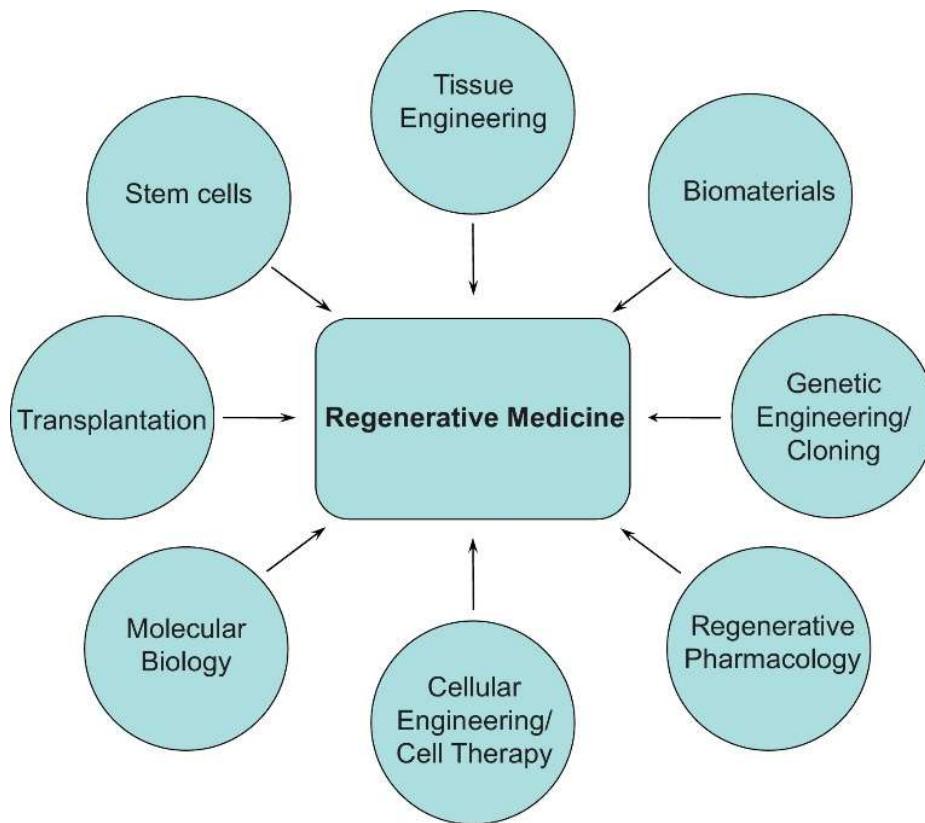


Regenerative medicine

Regenerative medicine



<https://doi.org/10.1016/j.maturitas.2013.10.007>

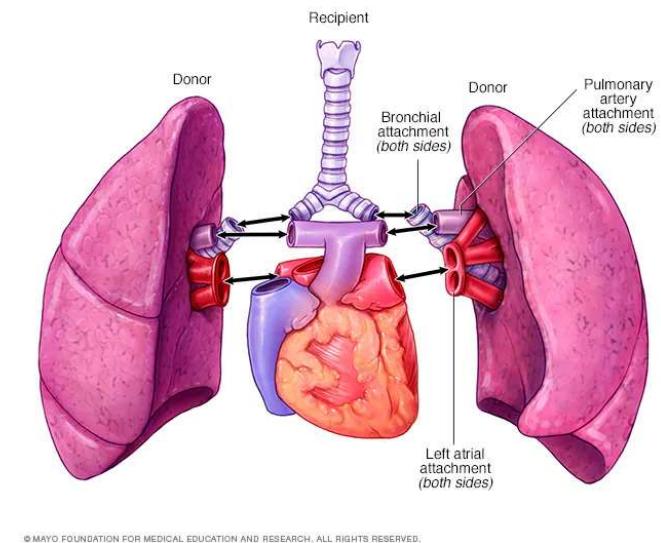
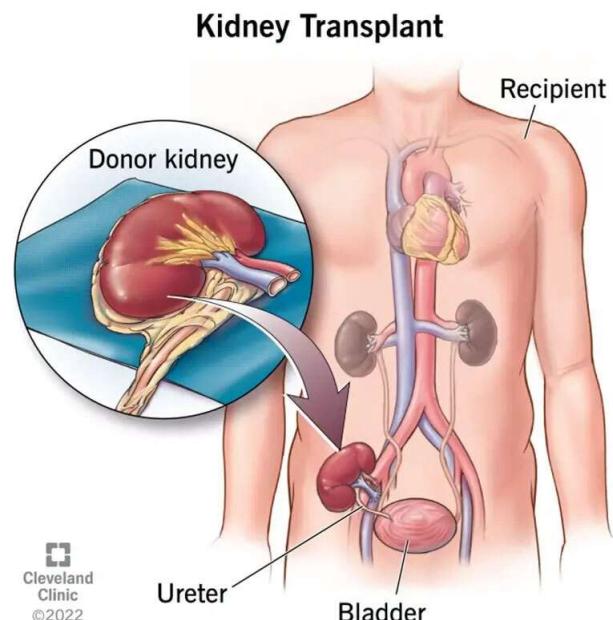
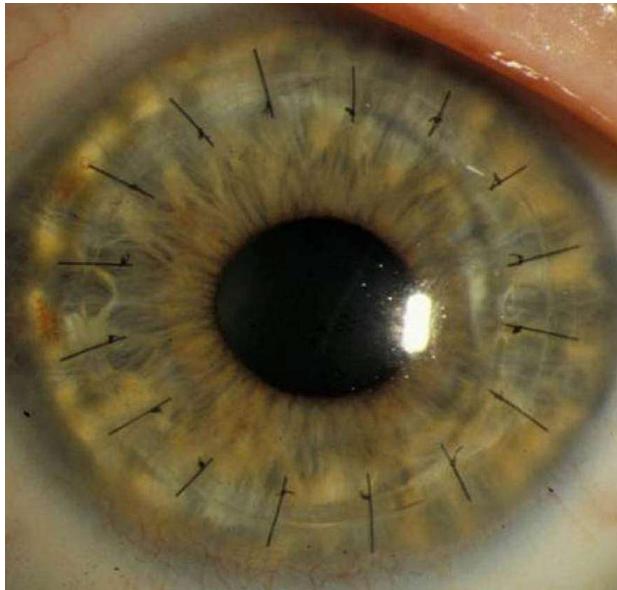
Regenerative medicine

- Regenerative medicine is a relatively new field that promises to improve health and quality of life by replacing or restore lost, damaged, or aging cells in the human body to restore function.
- Its principles revolve around harnessing the body's natural regenerative capabilities, using various approaches to stimulate healing, repair, or replacement of damaged tissues. Currently, the most prominent form of regenerative technologies attempts to use human stem cells.
- The field of regenerative medicine encompasses numerous strategies, including:
 - I. The use of materials and de novo generated cells to take the place of missing tissue,
 - II. Leveraging innate healing response to promote regeneration.

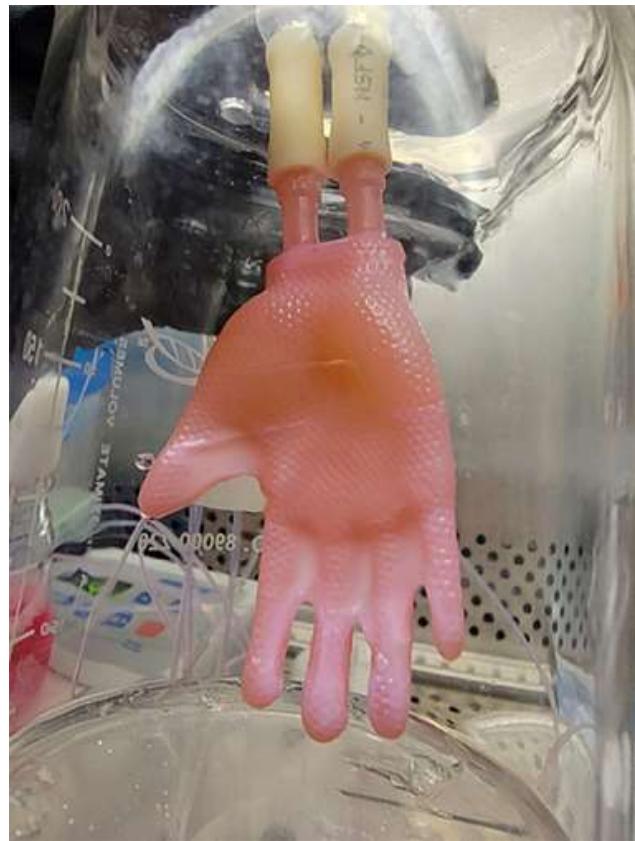
Why regenerative medicine?

- i. Reduce the need for transplants: Shortage of organ donors and graft rejection problems make transplantation an inefficient therapy
- ii. Potential long term solution: Some of the current available treatments treat only the symptoms, but regenerative medicine aims to provide long term solution.
- iii. Personalized medicine: by understanding an individual's unique genetic makeup and specific health conditions personalized treatment method can be developed.
- iv. Minimal invasiveness

