Co-funded by the
Erasmus+ Programme
of the European Union

EC2U Summer School

Transgenerational Aging & Gendered Life-Cycle Approach

18 - 25 September 2022

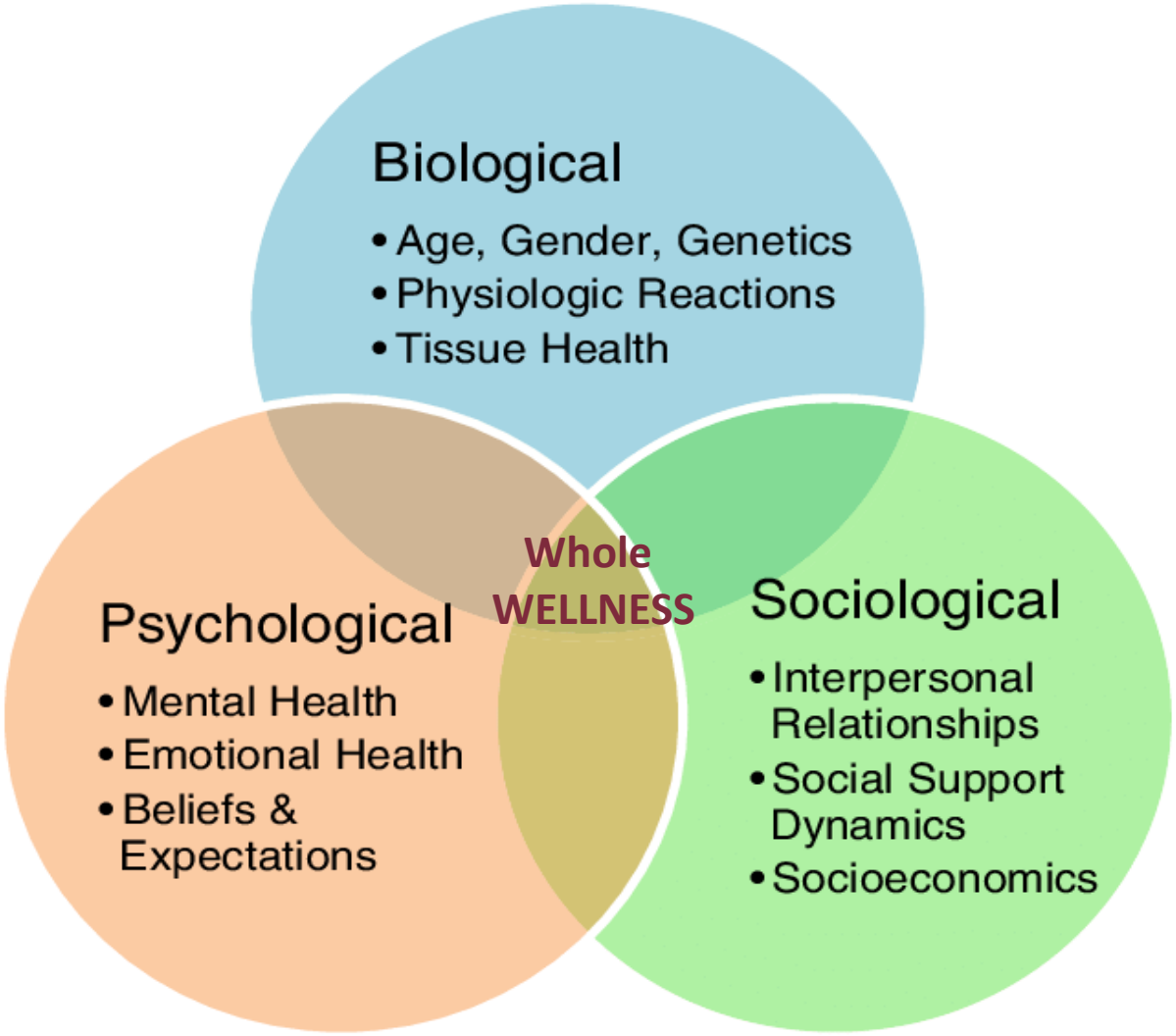
University of Pavia
Pavia (Italy)

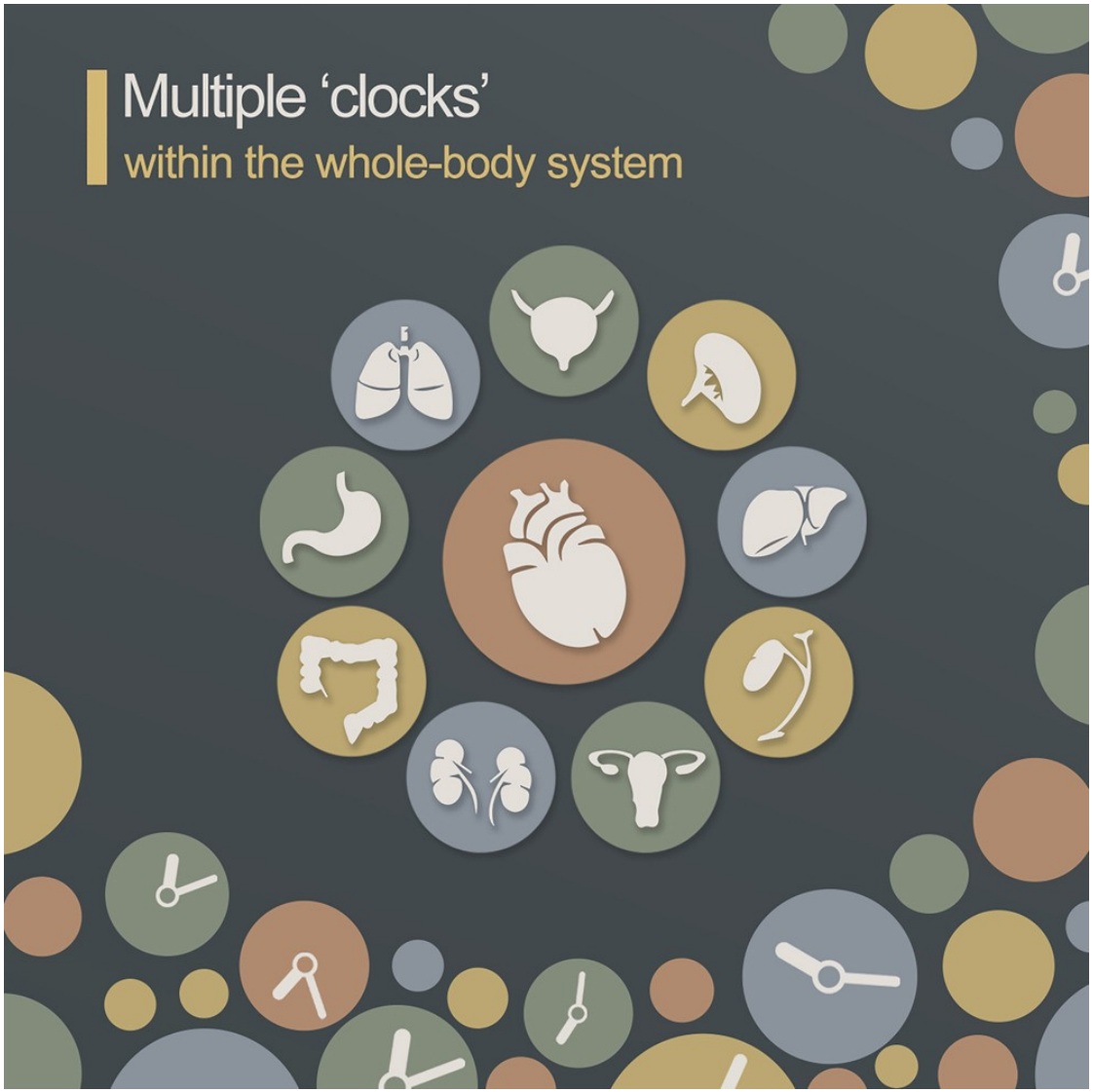
Regenerative Medicine: where do we stand?

