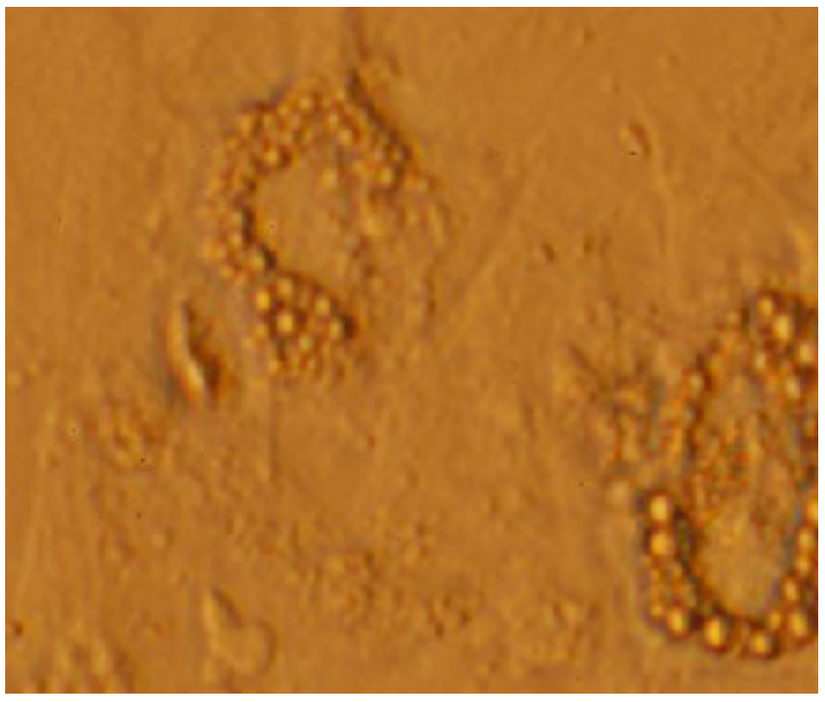


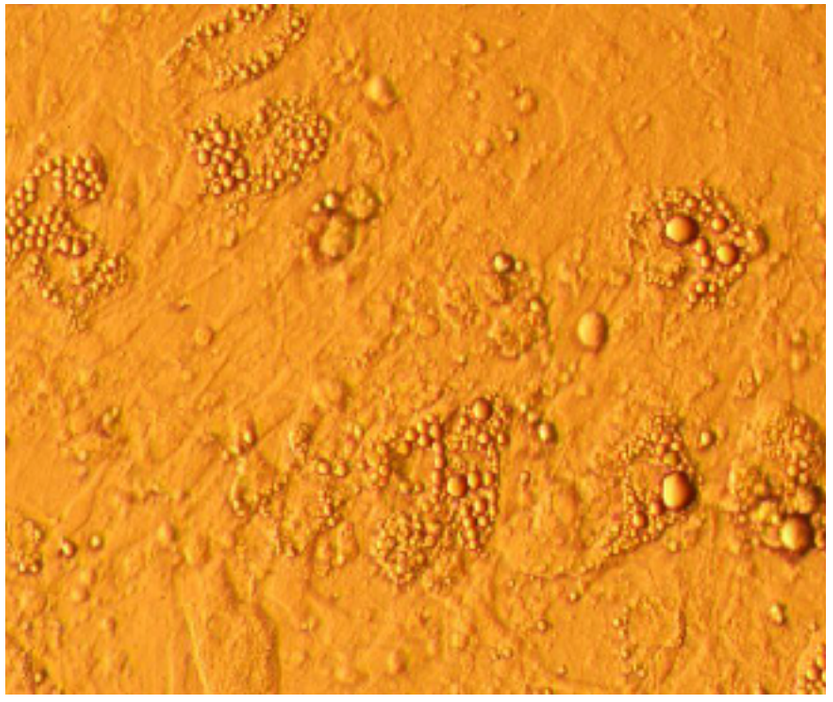
7 days



12 days





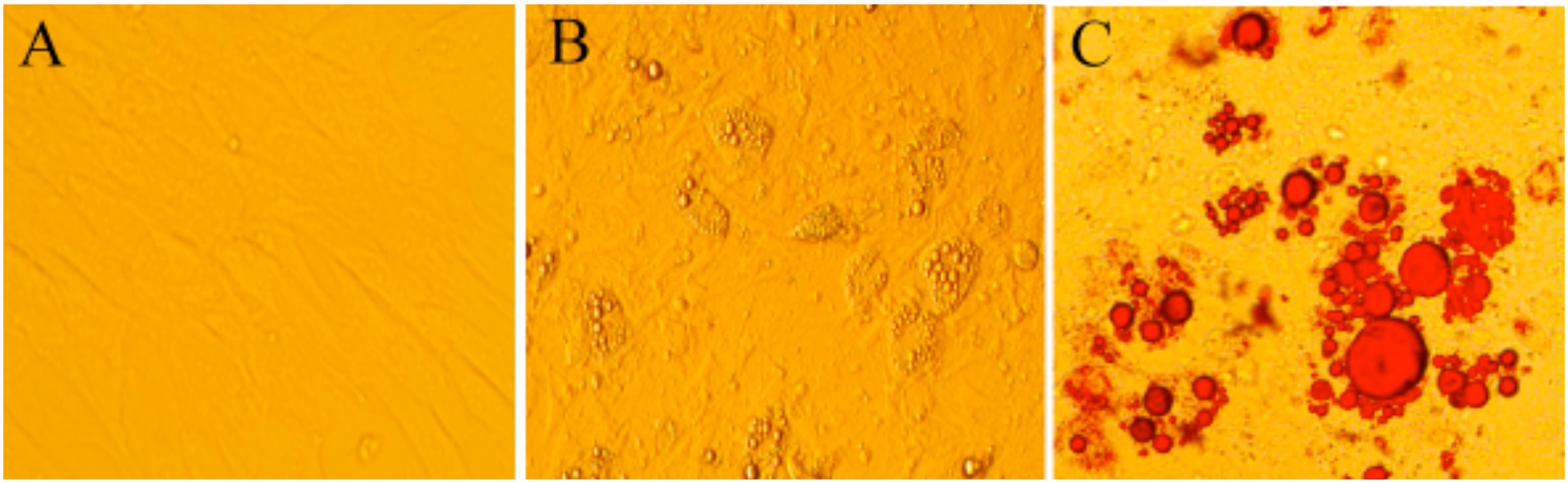


24 days

26-28 days

33-35 days

Differentiation of MSCs into adipocytes