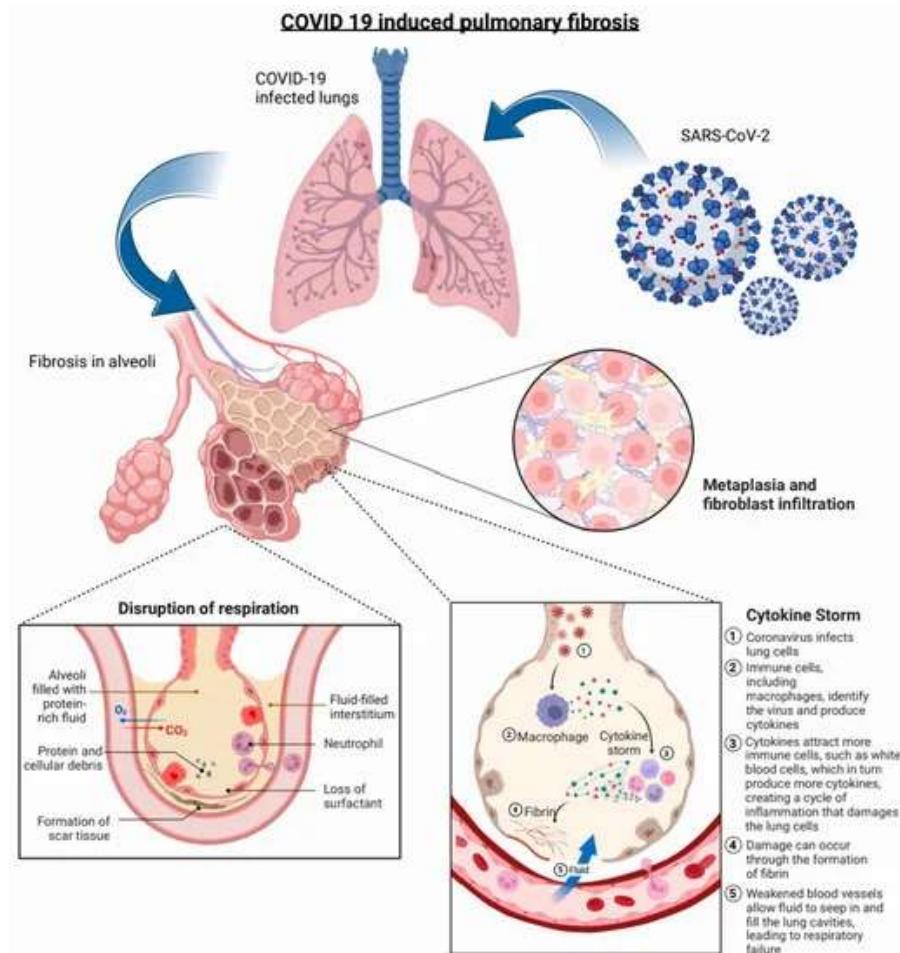
Immune compatibility and availability



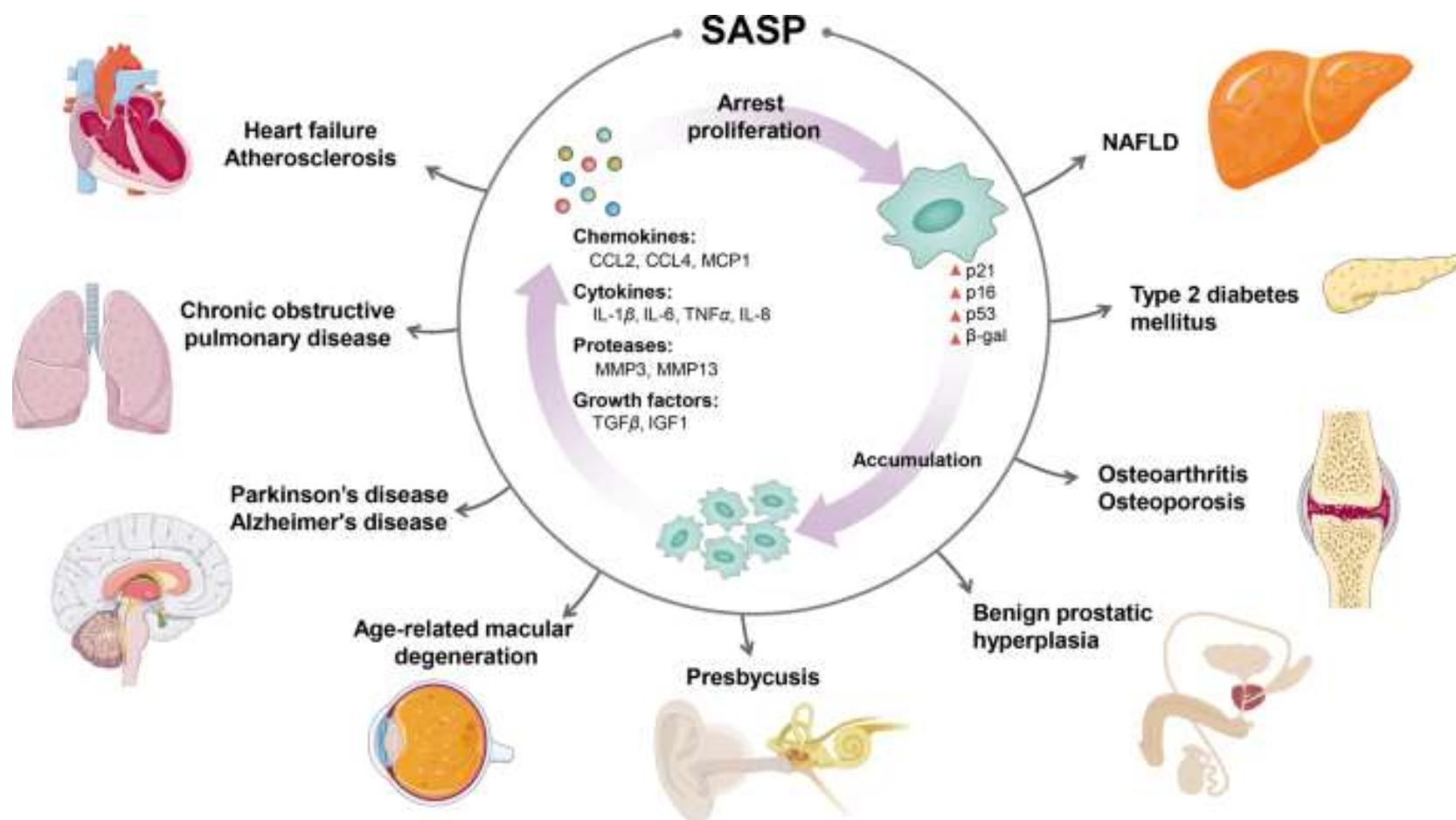
Complex structures



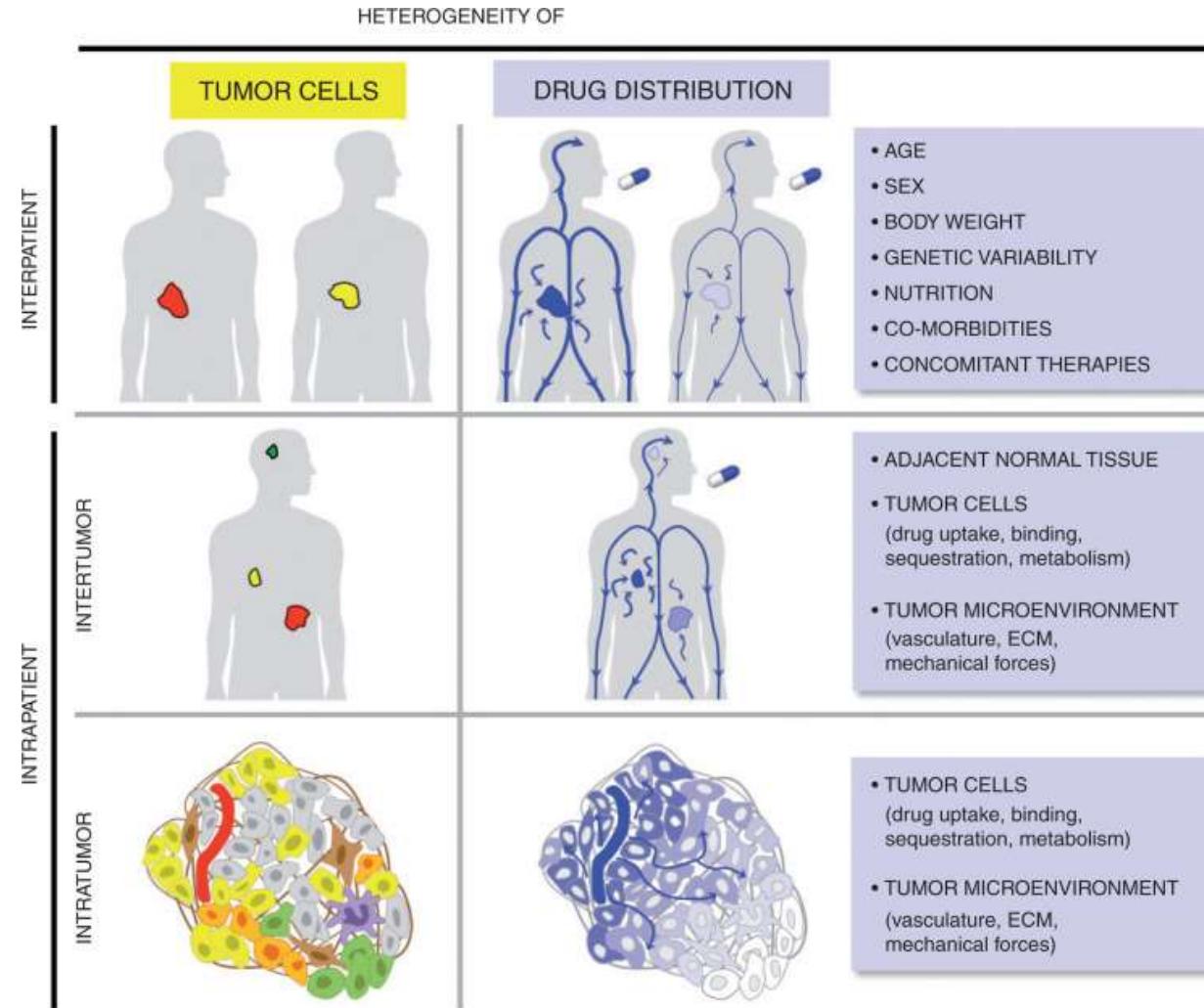
Regeneration versus symptomatic treatment