Federica Riva & Manuela Monti

BIO-PSYCHO-SOCIAL MODEL

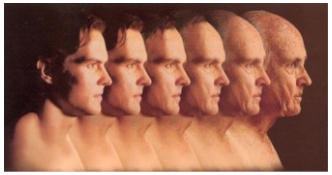
[Engels, 1977; Schwartz, 1982; Blascovich et al., 2004]





Volume 38, Issue 10, 8 March 2022

Longevity
Aging
Chronic deseases



Brain

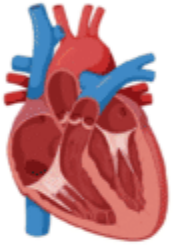
- Brain Volume [5]
- Deterioration of Myelin Sheath [12]
- Temporal Lobe [9]
- Hippocampus Volume [9]

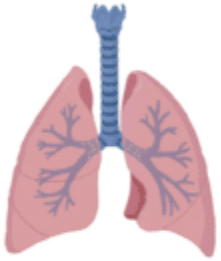
Degenerating Myelin Sheath

Healthy Myelin Sheath

Heart

- Reduced cell number of cardioyocytes and sinoatrial pacemaker [53,54]
- Decreased Strength and Elasticity of Cardiac walls [53,54]





Lungs

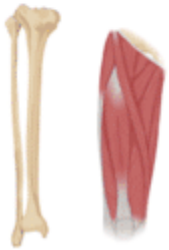
- Reduced Cough Strength [35,36]
- Reduced ability of cilia lining (upper and lower) [37]
- Decrease in Alveolus Elasticity [38-39]
- Increase in Alveolus size [38-39]

Common Features across Organs

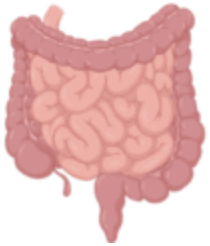
Decreased Cell Number and Function
Change in Tissue Structure
Increased Chronic Inflammation

Musculoskeletal

- Increased Brittleness: change in bone mineral density and protein matrix ([20-22]
- Decline in Muscle mass and formation [24-25]
- Decrease of fast myosin fibres
- Accumulation of fat tissues [27-29]