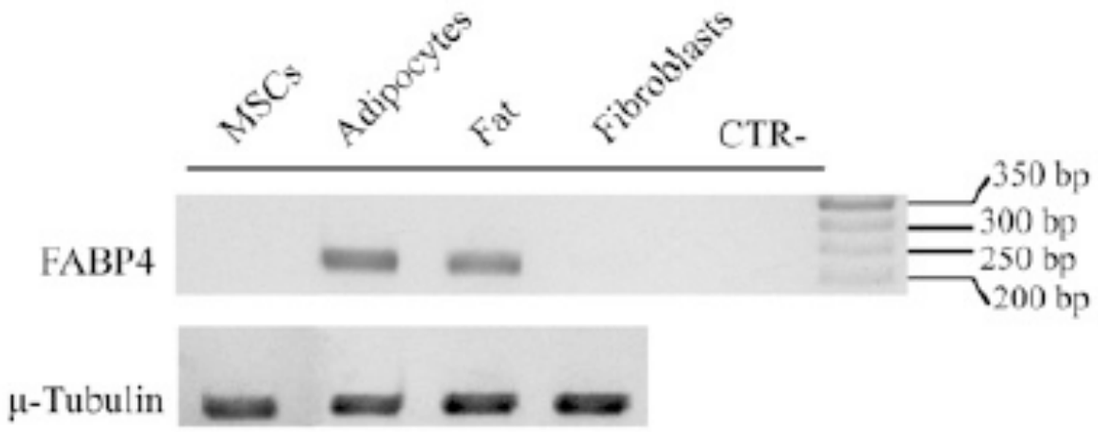
35 days in differentiation medium

Adipocytes:
Medium I

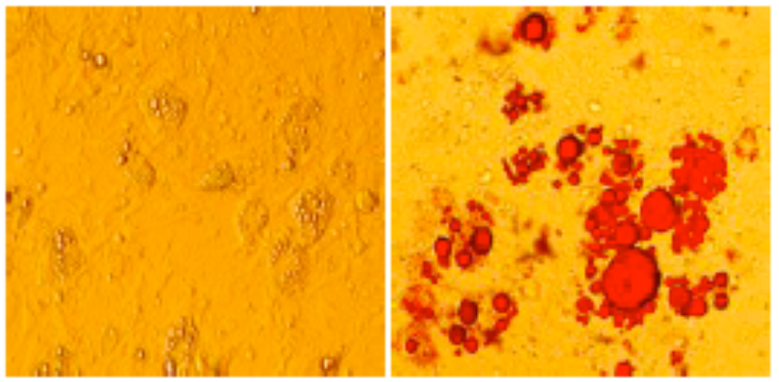
A) 1uM **Dexamethasone**
B) 100ug **1-methyl-3 isobutylxanthine**
C) 100mM indomethacin