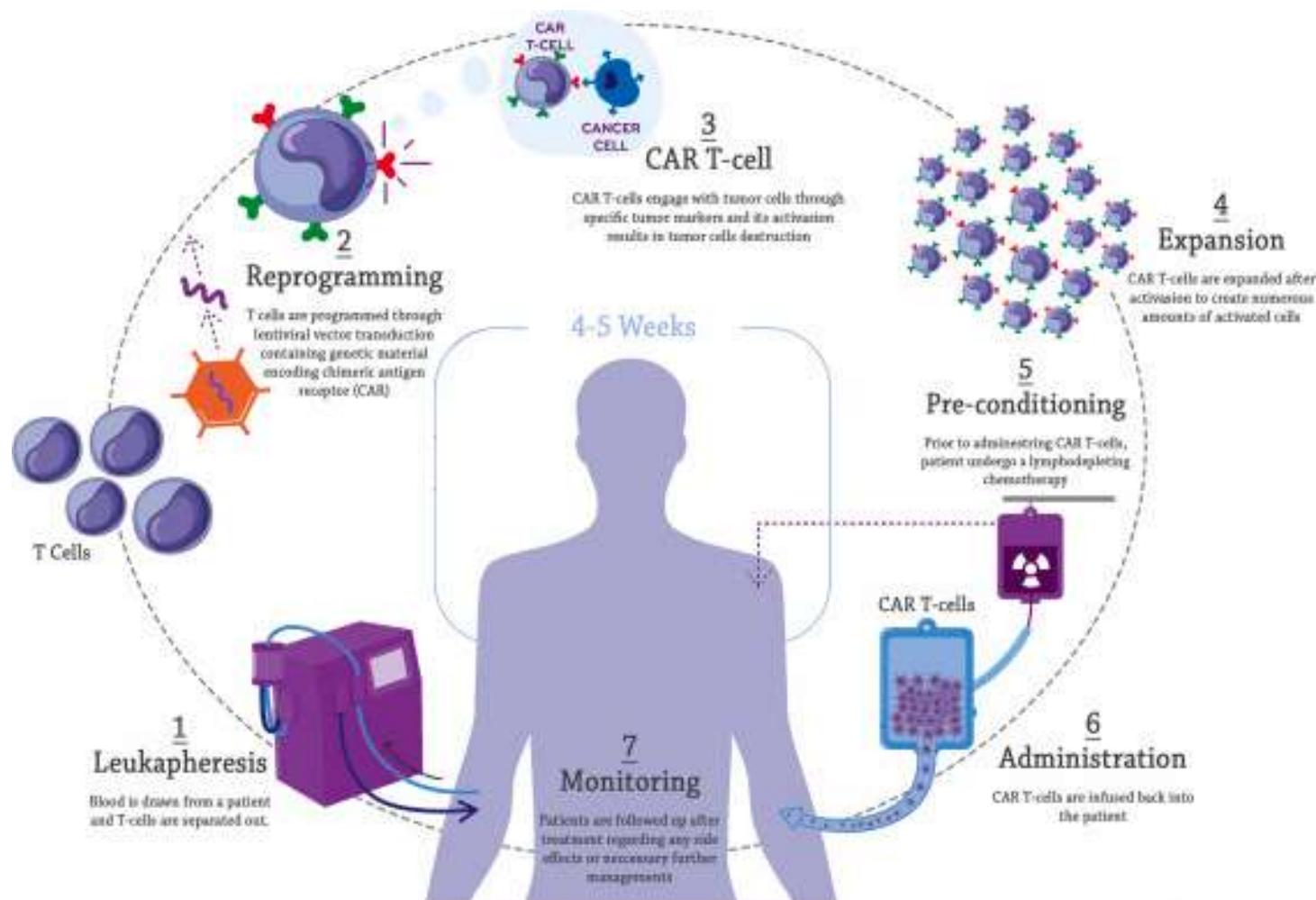
Age related diseases



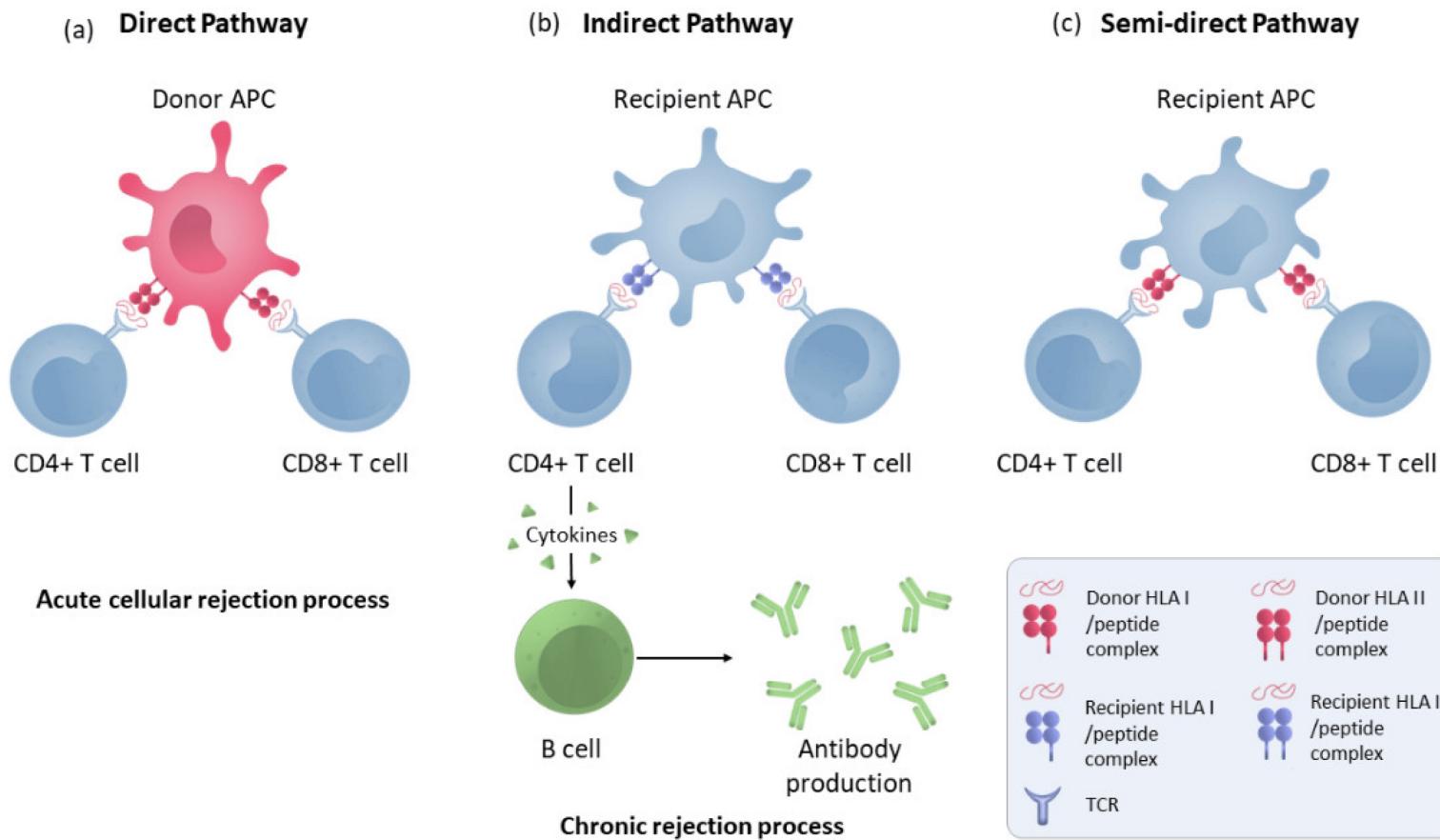
Personalized medicine



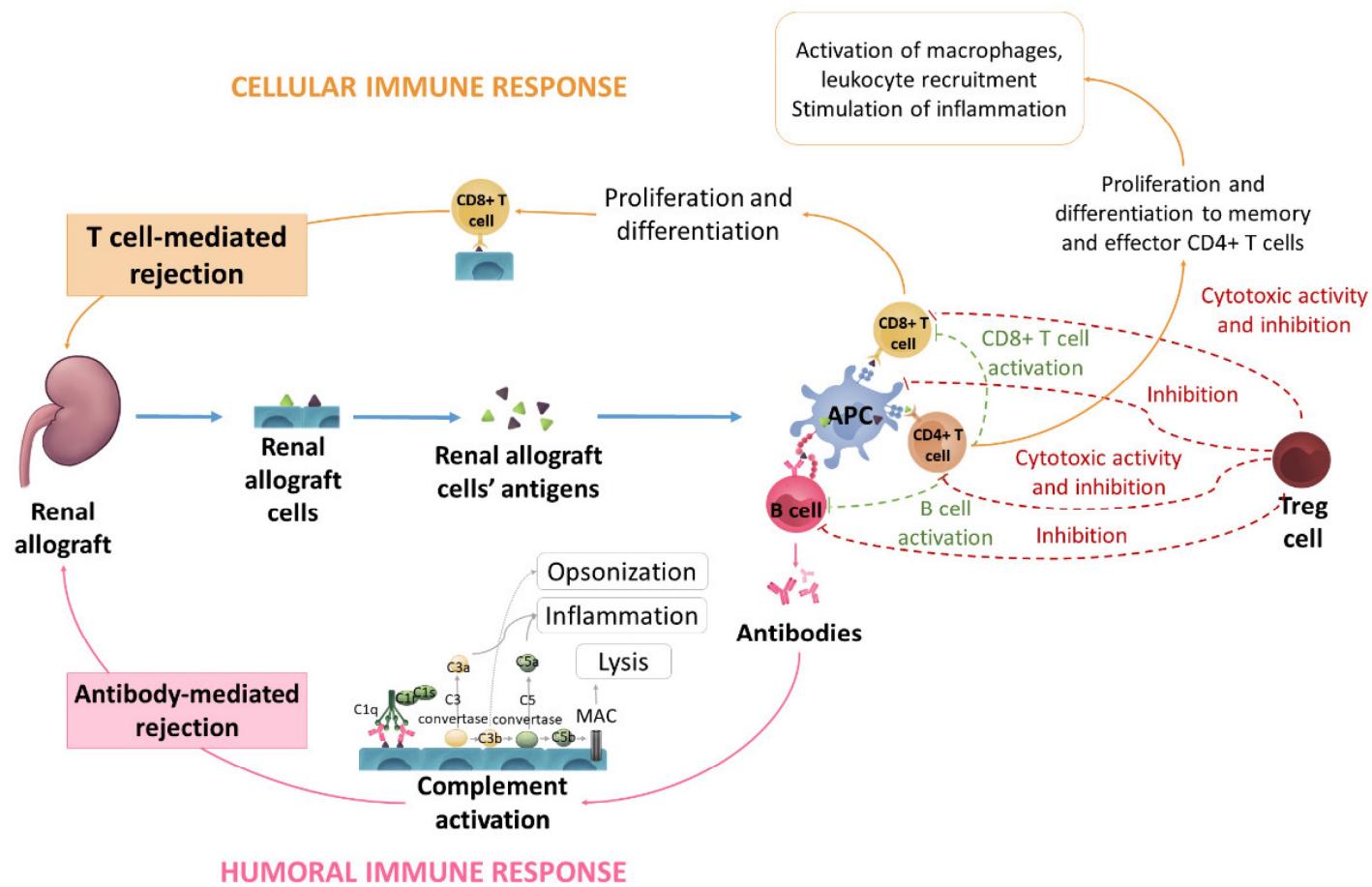
Personalized medicine



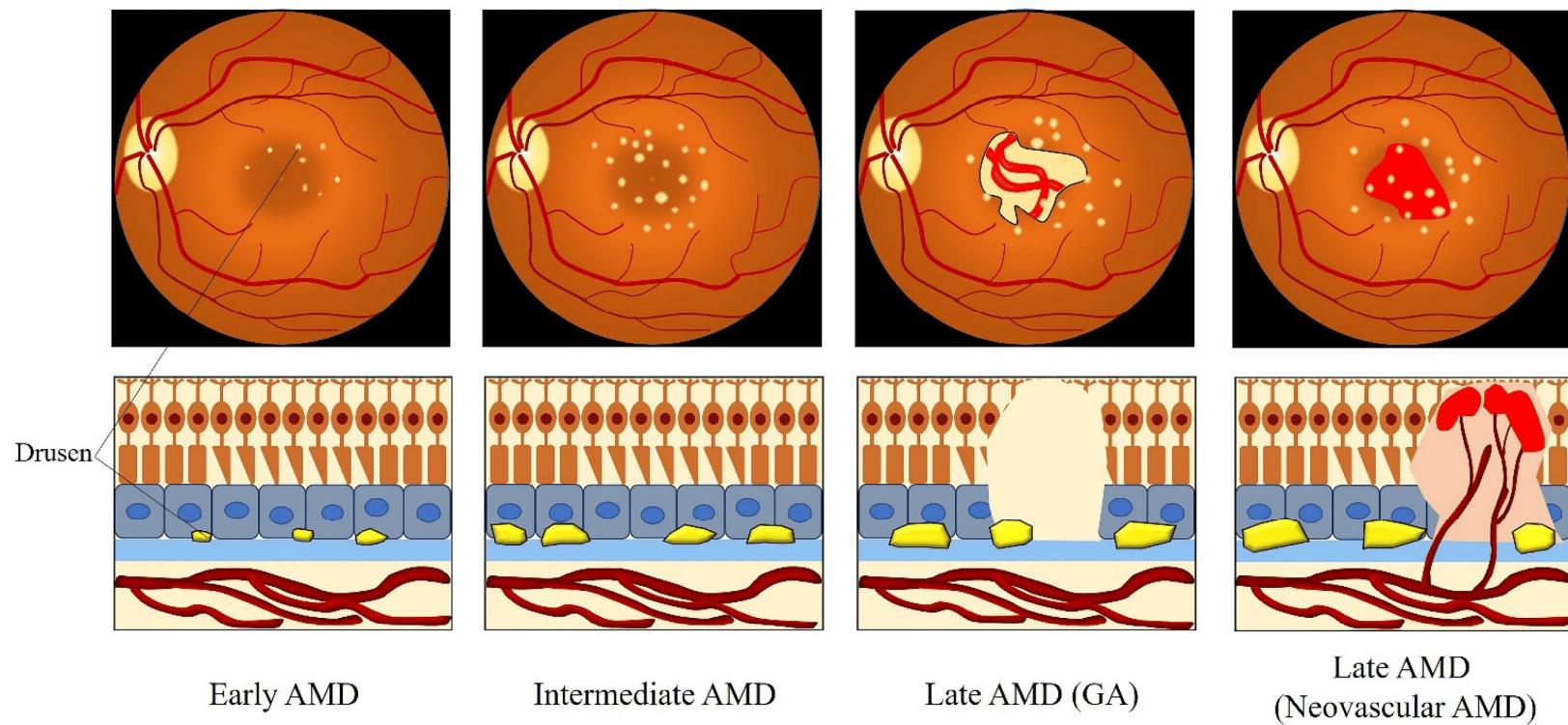
Immune incompatibility



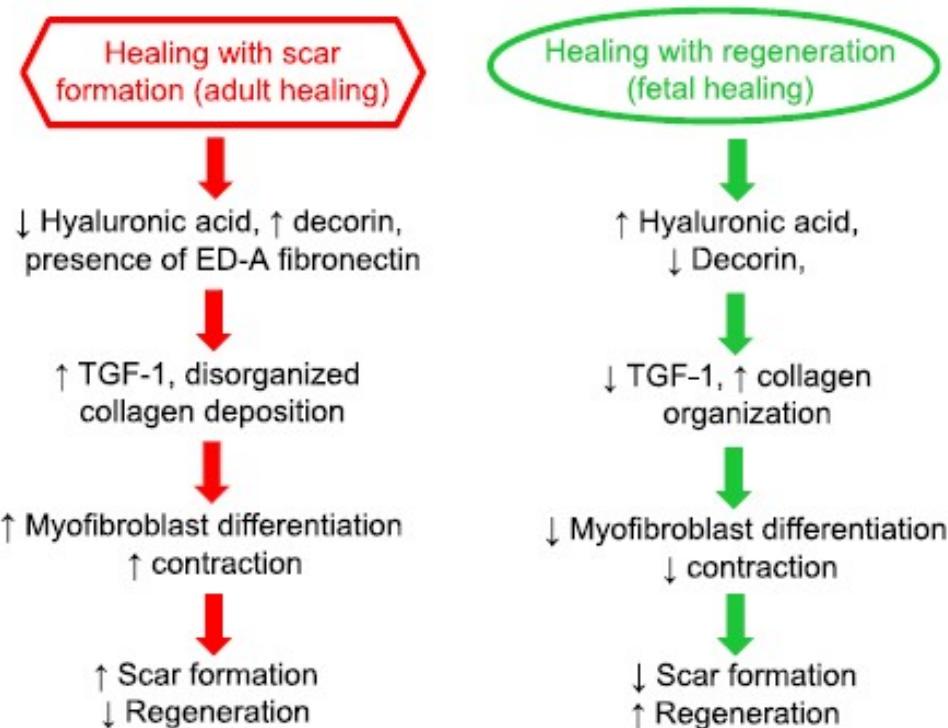
