



Lecture 3



Topics

Organoids

Aging

Cloning

Organoids



Overview

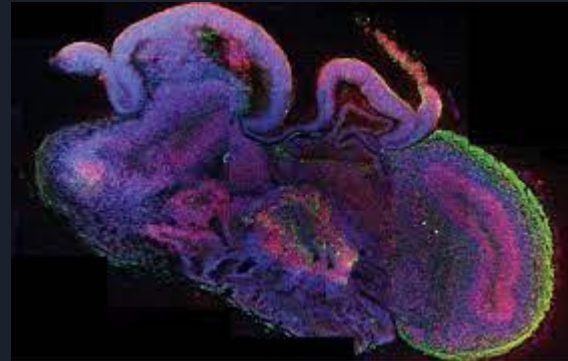
“3D structure derived from (pluripotent) stem cells, progenitor, and/or differentiated cells that self-organize through cell-cell and cell-matrix interactions to recapitulate aspects of the native tissue architecture and function in vitro”

Key features:

1. Self assembly
2. Self patterning
3. Self organization

2D cultures vs 3D cultures

1. 2D advantages
2. 3D advantages

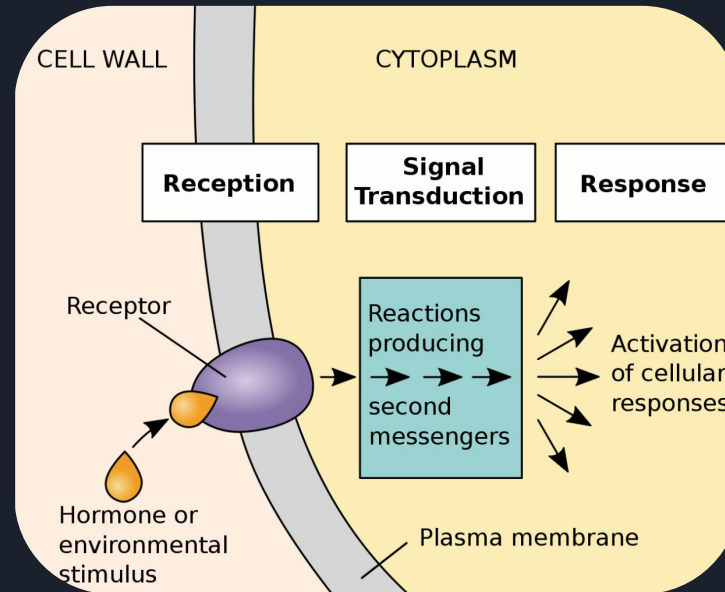


Self Assembly

Capability for cells to form structures without external direction.

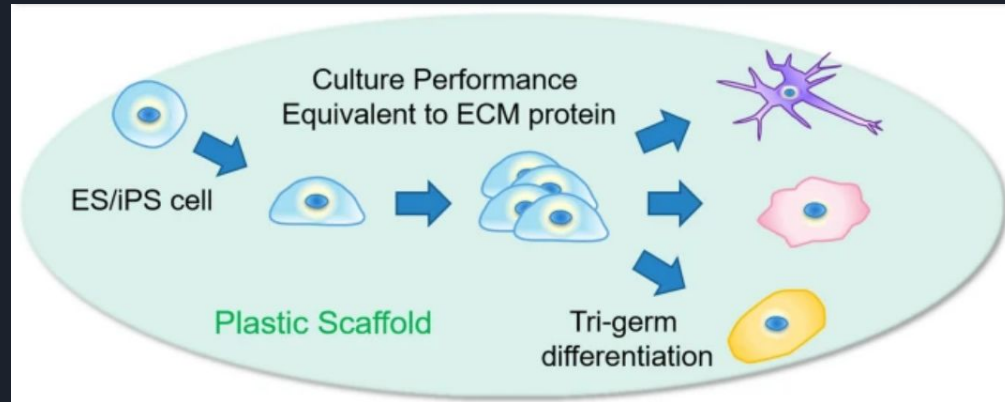
Process is driven by:

1. Cell-cell interactions
2. Cell-ECM interactions
3. Biochemical signals



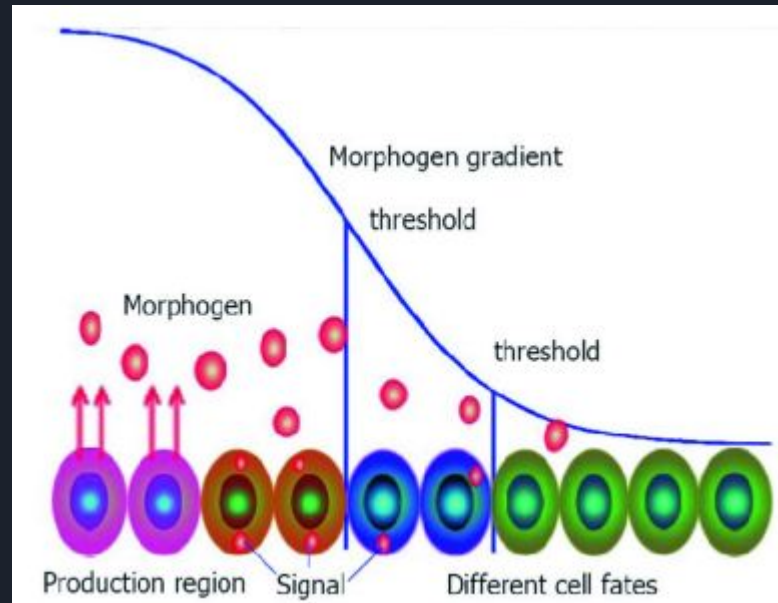
What is the ECM in research?

(The plastic surface cells
bind to)

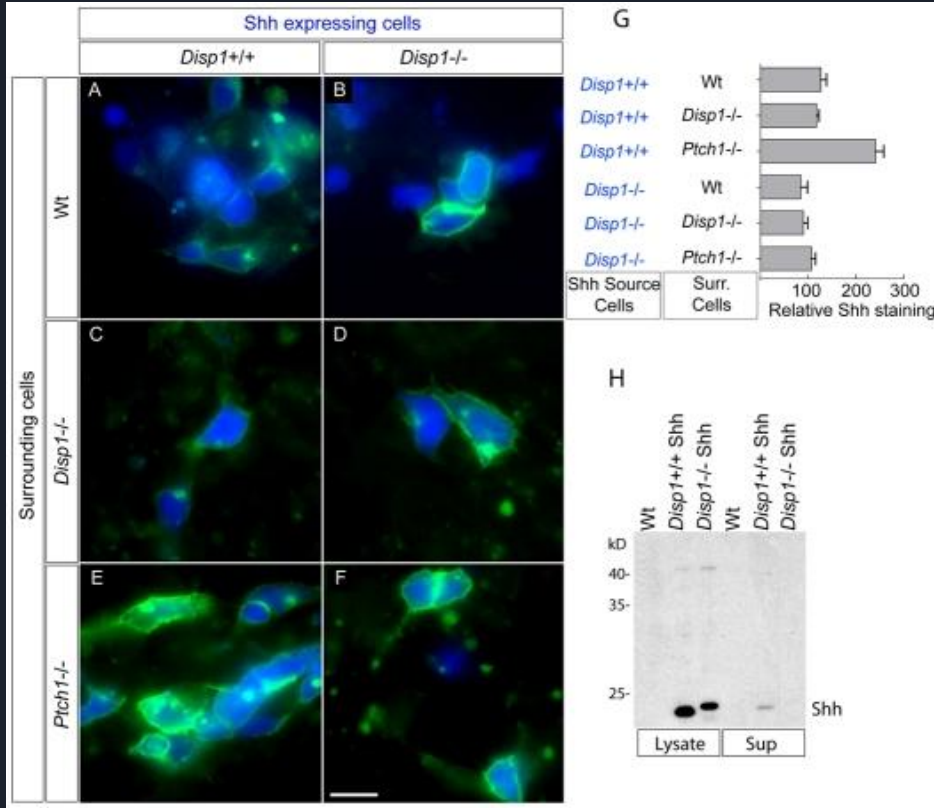


Self Patterning

Spatial organization of cells to form tissue-specific patterns, guided by morphogen gradients and signalling pathways which dictate differentiation