D) 1ng/ml insulin
E) 10% FBS
F) α – MEM medium for first 48h then exchange with medium II

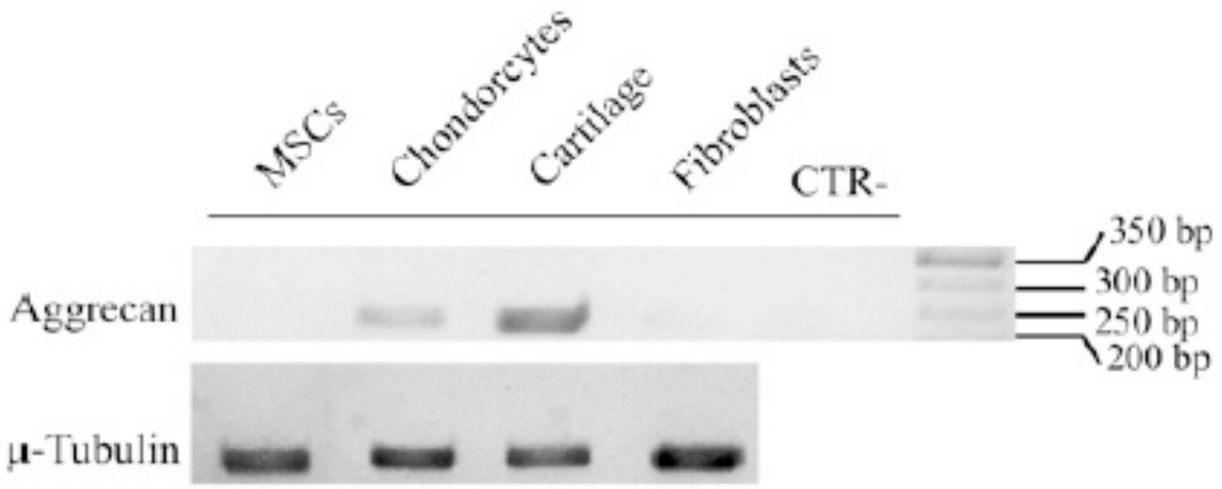
Medium II

G) 10g/ml insulin
H) 10% FBS
I) In α- MEM for 24h then exchange with first one.