Immune incompatibility



Age related diseases

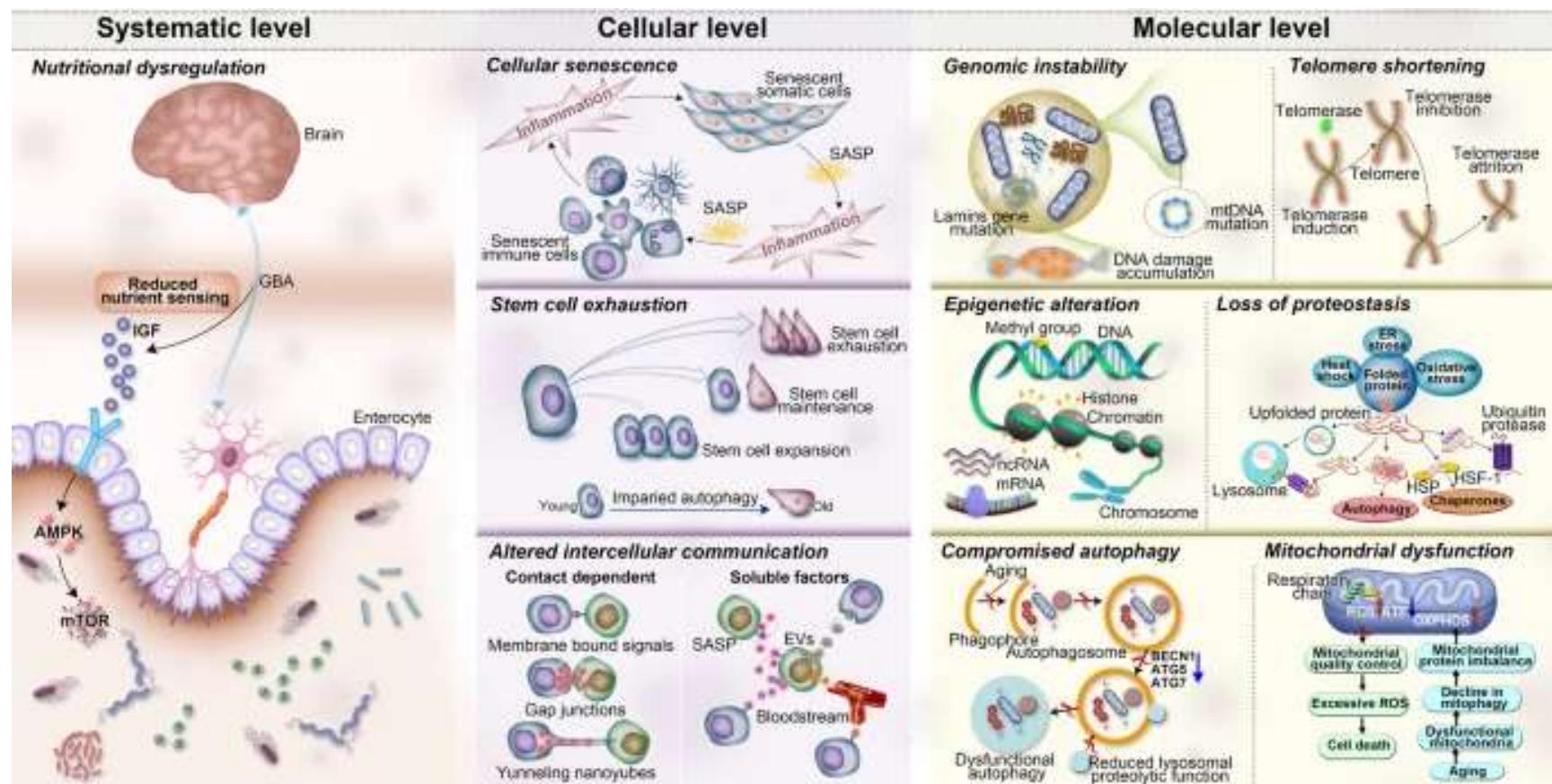


Wound healing-regeneration

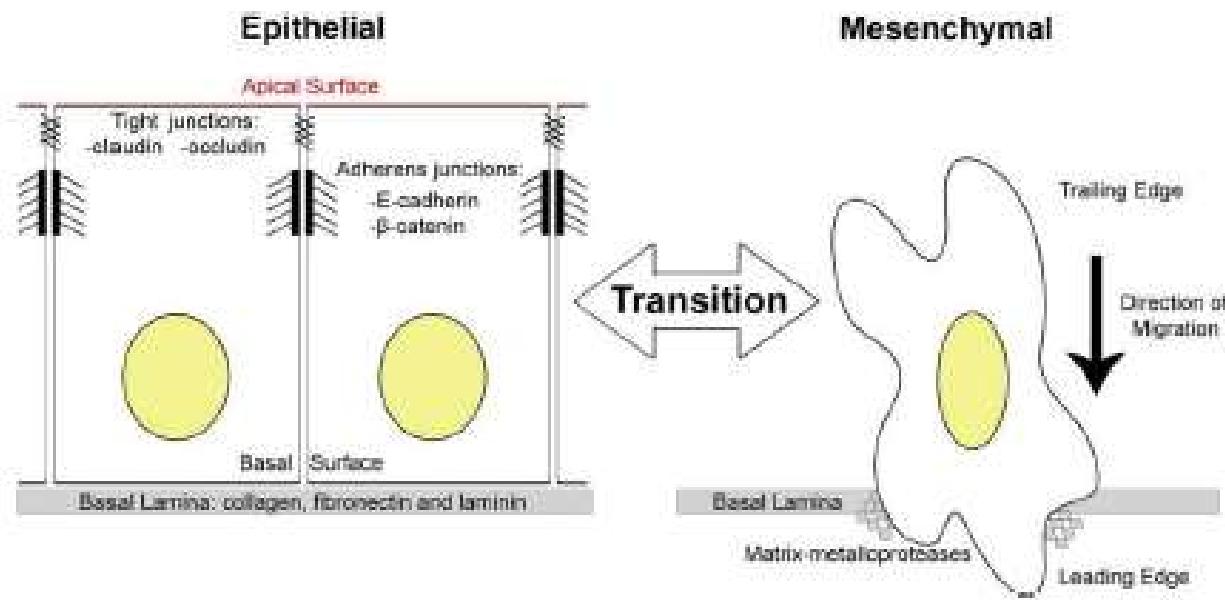


- Immune response
- ECM composition
- Signaling pathways
- Cell proliferation
- Differentiation abilities
- Tissue mechanical properties
- Apoptosis