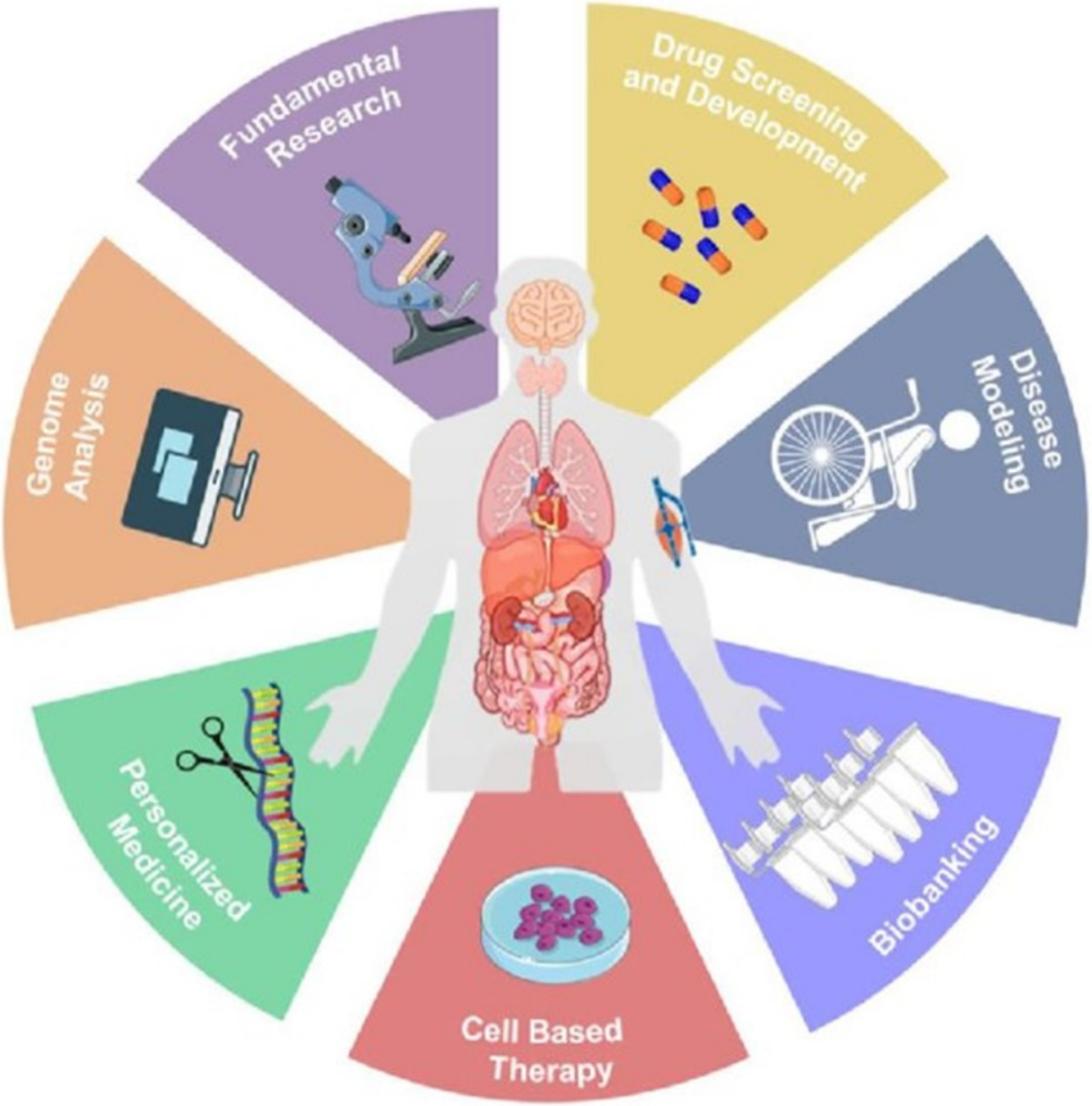


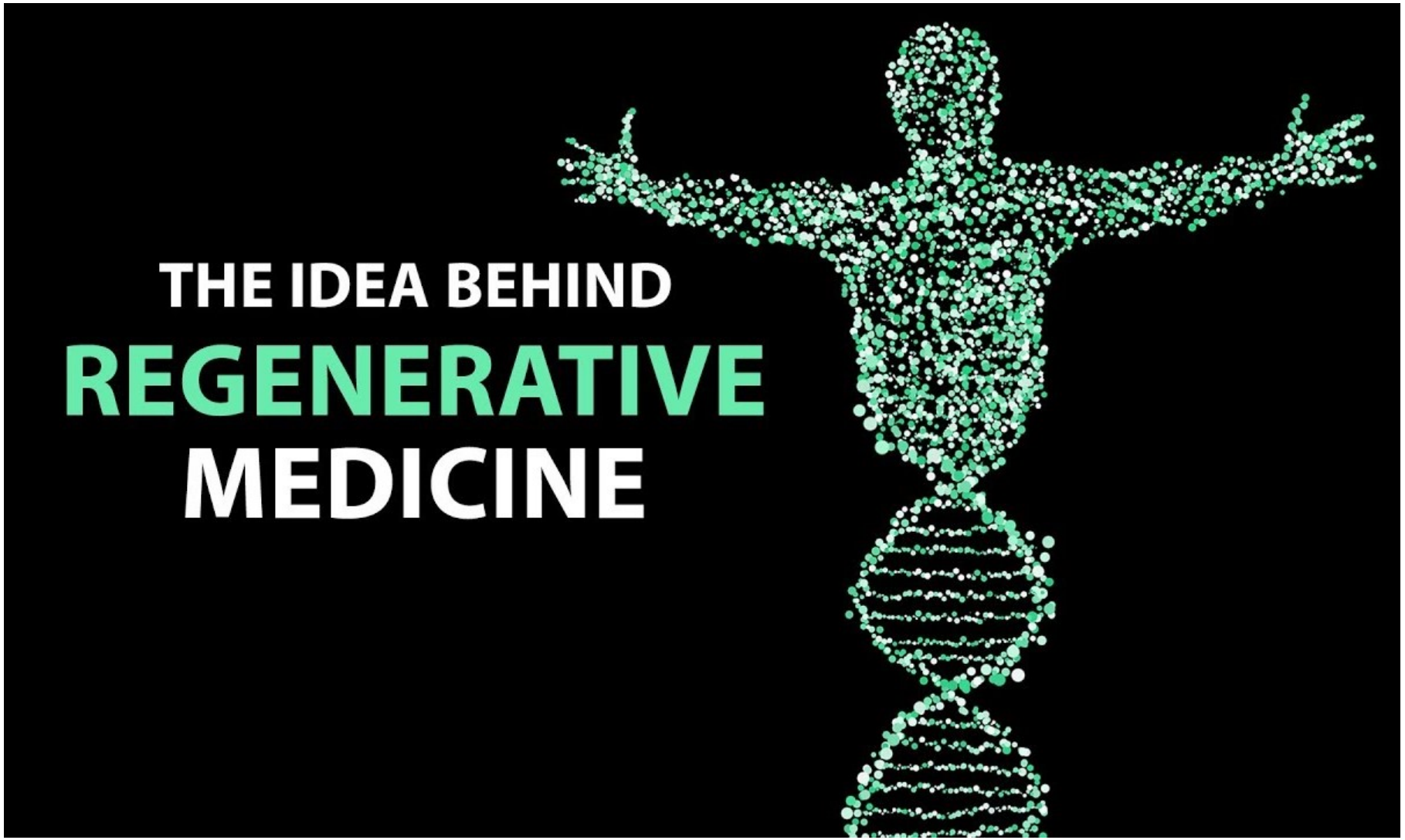
Gastrointestinal



- Decreased Microbiome Diversity [46,47]
- Reduced Gut Motility [48]
- Loss of Intestinal Barrier Integrity [48]

Seminars in Immunopathology 2020 42(5):559-572


Fundamental Research
Drug Screening and Development
Genome Analysis
Disease Modeling
Personalized Medicine
Biobanking
Cell Based Therapy