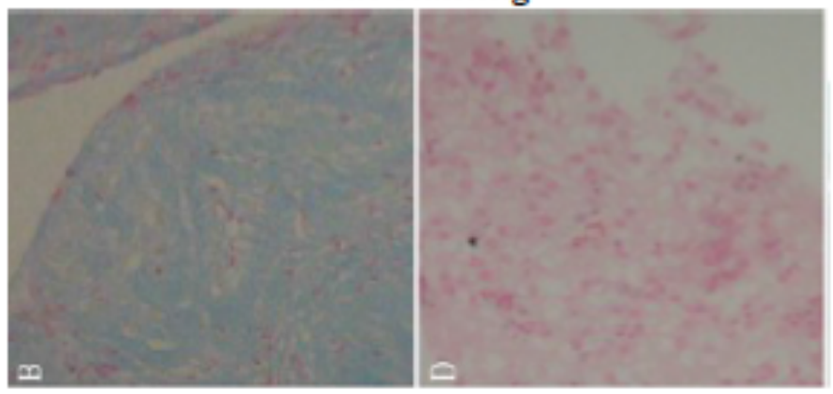


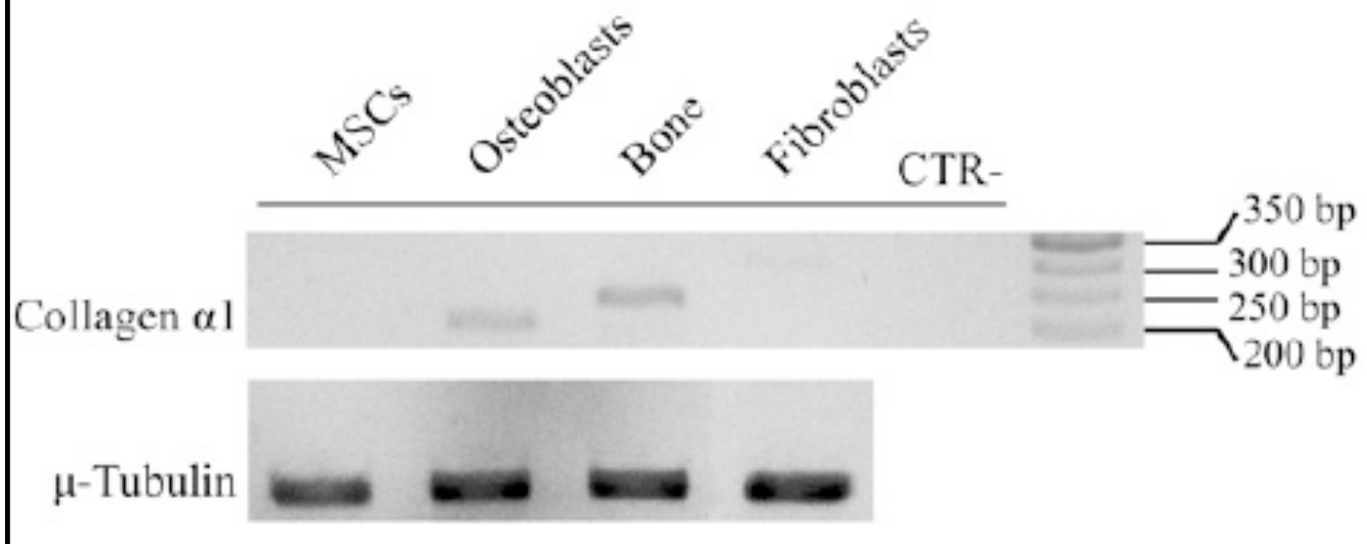
Accumulation of neutral lipid vacuols and Oil O Red.