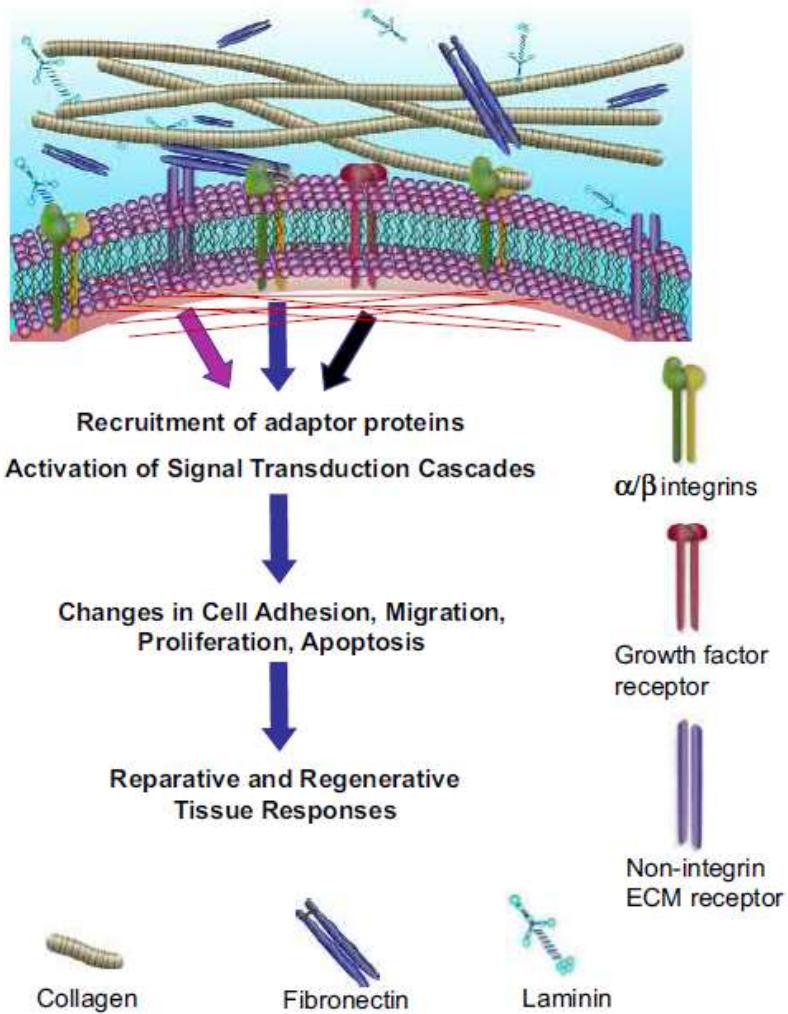
Aging



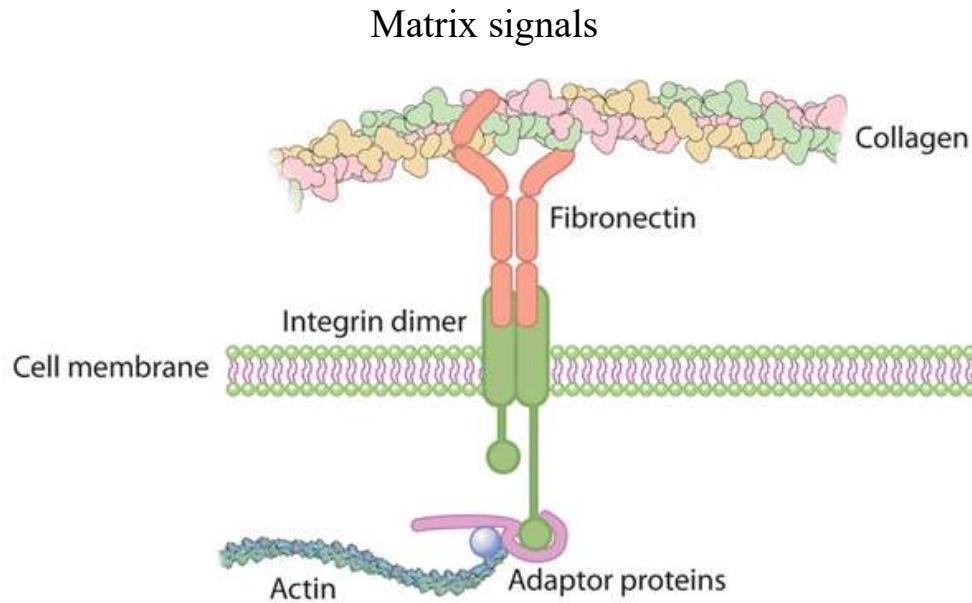
Cell types



Cell-ECM interactions

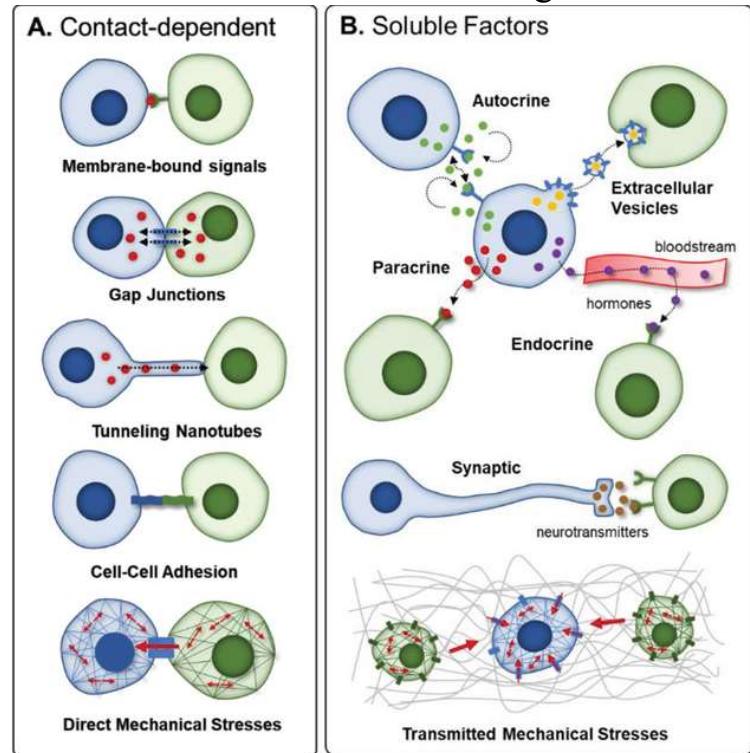


Cellular interactions with their environment



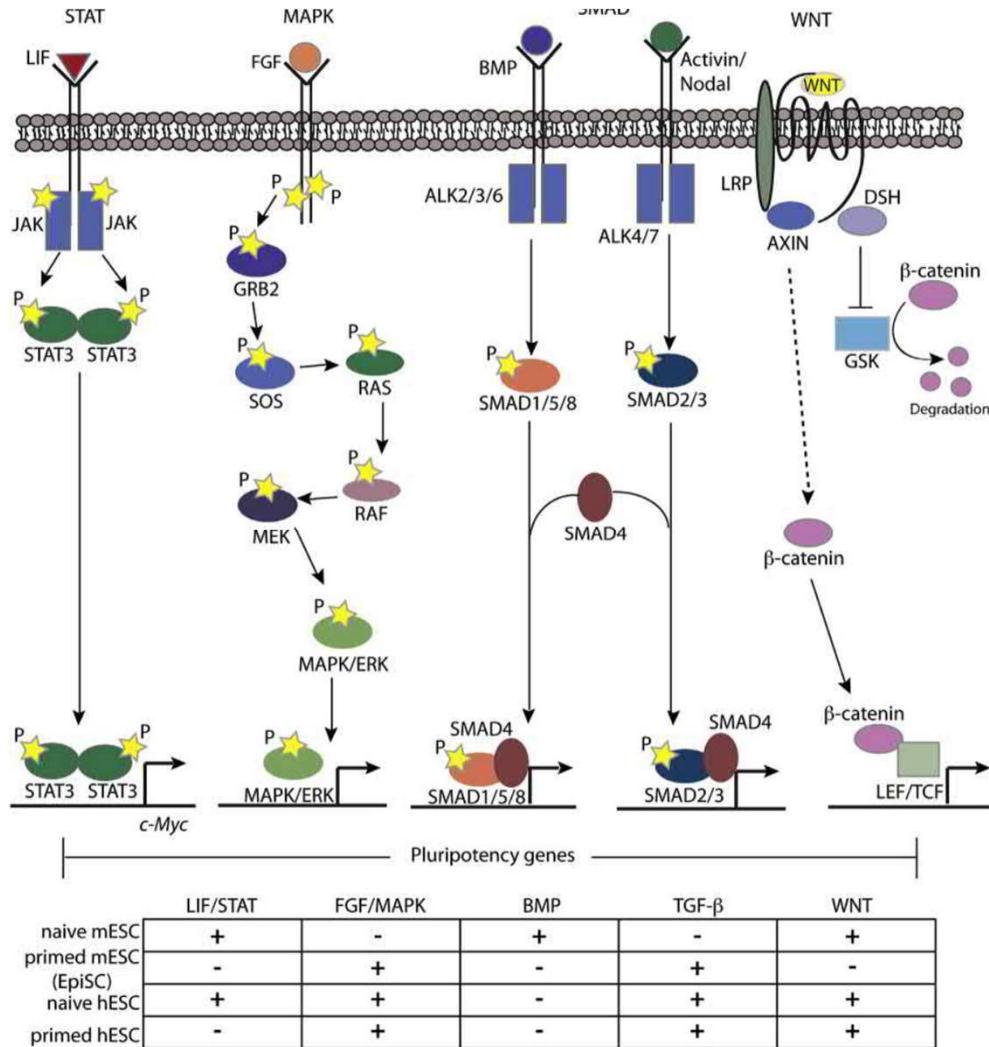
<https://www.nature.com/scitable/content/integrin-connects-the-extracellular-matrix-with-the-14707425/>

Soluble and intercellular signals



<https://doi.org/10.1002/advs.202002825>

Signaling pathways



Regeneration of the entire human epidermis using transgenic stem cells



Regeneration of the entire human epidermis using transgenic stem cells

History

The Early Years

- Early 1970s - Dr. W.T. Green, a surgeon at Children's Hospital Boston.
Experiments to generate new cartilage using chondrocytes seeded onto spicules of bone and implanted in nude mice.
Result – Unsuccessful
Conclusion - possible to generate new tissue by seeding viable cells onto scaffolds
- 1981 - Drs. John Burke and Iannas Yannos,
Tissue-engineered skin substitute using a collagen matrix to support the growth of dermal fibroblasts.
Patents [U.S. Pat. 4,418,691 (December 6, 1983)] granted to MIT
IntegraTM by Integra LifeSciences Corp

History

The Early Years

- 1983 - 1984 - Dr. Howard Green & Dr. Olaniyi Kehinde –
Test tube skin; Sheets of cultured keratinocytes transferred onto burn patients.
Formed a company - BioSurface Technology, later taken over by the Genzyme Corporation – Epicel® (cultured epidermal autografts).
- Dr. Eugene Bell seeded collagen gels with fibroblasts, referring to them as contracted collagen gels.
- 1991, a young patient with Poland syndrome first human to receive a tissue-engineered implant composed of a synthetic polymer scaffold implant seeded with autologous chondrocytes. Surgeons - Drs. J. Upton and J. and C. Vacanti.
- 1996, Integra's Artificial Skin was approved for as an in vivo, non-biological tissue regeneration product.
- 1998 - General and Plastic surgery approval of ‘Apligraf’, human skin equivalent for the treatment of venous leg ulcers.

Tissue engineering

Proliferation

Cells divide and form two daughter cells-cell cycle and Mitosis

Differentiation: - during development

A process by which the cells acquire a specialized structure and function in an organism. Differentiation is due to differential gene expression. Several signals (extrinsic or intrinsic) control the gene expression in a cell. The cells respond according to the nature of the signals (Qualitative, quantitative, spatial, temporal) as well as based on its cellular context and history.

Cell death:

Different forms of cell death regulate the tissue homeostasis as well as during injury or infection.

Apoptosis, necrosis, autophagy, necroptosis, pyroptosis and senescence.

Stem Cells

Stem cells are defined as cells with self-renewal and multipotent differentiation ability.

Thus, stem cells are functionally defined.

Why do we need stem cells: For repair and regeneration and regeneration is limited in higher organisms. Stem cells can be transplanted from one individual to the other.

Loss of stem cells: Degenerative diseases – neurodegenerative diseases, muscular degenerative diseases, macular degeneration, etc

Cells that can undergo unlimited population doublings without oncogenic transformation. Can be tested in the lab where the cells show extensive population doublings, commonly seen in embryonic stem cells. No replication arrest or senescence.

Telomere: How is it maintained- through telomerase-how is the gene activated?

Proliferation of adult stem cells are tested in vivo in experimental animal models by serial transplantation.

Has clonal propagation capacity.

Progenitor cells have differentiation capacity with limited proliferation ability

Differentiation ability: multiple lineages or multiple cell types of a lineage

Stem cells can be totipotent (?), pluripotent or multipotent

Stem cells can be isolated from embryonic, fetal or adult stage or iPSC

Thus, stem cells have a different set of gene activation mechanisms and gene expression profile. Molecular signature of stem cells

Stem Cells

What is the origin of stem cells:

- i. Uncommitted cells during development – occupy a niche to become stem cells
- ii. Committed cells occupy a particular niche to become stem cells

Stem cell niche might impose a lineage restriction on the stem cells

Stem cells identification:

Stem cells are identified based on their characteristics: that is, the functional characteristics

Embryonic stem cells: derived from the inner mass of the blastocyst

Self-renewal, multilineage differentiation in vitro and in vivo, clonogenicity, normal karyotype

Ability to give rise to all lineages including germ line

Teratoma formation, embryoid bodies formation, chimera formation after transplantation into non-human blastocyst

Expression of molecular markers of pluripotent stem cells

Adult stem cells

Clonal cell that self-renews and has ability to differentiate
transdifferentiation abilities (?)