THE IDEA BEHIND
REGENERATIVE
MEDICINE

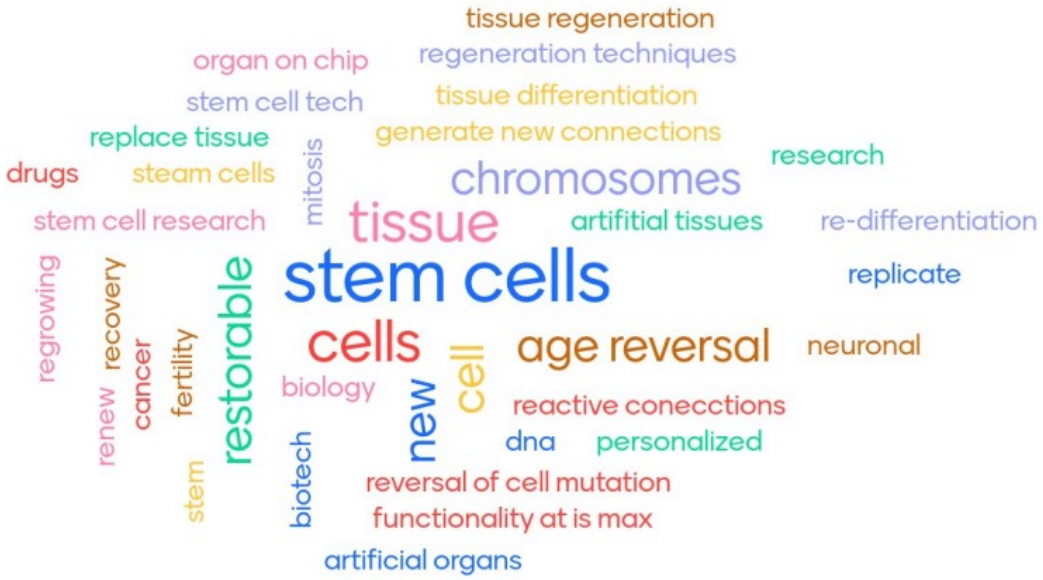
Online Question

https://www.menti.com

the voting code 3249 7119

3 terms or concepts that, in your opinion, refer to REGENERATIVE MEDICINE

Mentimeter



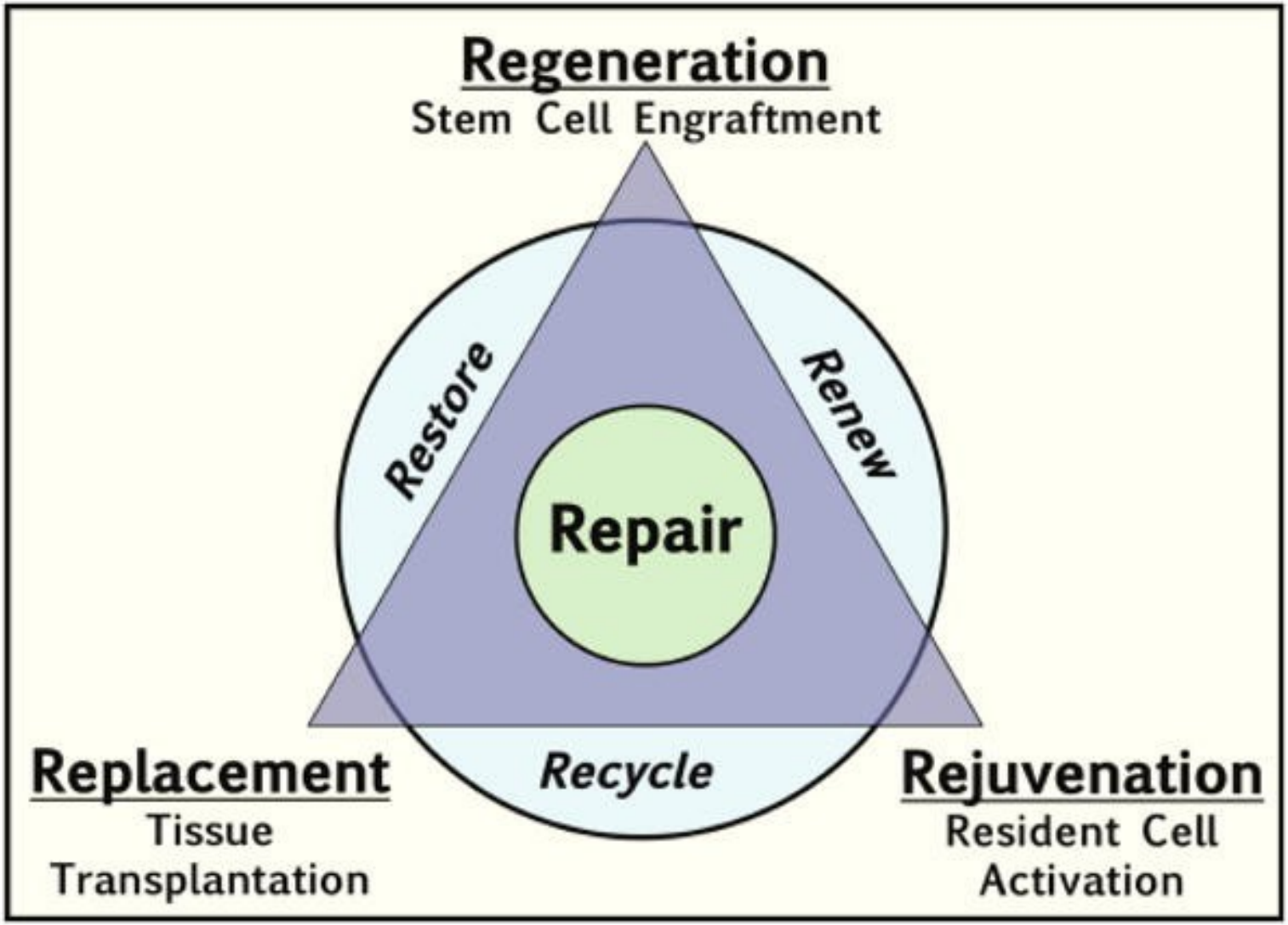
REGENERATIVE MEDICINE (RM)

"Regenerative medicine is an emerging interdisciplinary field of research and clinical application focused on the repair, replacement or regeneration of cells, tissues or organs to restore (using the natural ability of human body to heal itself) impaired function resulting from any cause, including congenital defects, diseases, trauma and aging."

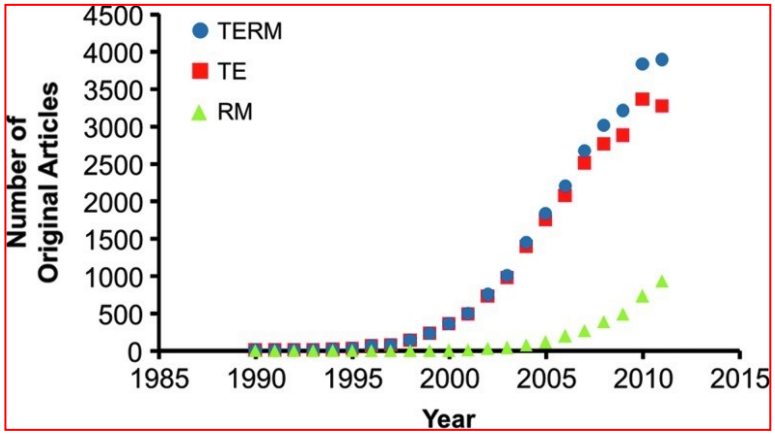
W. Haseltine 1999

Greenwood H. et al. *Int. J. Biotechnol*. 8, 60–77 (2006)

REGENERATIVE MEDICINE PARADIGM R^3



Clin Transl Sci. 2008 Sep 10; 1(2): 168–171



The rise of tissue engineering (TE) and regenerative medicine (RM) (=TERM). (Results obtained via Scopus® search using key words “tissue engineering” OR “regenerative medicine”). Color images available online at www.liebertpub.com/teb.