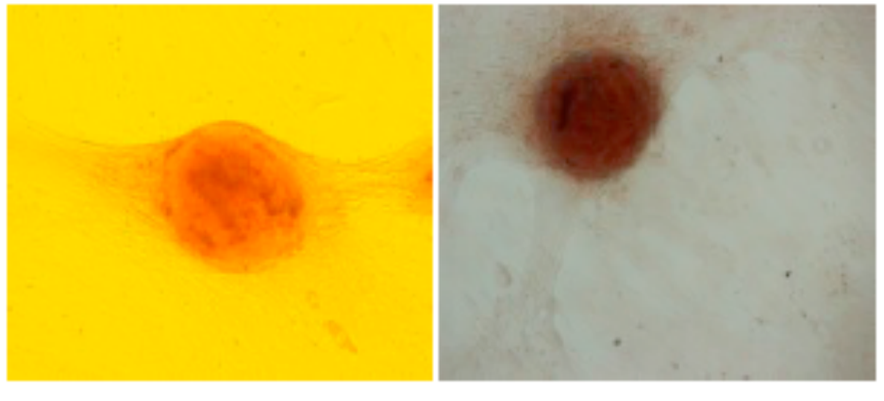


Presence of acidic proteoglycans by Alcian blue staining





Presence of mineralized deposits by Alizin red



PART 2. Cells Counting

Cells counting

https://www.youtube.com/watch?v=qfT9uqqme8c

https://www.youtube.com/watch?v=WWS9sZbGj6A

https://www.youtube.com/watch?v=V9bQW7yn1cI

Video 1. Cells counting

PART 3. Freezing of Cells

https://www.youtube.com/watch?v=tCNtKrxlZPs

https://www.youtube.com/watch?v=jehmGUvsr_I

freezinghttps://www.youtube.com/watch?v=qybFQJ4-KEY

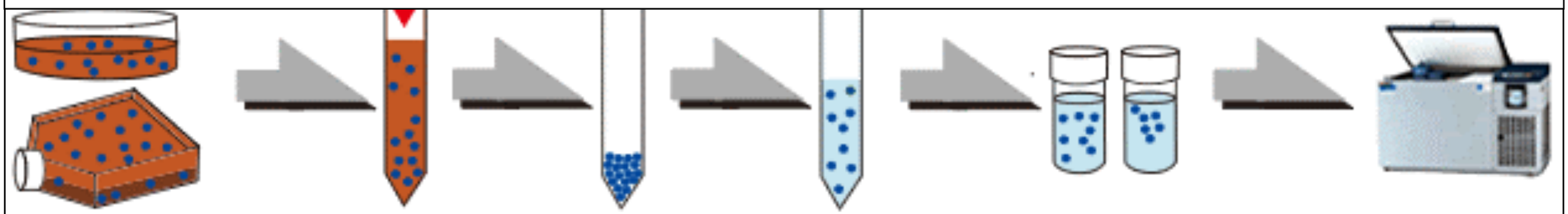
Cells Freezing principal info:

- **Freeze slowly** to allow water to leave the cell but not so slowly that ice crystal growth is encouraged

- The best cells concentration is from **1 x 10^5 /mL to 1 x 10^7** frozen in 1 mL of freezing medium (**20% FBS, 20% DMSO in culture medium**).

- Chamber for freezing cells - Mr. Frosty - allow to achieve a rate of cooling very close to -1°C/minute, the optimal rate for cell preservation and give the best results in term of cell viability.

FREEZING PROCEDURE



Trypsinize cells

Pelette cells by spining at 1200rmp

Resuspend cells in culture medium

Aliquot in cryovials 0.5 ml (1 x 10^6 cells) / vial

Store in Mr Frosty in -80C for 24h

Remove culture medium

↓

Wash with PBS

↓

Add trypsin

↓

Add medium with serum

↓

Transfer cell into tube

↓

Count cells

↓

Add **drop by drop** 0.5 ml freezing medium into each cryovial

↓

Transfer into Liquid Nitrogen

Frezzing medium:
20% DMSO
20% FBS
60% Culture medium

Video. Freezing cells

The end …