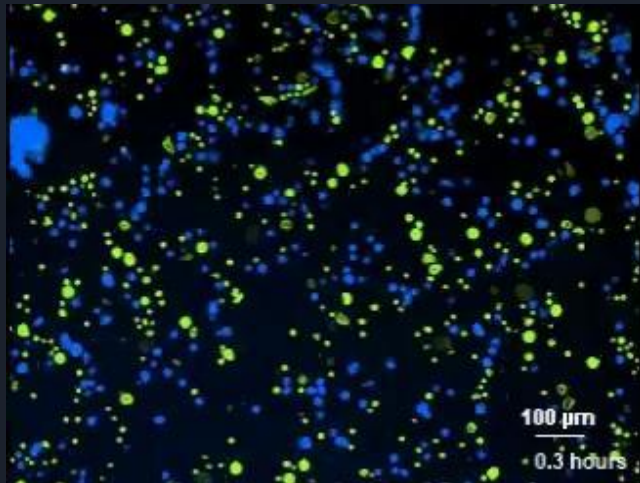
Shh Pathway & Conboy



Research from the Conboy lab, showing how Shh (a morphogen) measurements in cocultures showed a contact-dependent response and transport-dependent response

Self organization

- Broader process of coordinating behavior to form complex structures and patterns.
- Based on the collective behavior of cells within the system.
- Allows exhibition of emergent properties.



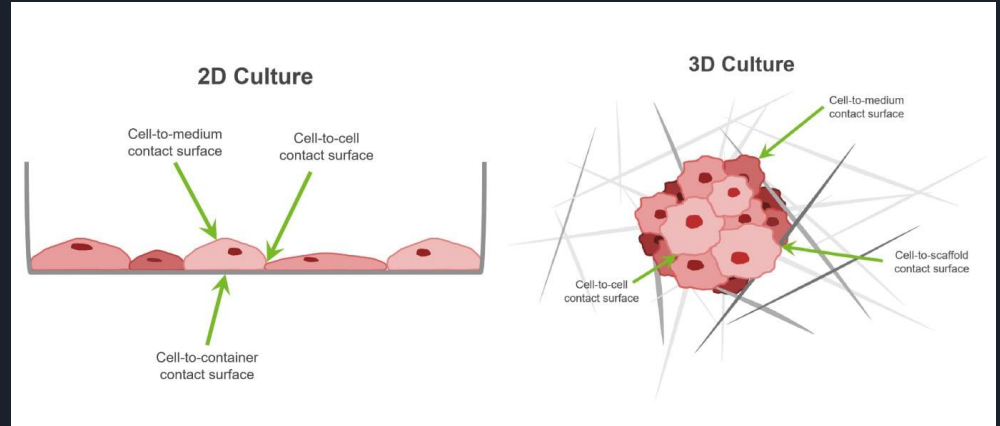
2D Cultures

Advantages:

- Homogeneity
- Reproducibility
- Efficiency

Disadvantages

- Fail to recapitulate 3D environments



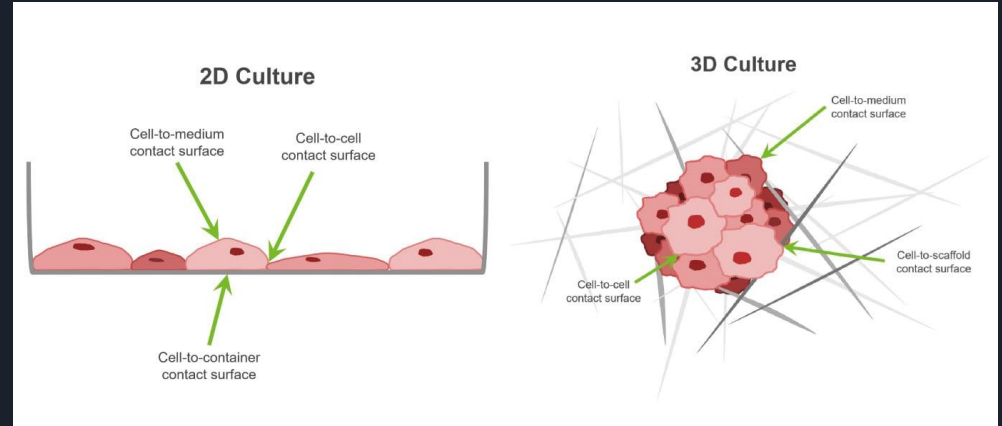
3D Cultures

Advantages:

- Self-organization
- Recapitulation of stratified structures

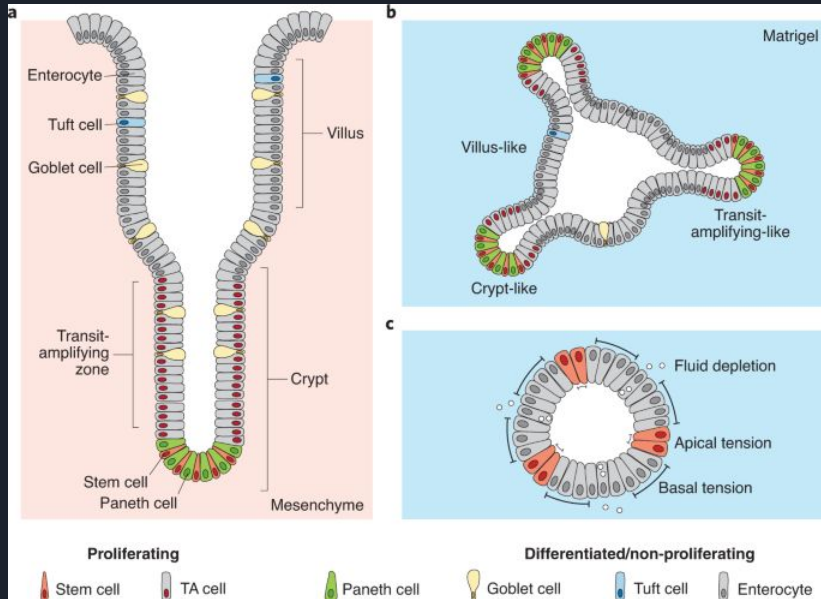
Disadvantages

- Disruption of collective behavior in tissue-like context
- Don't capture all aspects of native tissue architecture



Structural Aspect of Organoids

Structure is conducive to function



Structure-Function relationship

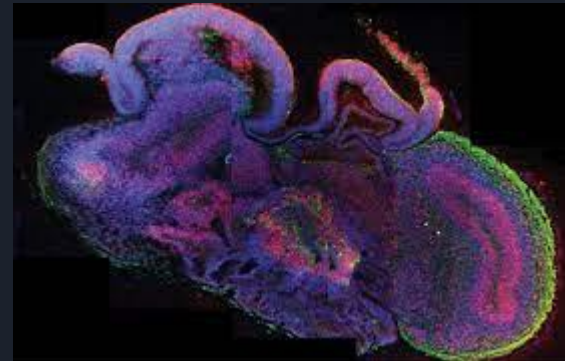
Gut Organoid

- Need to recapitulate CRYPTs and VILLI to accurately capture the key functional aspects of the gut



Brain Organoid

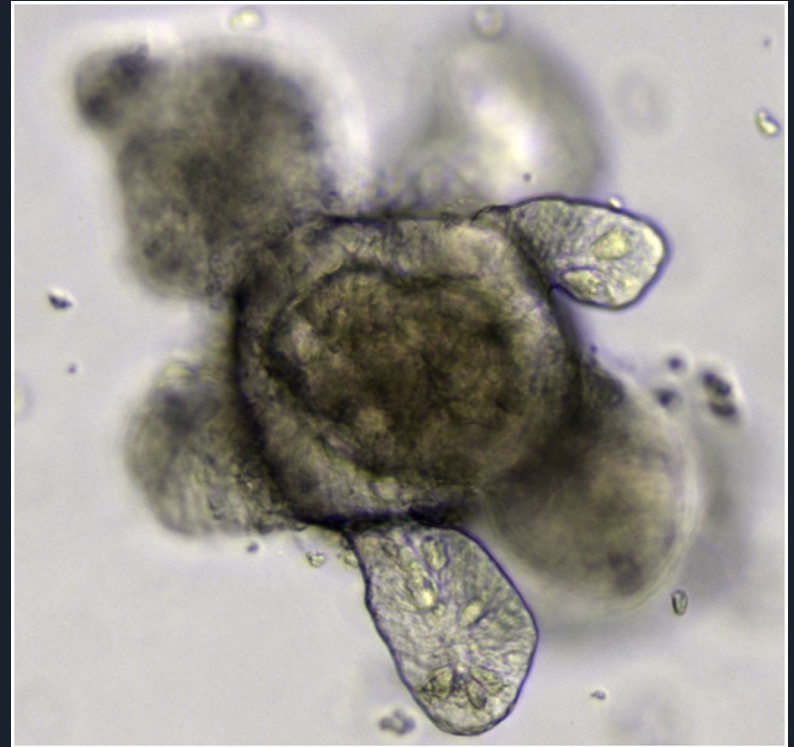
- Spheroids
 - Recapitulating wrinkles - inner structure, requires imaging
- iPSC derived