National Library of Medicine
National Center for Biotechnology Information

Posta in arrivo (249)... Maps Università degli Stu... Home page di Micr... zoom LT BIOTECH Pagina di accesso Argo - Famiglia

PubMed.gov

REGENERATIVE MEDICINE 2022 Search

Advanced Create alert Create RSS User Guide

Save Email Send to Sorted by: Best match Display options

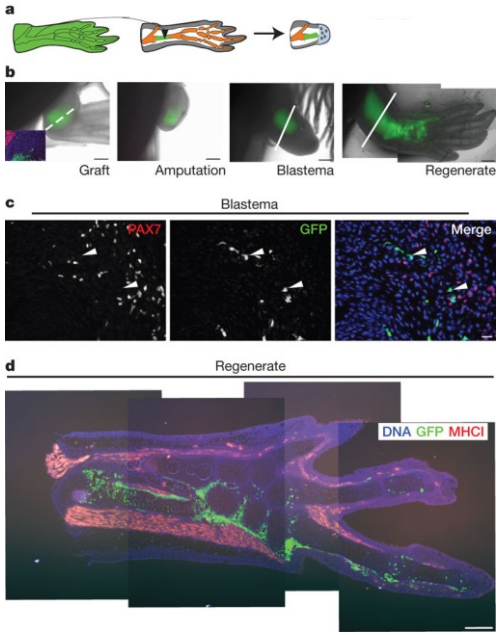
MY NCBI FILTERS 86,494 results Page 1 of 8,650

RESULTS BY YEAR

Regenerative medicine, organ bioengineering and transplantation.
PMID: 27616171 Review. Japanese. No abstract available.
Share

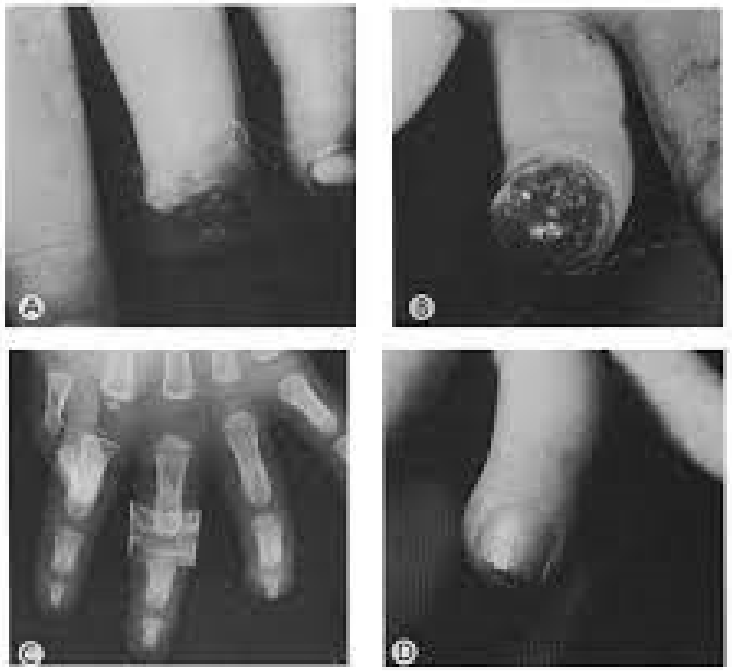
Tissue engineering and **regenerative medicine**: history, progress, and

A salamander can regenerate an amputated limb after several days…

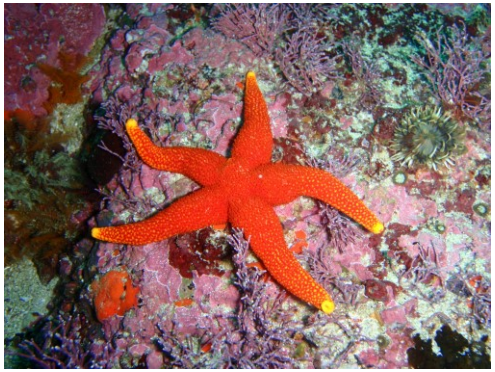


M Kragl *et al. Nature* **460**, 60-65 (2009) doi:10.1038/nature08152

…a human severed fingertip can regenerate until 11 years of age!