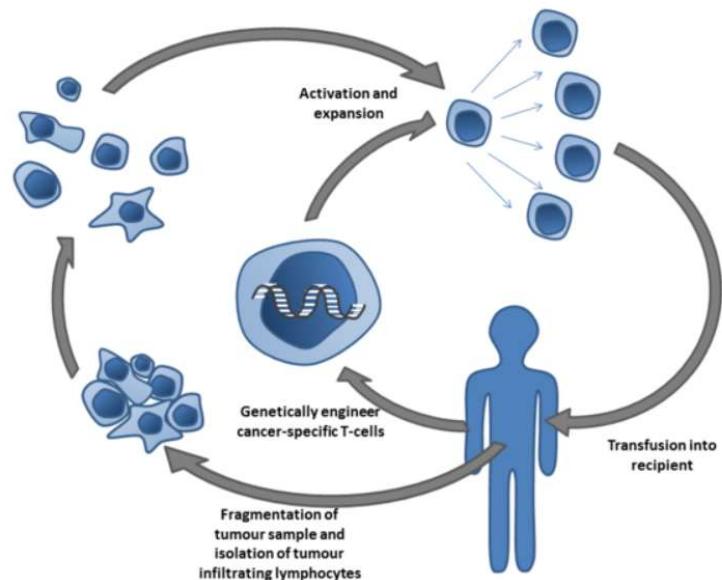
Reconstitution in the in vivo assay

Stem Cells

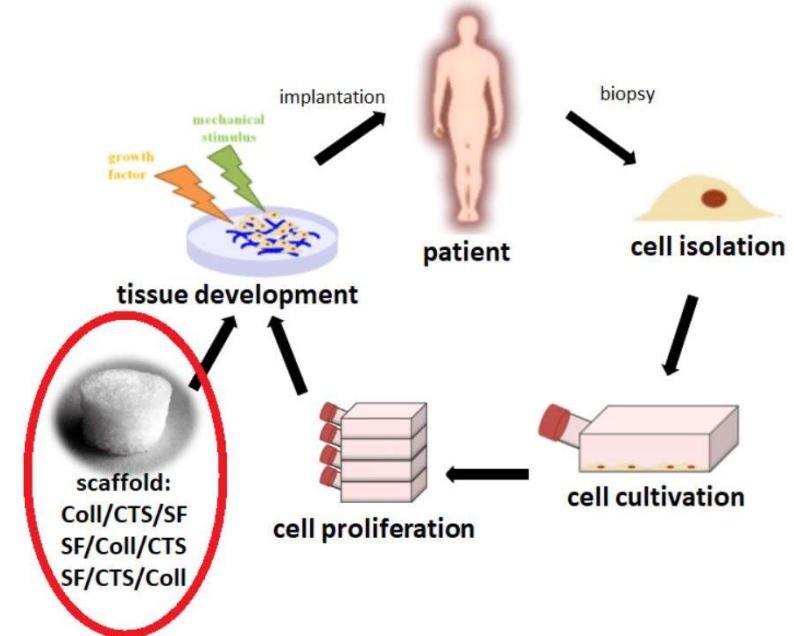
Characteristic features common to stem cells:

- Gene expression profile
 - Signal transduction pathways
 - Cell cycle regulators
 - Telomerase maintenance
 - Epigenetic profile
 - Transcriptional regulatory mechanisms
 - Multidrug resistance
 - Protein folding machinery
 - Ubiquitin system
- Quiescence
- Hierarchical arrangement
- Asymmetric/symmetric cell division

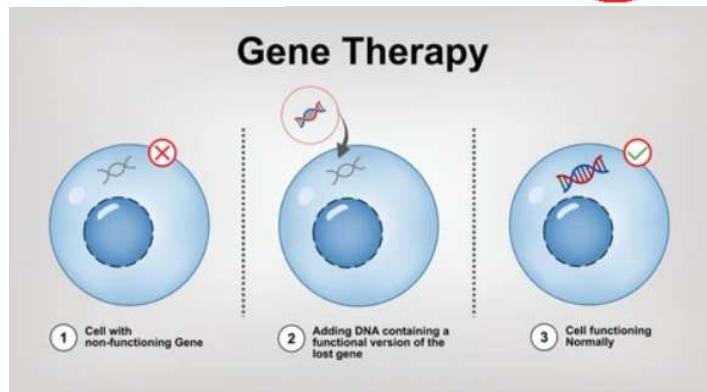
Techniques in regenerative medicine



Cell Therapy

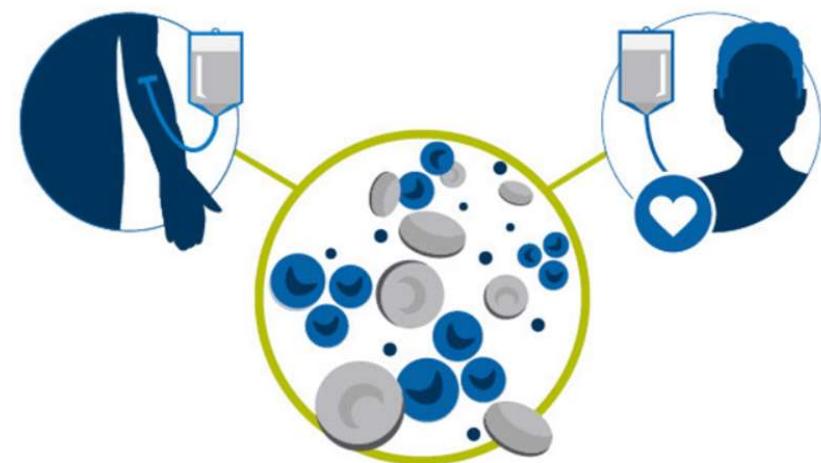


Tissue Engineering



Gene Therapy

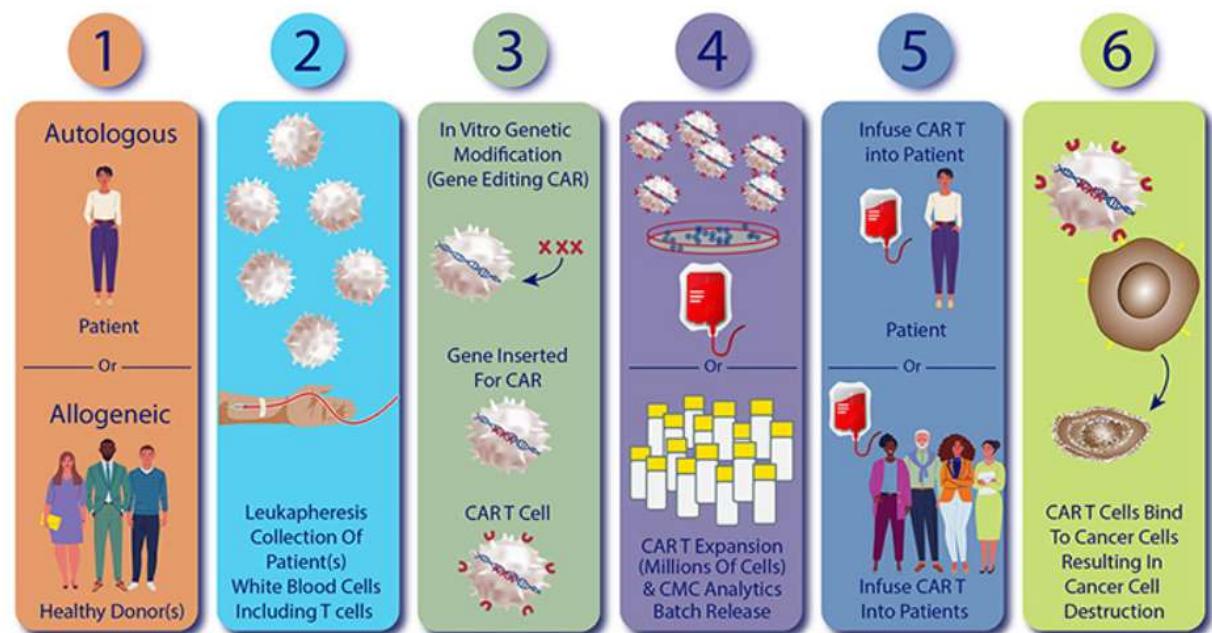
CELL THERAPY



<https://www.drugdiscoverytrends.com/how-first-generation-outcomes-data-can-advance-future-cell-therapies/>

1. Cells are isolated from donors.
2. Isolated cells are cultured manipulated, and expanded.
3. Cells are then transplanted into the patient.

CELL THERAPY PROCESS OVERVIEW



<https://www.eurofins.com/biopharma-services/product-testing/services-by-modality/cell-therapy-testing-services/>

CAR-T cell therapy

Cell therapy cont.

Autologous cell therapy are used :

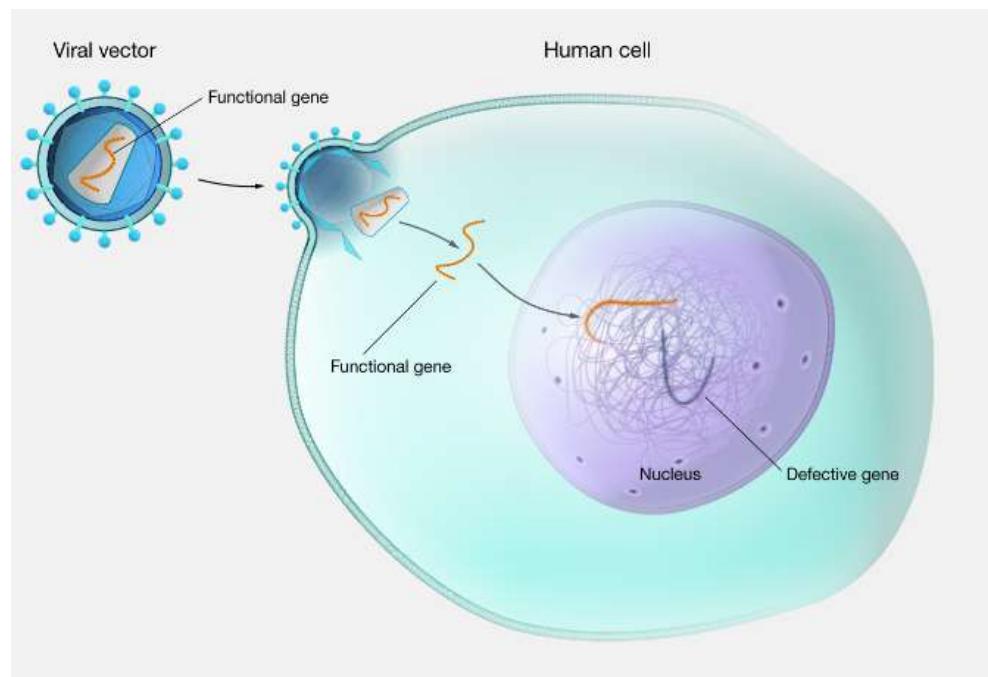
1. Malignancy in which disease free bone marrow is preserved before the massive myeloid ablation that occurs after radiotherapy, these bone marrow stem cells can also be collected and genetically corrected, for later reinfusion into a patient.
2. Large burn on the skin – This was made possible by the discovery that stem cells from the epithelial can be clonally expanded in culture. This procedure can be used to generate large sheets that are transplanted on same patient to cover the surface previously burnt.

Gene therapy

- Gene therapy is based on delivery of genes as medicines for a growing variety of clinical indications.
- Gene therapy aims to correct a genetic defect in a given cell type, to restore function, or to provide novel function.
- Gene therapy could be used to provide cells with a novel function for a specific goal (eg, express antigens that enhance immunogenicity of cancer cells or enhance lymphocytes with mechanisms to kill tumour cells). more efficiently).
- Gene therapy is divided into two main categories: in vivo, in which genes (or their products) are introduced directly into a patient's cells, and ex vivo, in which a patient's cells are grown in culture, genetically modified, and then reintroduced into the body.
- Genes—or more commonly their cDNA—are very large, highly hydrophilic, and electrically charged molecules that do not cross the cell membrane by themselves. For this reason vectors are used, mainly viral vectors.

Gene therapy Cont.

- Viral vectors currently used in patients are –
 1. Adenovirus
 2. Adeno-associated virus
 3. Lentivirus
 4. Retrovirus
- Adenovirus and adeno associated viruses are mainly used *in vivo* in patients. Retroviral vectors are mainly used *in ex vivo* gene therapy.
- Although adenovirus vectors can accommodate large cDNAs, they are highly immunogenic. For this reason they are mainly used in cancer gene therapy in which immunogenicity will enhance the host immune response—something to be avoided in the long term correction of genetic defects, for which adeno-associated viruses are the vectors of choice.