Applications

Colon Cancer

- Dysregulation of Wnt signaling causes cells to retain stemness too far (physically) up the crypt/villus
- Formation of a polyp because it just keeps on growing, without getting pushed up the villi



Cloning





Overview

Types

- Reproductive
- Therapeutic
- Molecular

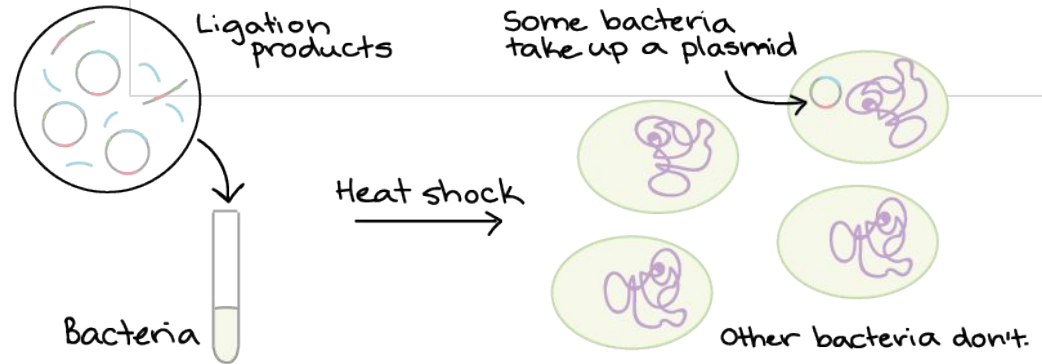
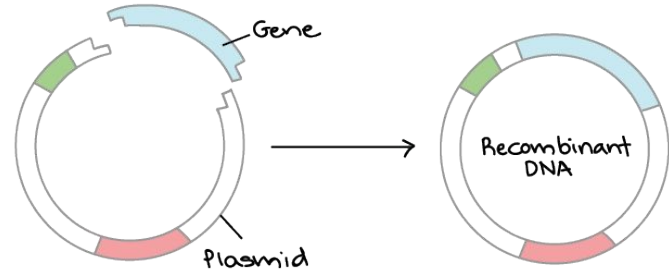
Applications

- Disease modelling
- Drug development and testing
- Transplantation

Molecular Cloning

Through CRISPR/Cas9, we can make precise genetic modifications in stem cells

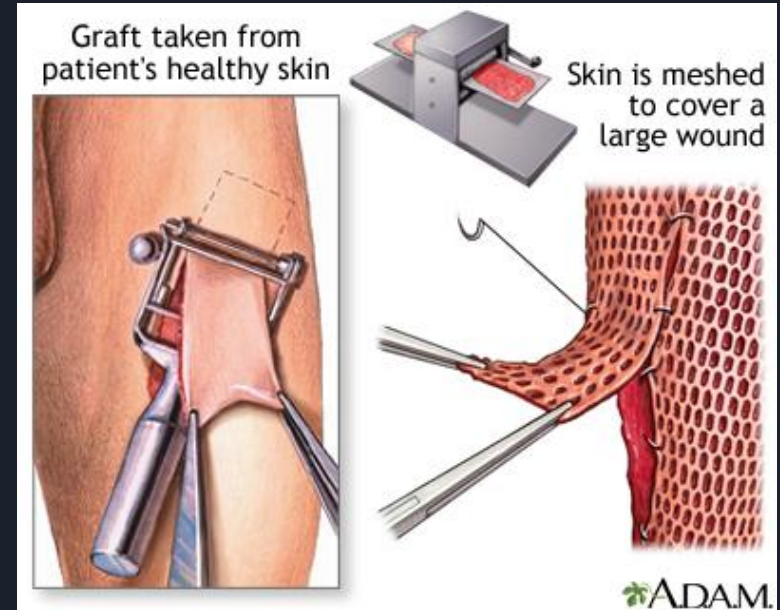
- Gene editing
- Drug development
- Improved therapies



Therapeutic Cloning

Create patient specific research targets

- Nucleus transferred to an enucleated oocyte
- Resulting embryo can make ESCs that are genetically identical to the parent