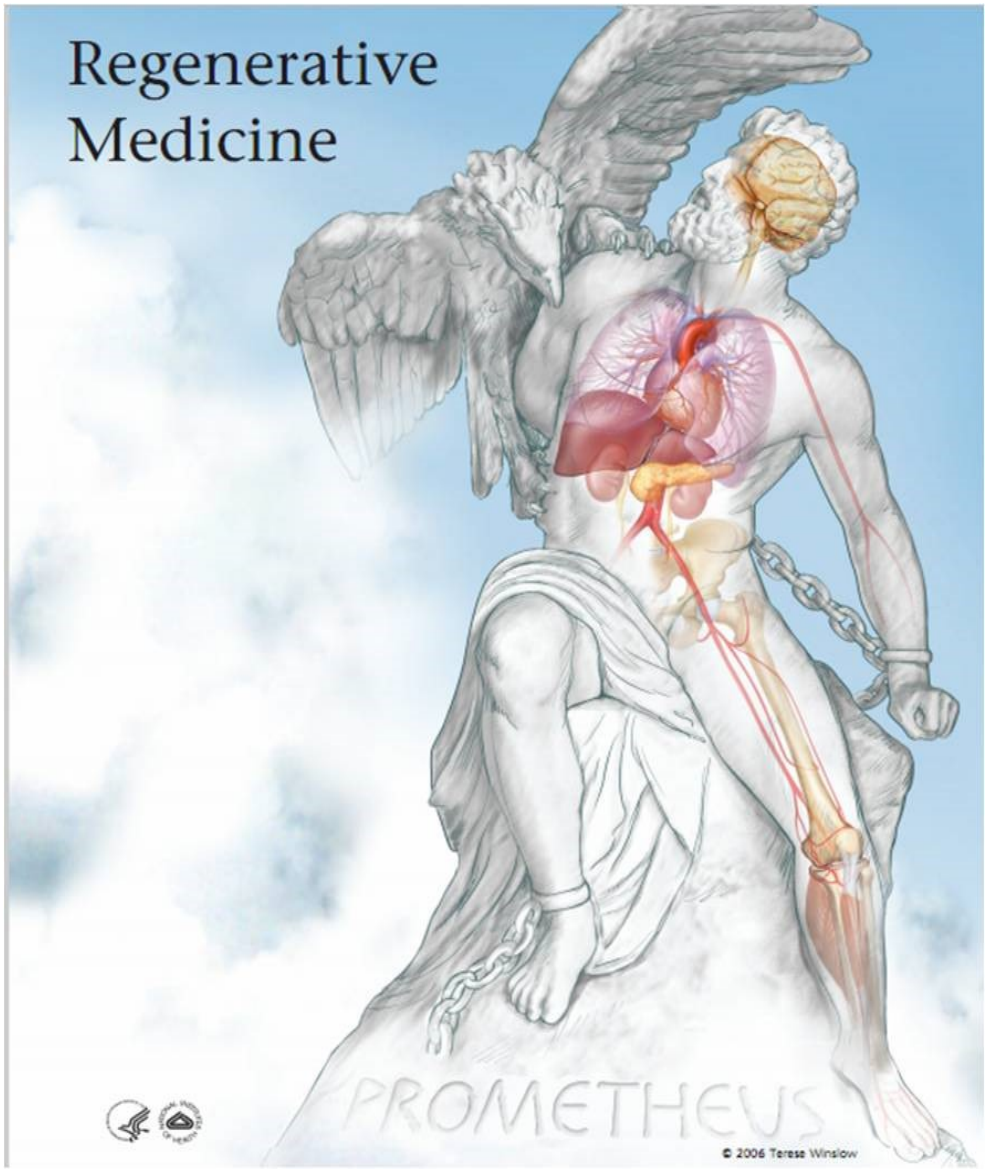


Journal of Pediatric Surgery 9, 1974, 853-858

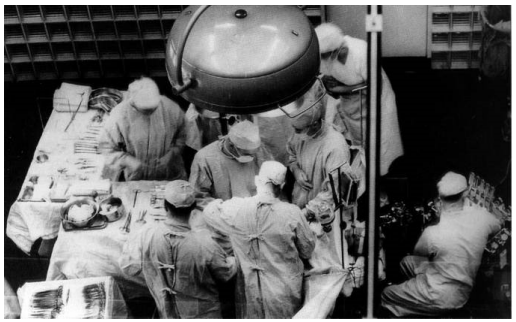


The regeneration of starfish arm

Wound Repair Regen . 2015 Jul-Aug;23(4):623-34.

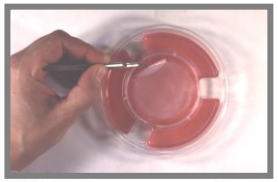

Regenerative
Medicine
PROMETHEUS
© 2006 Terese Winslow

Timeline of the recent history of RM



Joseph Murray and his team (1954)

Apligraf Skin Equivalent



1954 First human transplant (*1*)

1960 Boston researchers attempt to regenerate skin and cartilage tissue (*2*)

1979 Epicel is approved as the first cell-based product (*16*)

1980 1981 Apligraf is invented (*16*)

1998 Discovery of a method to grow embryonic stem cells in the laboratory (*2*)

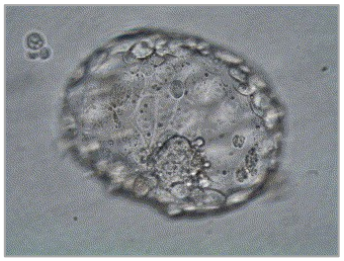
1999 William Haseltine coins the term regenerative medicine (*2*)

2000 2002 Capital value of the industry plummets (*17*)

2004 Start-up companies dominate the field (*17*)

2006 First engineered tissue is implanted (*3*)

2019 RM is at the forefront of healthcare



Human embryo at blastocyst stage

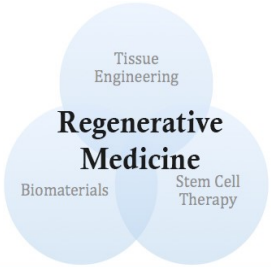
Clin Transl Sci (2020) 13, 440–450

Timeline of the recent history of RM

A partial list of firsts in RM.

Year	First
1968	First cell transplantation: bone marrow transplant
1978	Discovery of stem cells in human cord blood
1981	First in vitro stem cell line developed from mice
1981	First engineered tissue transplantation: skin
1996	Creation of the first cloned animal: a sheep, named Dolly
1998	Isolation of human embryonic stem cells
1999	First laboratory-grown organ: an artificial bladder implanted in a patient suffering from myelomengicocele
2004	Implantation of first engineered tubular organs (urine conduits)
2007	Discovery of stem cells derived from amniotic fluid and placenta
2009	First solid organ engineered by recycling donor live

J Microsc Ultrastruct. 2015 Jul-Sep; 3(3): 101–107



Alternative to organ transplants to regenerate tissues or organs biologically

STRATEGIES:

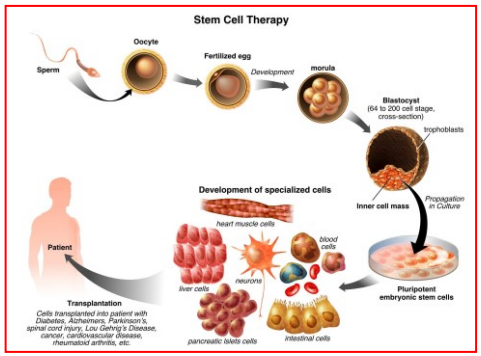
- Tissue regeneration *in situ*
- Cell implant
- Tissue/organ 3D construct *in vitro*
- Grow factors delivery by scaffolds

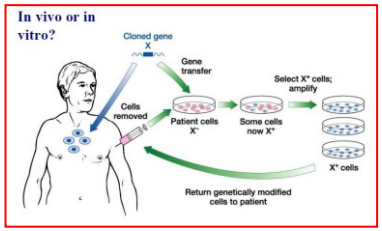
CELL THERAPY

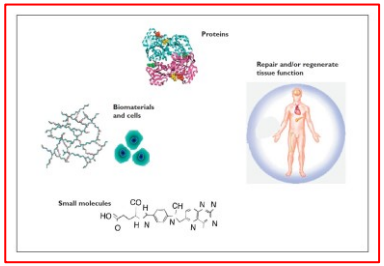
GENE THERAPY

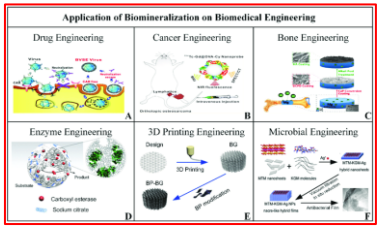
TISSUE ENGINEERING

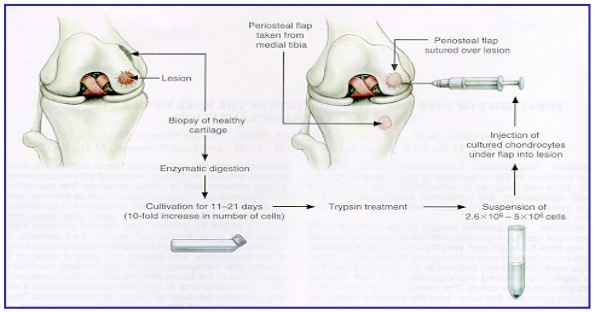
BIOMEDICAL/PHARMACOLOGICAL ENGINEERING APPROACHES