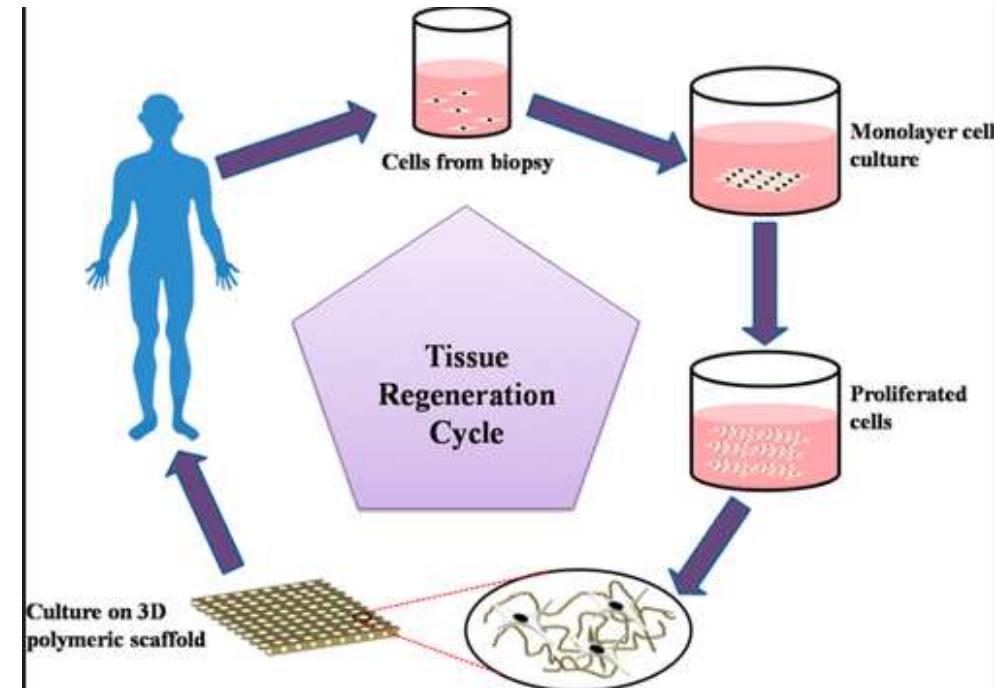
<https://www.genome.gov/genetics-glossary/Gene-Therapy>

Tissue engineering

- Tissue engineering combines the principles of cell transplantation, material science, and bioengineering to develop new biological substitutes that may restore and maintain normal organ function. Tissue engineering strategies generally fall into two categories:

- (i) the use of acellular matrices, which serve as guides for proper orientation and direction of new tissue growth but depend on the body's natural ability to regenerate, and
- (ii) the use of the matrices seeded with cells.

Acellular tissue matrices are usually prepared by manufacturing artificial scaffolds, or by removing cellular components from tissues via mechanical and chemical manipulation to produce collagen-rich matrices.



<https://doi.org/10.1021/acs.iecr.8b05334>

Components of tissue engineering

3 main components

- Cells – in vivo and in vitro
 - Autologous
 - Allogenic
 - Xenogenic
 - Progenitor cells

Signals – From tissue microenvironment such as

- Growth factors
- Cytokines
- Transcription factors
- Mechanical stimulation
- Nutrients

Scaffolds – Synthetic and Natural

- ECM components (cell and tissue derived)
- De-cellularized scaffolds
- Synthetic polymers
- Hydrogels
- Composite scaffolds

Sources of cell for tissue engineering

Tissue specific cells

- Chondrocytes for cartilage
- Osteocytes for bone
- Schwann cells for nerves
- Fibroblasts for ligament and tendon engineering

All these have significant proliferative potential in vitro

- Adult cardiomyocytes, hepatocytes, and adipocytes

Challenges

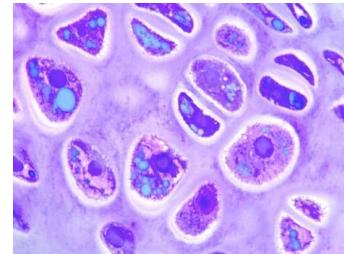
Difficult to culture and expand in vitro

Collection of cells – biopsy; uncomfortable and impossible due to diseased state of the tissue

Solution

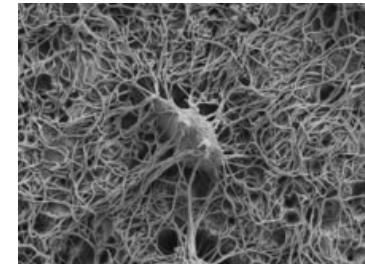
Utilize stem cells; expanded and differentiated ex vivo.

Chondrocytes



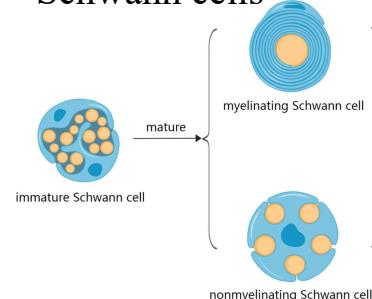
https://medcell.org/histology/connектив_tissue_lab/chondrocytes.php

Osteocytes



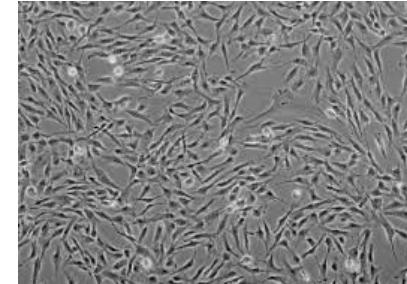
<https://en.wikipedia.org/wiki/Osteocyte>

Schwann cells



<https://doi.org/10.3389/fncel.2023.1228282>

Fibroblast

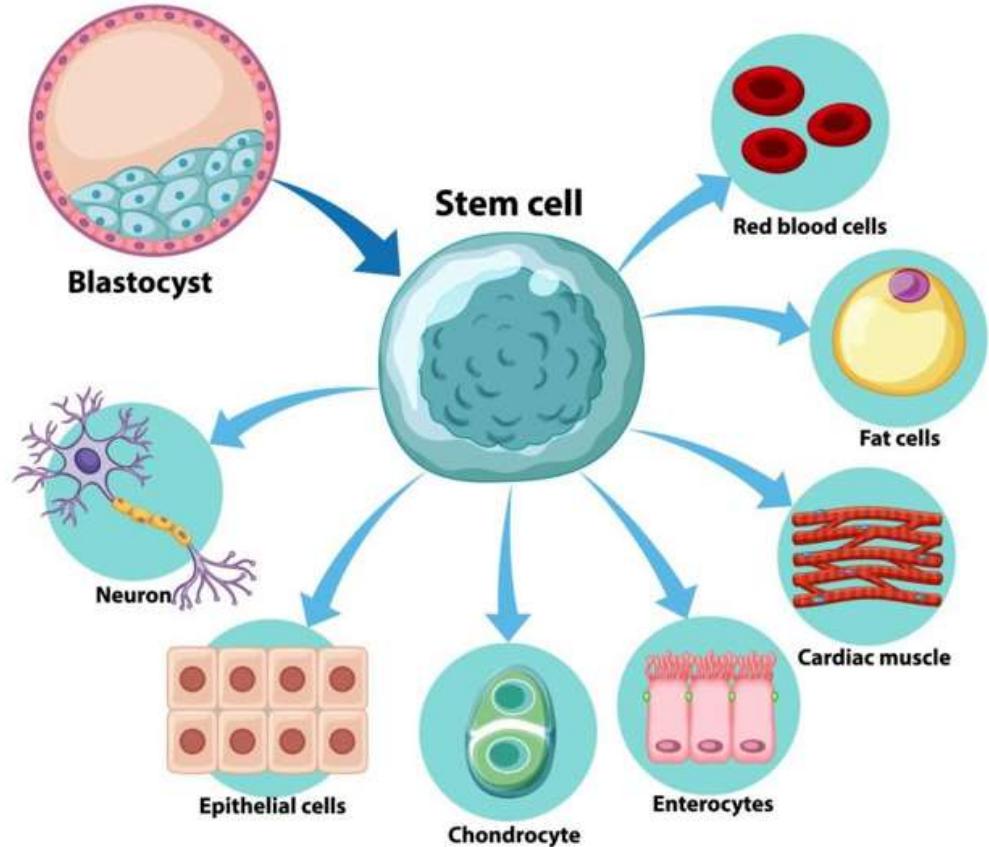


<https://doi.org/10.5812/jssc.69080>

Sources of cell for tissue engineering

Stem cells

- Stem cells are the unspecialized cells which are able to self renew and differentiate into mature cells (potency).
- Stem cells are cells that have the ability to branch out and change, or differentiate, into two or more different cell types, ability to develop into functional, differentiated cells.
- In general, there are two broad categories of stem cells: adult stem cells and embryonic stem cells. Artificial stem cells called induced pluripotent stem cells (iPSCs) also exist.

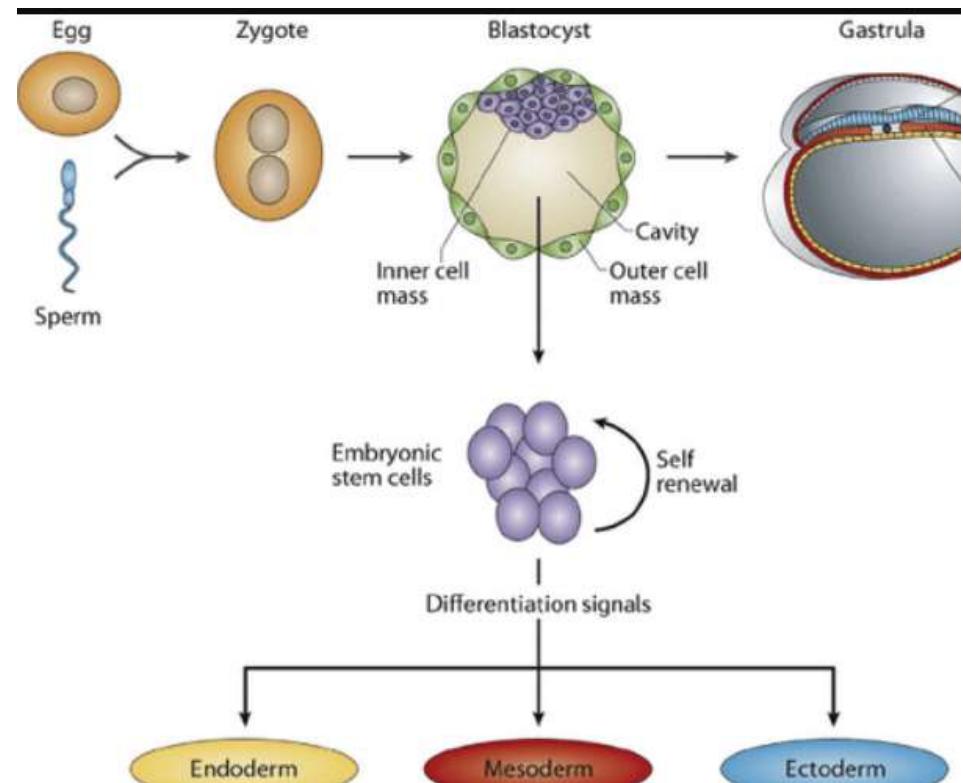


<https://www.geeksforgeeks.org/stem-cell/>

Sources of cell for tissue engineering

Embryonic stem cells

- Embryonic stem cells are a group of cells that are present in the inner cell mass of blastocyst.
- These cells are pluripotent, meaning they can develop and differentiate into all the cell types of the three germ layer.
- Due to their ability to differentiate into various cell types, embryonic stem cells hold promise for regenerative medicine.
- The use of embryonic stem cells has been a subject of ethical debate because their derivation typically involves the destruction of embryos.