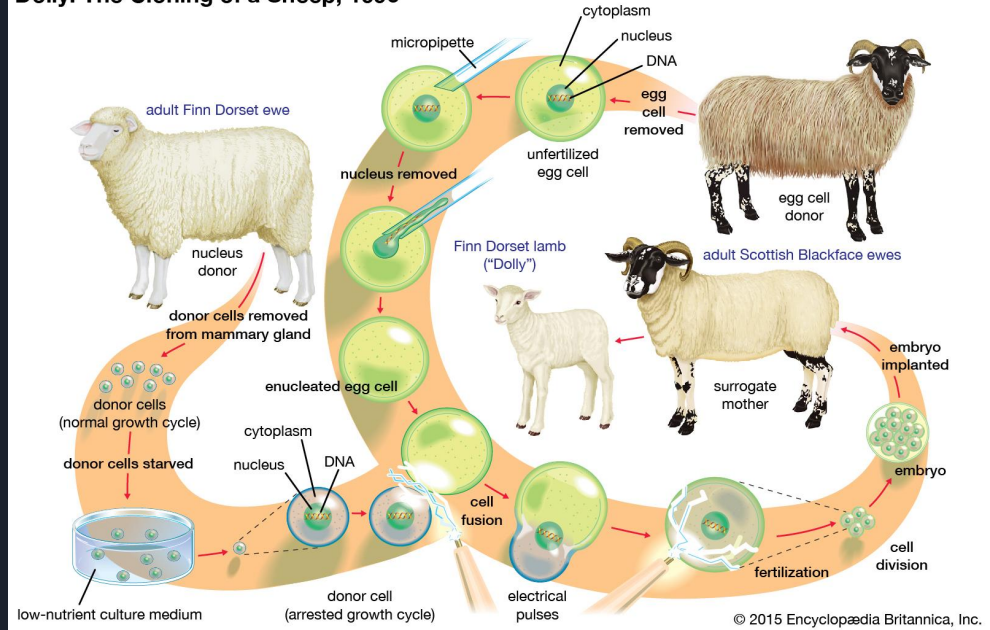


Reproductive Cloning

Creating an identical copy of an organism (Dolly the Sheep)

- Created through adult somatic cell nuclear transfer
- Proved that adult cells could be reprogrammed to a totipotent state