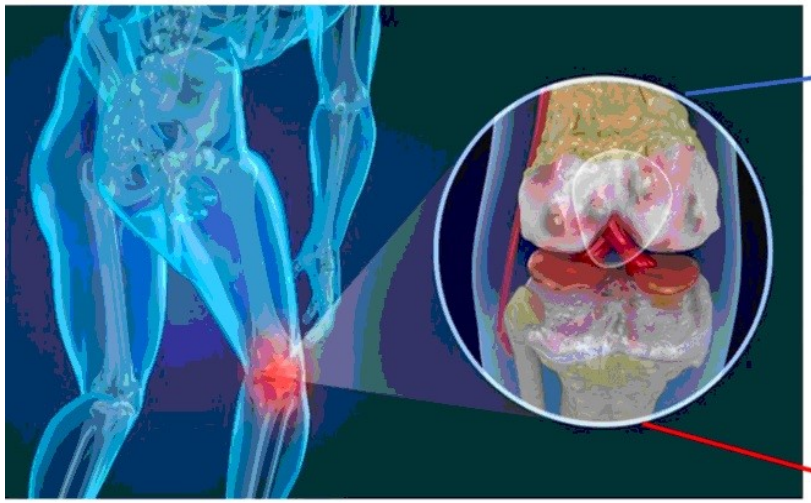




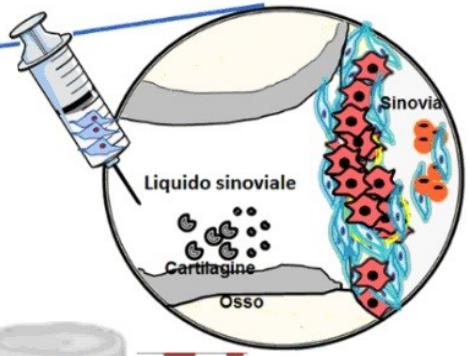


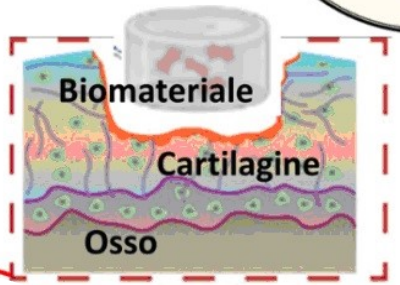


Osteoarthits (cruciated ligaments)



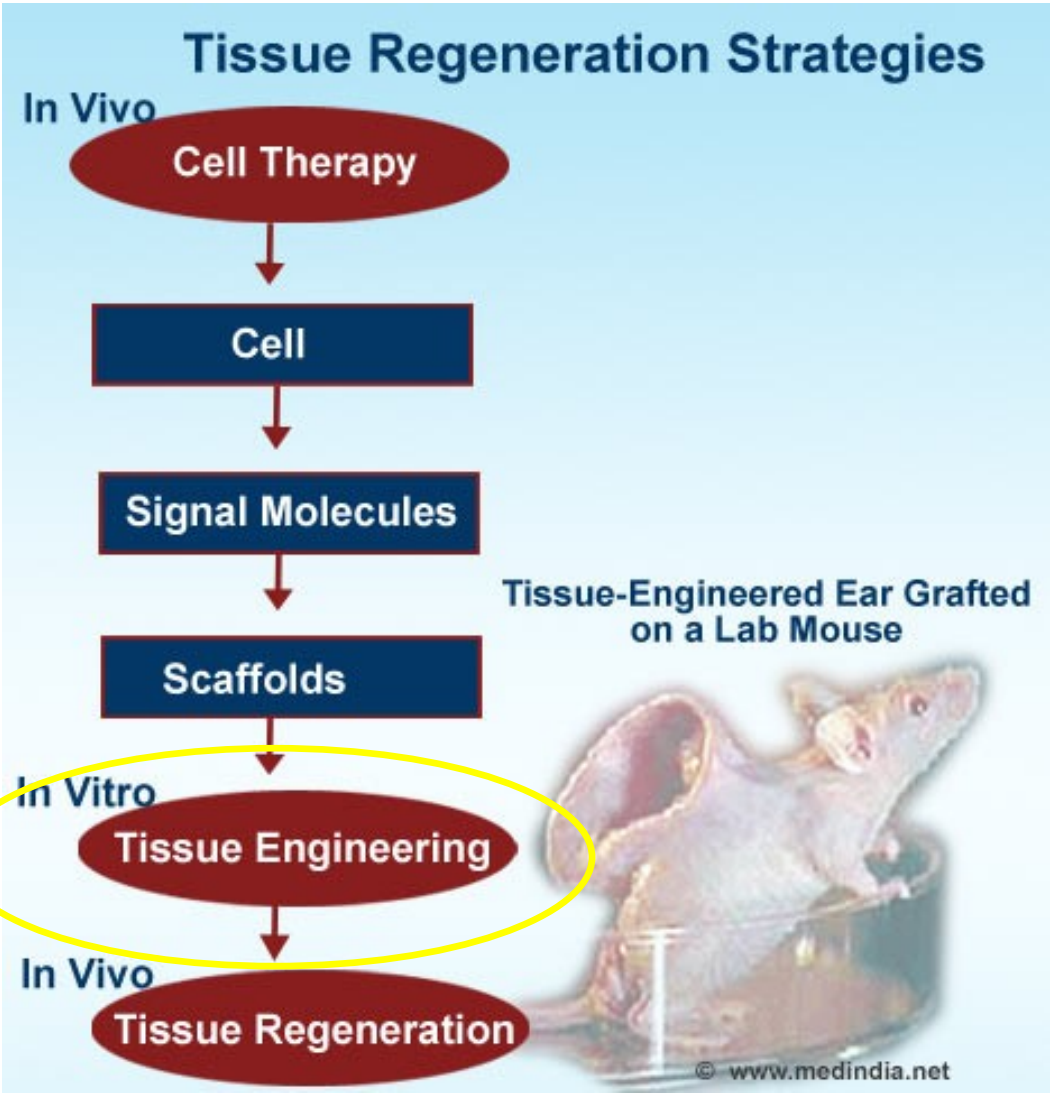
CELL THERAPY





TISSUE ENGINEERING

Summarizing…

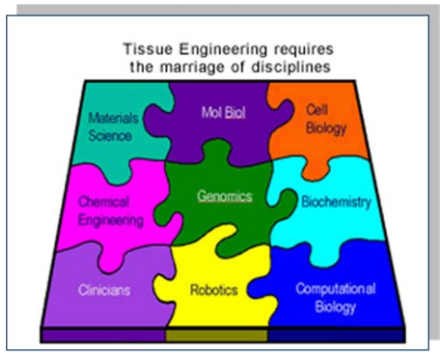


TISSUE ENGINEERING (TE)