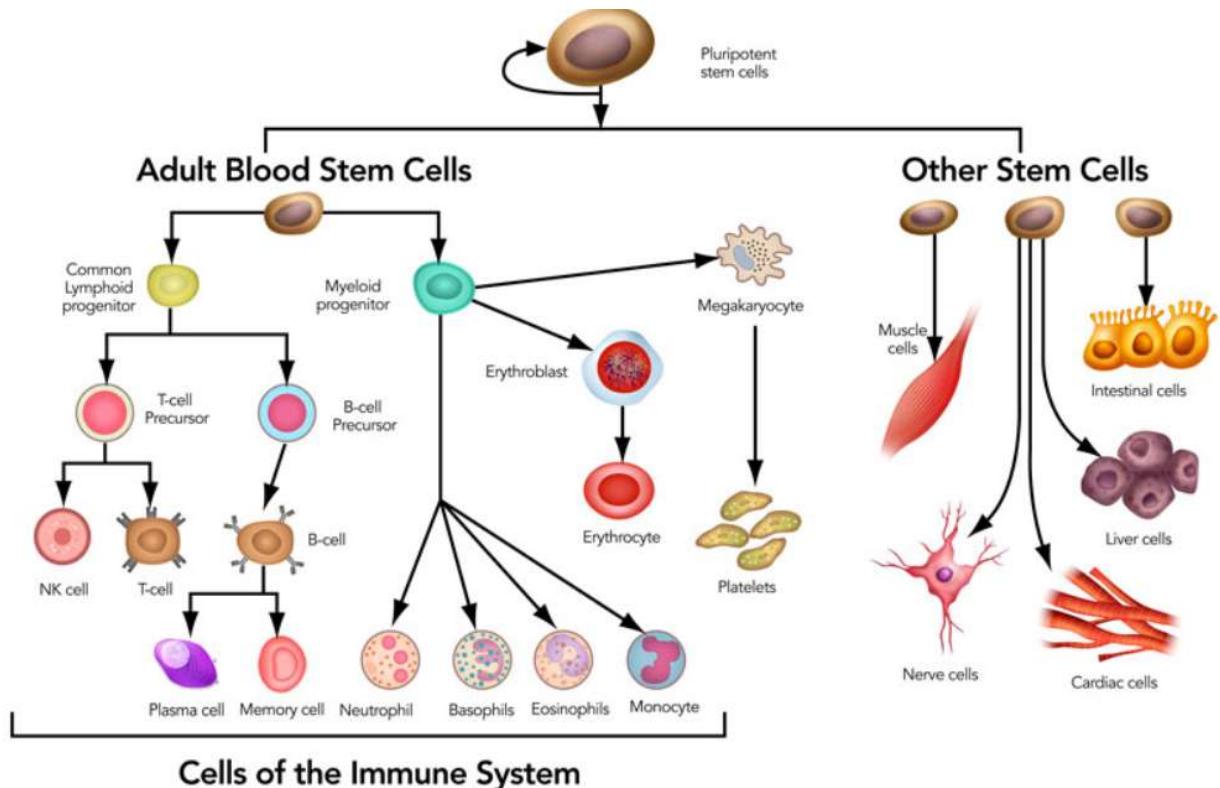


DOI: 10.1016/bs.pmbts.2015.07.005

Sources of cell for tissue engineering

Adult stem cells

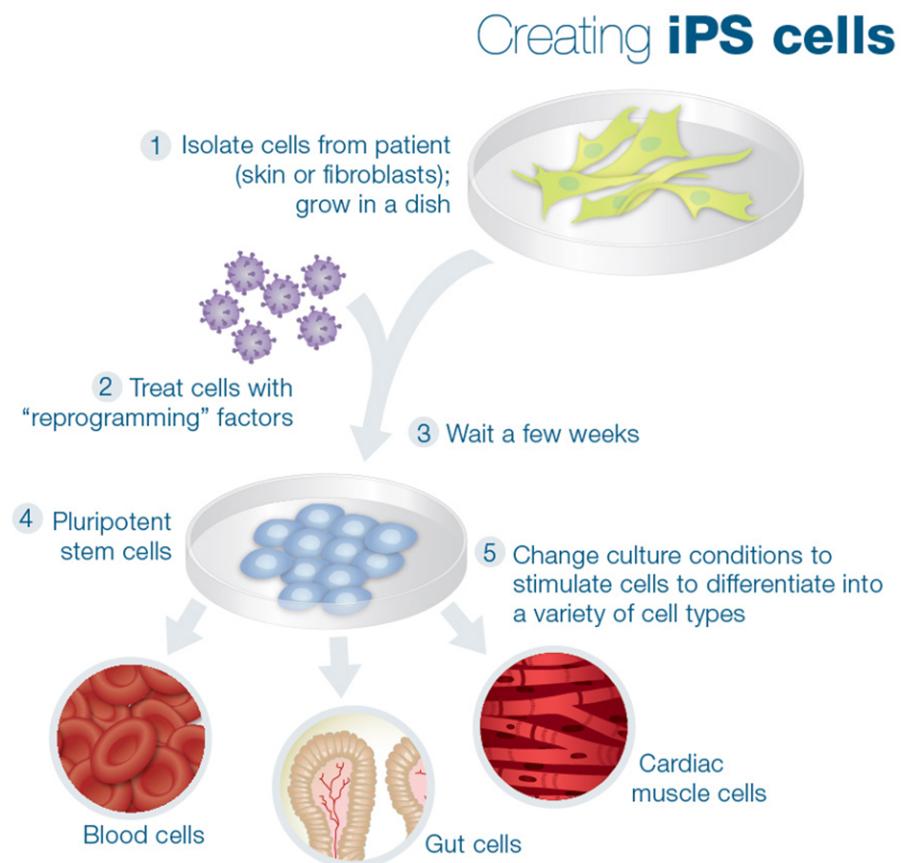
- Adult stem cells are derived from various tissues in the human body.
- Because they can branch out into many different cell types, they are referred to as “multipotent.”
- These cells are considered less potent than embryonic stem cells as they cannot differentiate to all the cell types of the three germ layer.
- Adult stem cells exist in niches or areas created by other cells which secrete fluids and nutrient for the stem cells to remain alive on.



<https://microbenotes.com/stem-cells/>

Sources of cell for tissue engineering iPSCs

- iPSCs are created by reprogramming adult cells using specific factors to induce a pluripotent state. This resets the adult cell and reverts it back to a more embryonic-like state.
- Four transcription factors initially used were Oct4, Sox2, Klf4, and c-Myc.
- iPSCs have the potential to differentiate into cells from all three germ layers.
- iPSCs can be derived from a patient's own cells, such as skin cells, allowing researchers to generate cells and tissues that genetically match the patient.
- One of the significant advantages of iPSCs is that their creation does not involve the destruction of embryos, thereby circumventing the ethical issues associated with the use of embryonic stem cells.



<https://www.ipsc21.com/ipscs/>

Sources of cell for tissue engineering

Challenges associated with adult stem cells

Advantages

- An autologous and/or non-immunogenic source of cells.

Limitations

- Patient-to-patient variations in their prevalence, proliferative capacity, and differentiation potential
- Additionally, their utility is also a factor of age and disease state of the donor
- Exit the cell proliferation cycle (prematurely senesce) or prematurely lose differentiation potential during ex vivo expansion

Challenges associated with iPSCs

- Not immune privileged requiring collection, induction, expansion, and differentiation of autologous cells.
- Costly and time-consuming

Cellular interactions with their environment

- Many cell types are exquisitely sensitive to stimuli present in the environment.
- **Soluble signals:** Soluble biomolecules eg: Metalloproteins, growth factors, chemokines, play vital roles locally and systemically in repair and tissue development.
- **Matrix signals:** Cells also possess receptors such as integrins and syndecans that bind to a variety of ligands present in the extracellular matrix (ECM). The ECM binds and sequesters growth factors that also drive cellular function.
- **Intercellular signals:** Cells also interact with neighboring cells in both native tissue and in ex vivo culture.

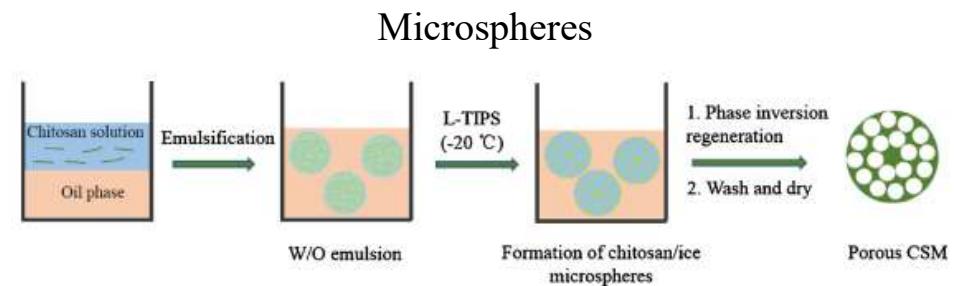
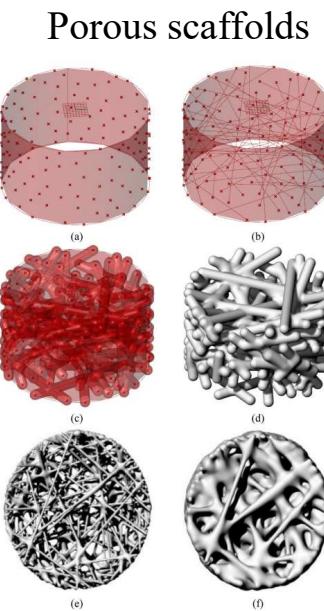
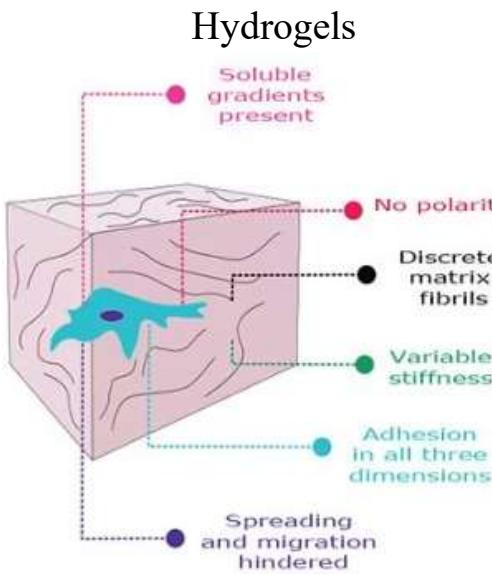
Two main methods:

- Direct contact via receptors such as cadherins
- Soluble signaling through paracrine factors

- Co-culturing cells with other types prior to implantation can facilitate their survival and function.

E.g. MSCs for paracrine effects or for their ability to incorporate into developing tissues *in vivo* like blood vessels

Mechanics and structure of the environment



<https://doi.org/10.1016/j.carbpol.2018.09.021>

<https://www.sigmaaldrich.com/IN/en/technical-documents/technical-article/cell-culture-and-cell-culture-analysis/3d-cell-culture/3d-hydrogels>

<https://doi.org/10.1016/j.matdes.2022.111467>

Mechanics and structure of the environment

- Cells respond to the mechanical properties and dimensionality of their environment. Response of cells to environmental stiffness and dimensionality is a tool that can be used to direct cell function.
- E.g. chondrocytes change morphology and lose their chondrogenic capacity in 2D monolayer culture but can maintain these features in 3D culture
- 3D cultures on biomaterial supports such as
 - Porous scaffolds
 - Hydrogels and
 - Microspheres
- Development of new biomaterials with tailored properties and direct the fabrication of these materials into three-dimensional scaffolds to maximize the healing process