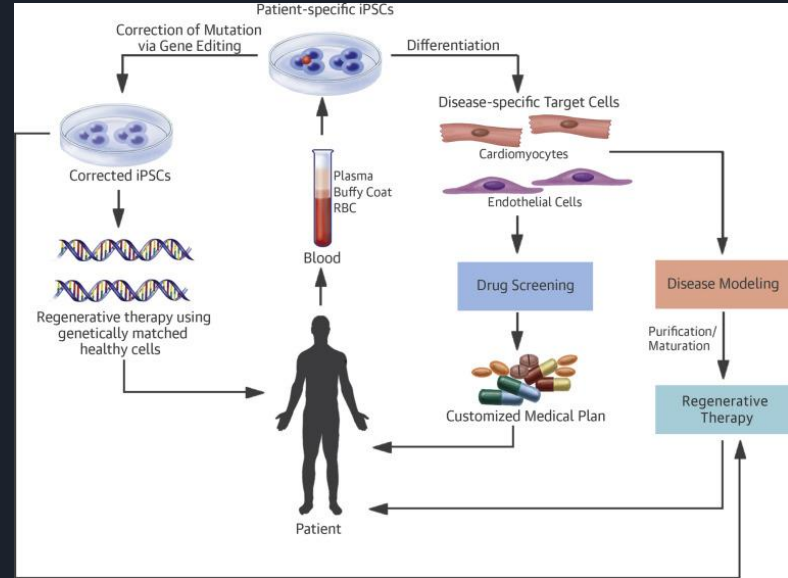
Dolly: The Cloning of a Sheep, 1996



Applications

Applications

- Disease modelling
- Drug development and testing
- Transplantation



Aging



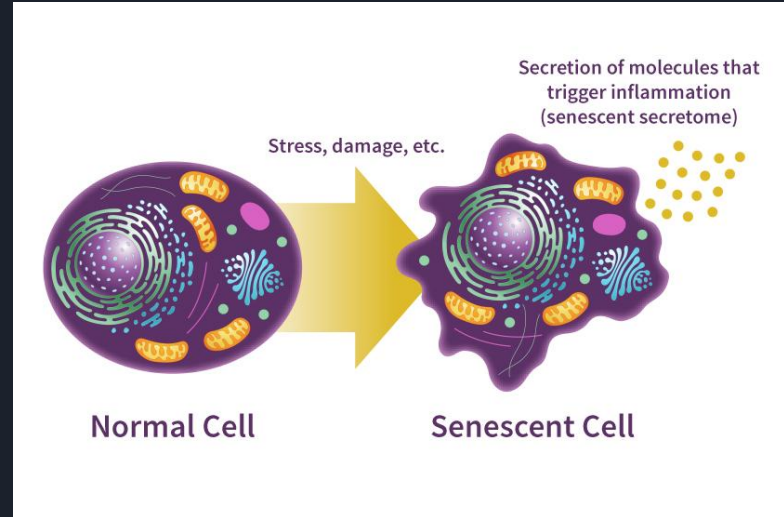


Overview

- What is aging? (Biological perspective of stem cell exhaustion)
 - Cell senescence
 - Telomere shortening
 - DNA damage accumulation
- Therapies
 - Macular degeneration
 - Osteoarthritis
 - Alzheimer's
- Parabiosis

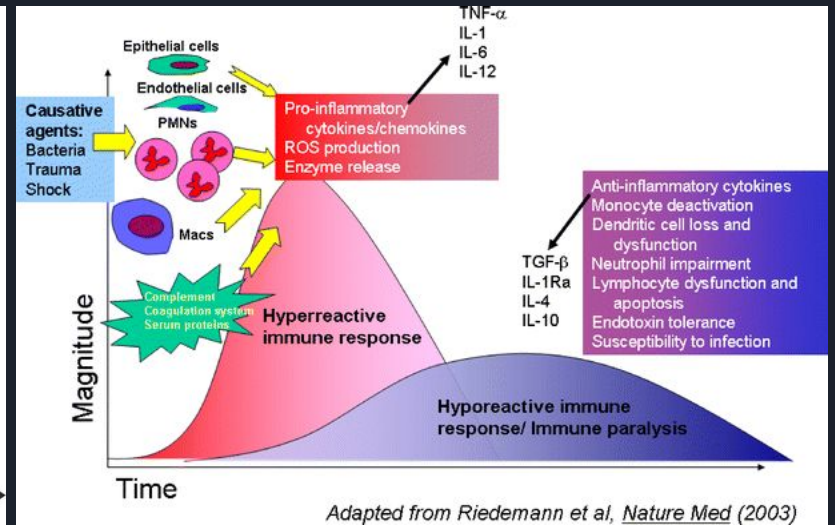
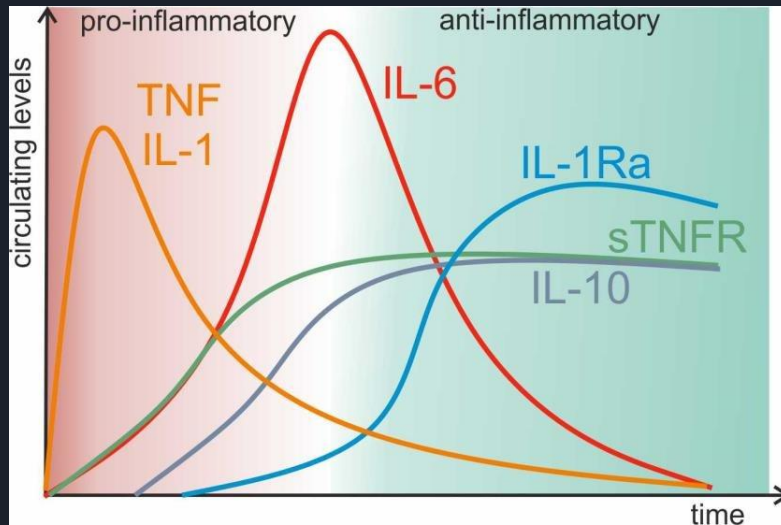
Cell Senescence

- Loss of ability to self-renew and differentiate
- SASP releases pro-inflammatory factors
- Accumulation causes age-related decline in organ function



Cytokines and sepsis

Cytokines are a broad and loose category of small proteins important in cell signaling.



Telomere Shortening

Telomeres help in the process of DNA duplication, but they also shorten over time

This process in stem cells has been linked to reduced self-renewal capacity and impaired differentiation potential