Robert Langer and Joseph Vacanti

Over 1000 articles
Over 1000 patents
Chemical Engineer who studied with the "father of angiogenesis" as a post-doc

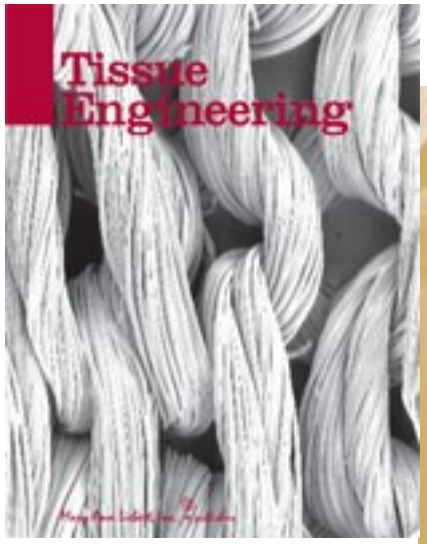
Surgeon at MGH
Has worked on 27 tissues/organs

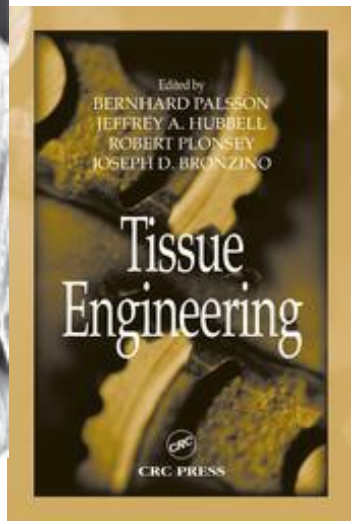


Mike Sefton, http://www.utoronto.ca/IBBME/research/tissue.htm

Tissue engineering is "an interdisciplinary field that applies the principles of engineering and life sciences toward the development of biological substitutes that restore, maintain or improve tissue function or a whole organ".

R. Langer and J Vacanti *SCIENCE* 1993, 260, Issue 5110 pp. 920-92





TISSUE ENGINEERING (TE)

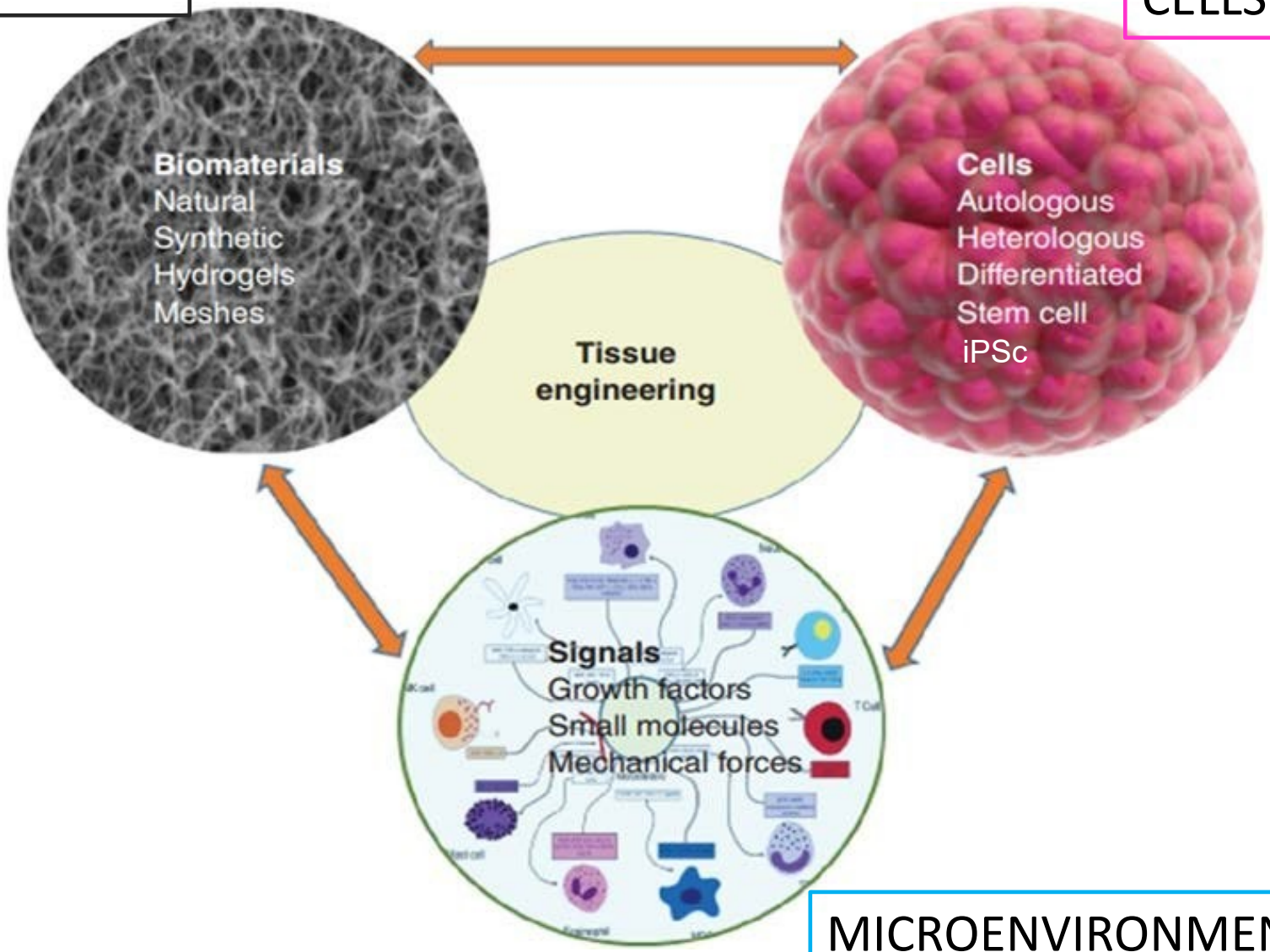
Create biological organs/tissues (known as neo-organs) in «a short time», similar to the original natural organs, to be used to overcome **rejection** and **availability** problems associated with **transplants** and with the use of artificial prostheses, used for the recovery of physiological functions of damaged organs and tissues.

TISSUE ENGINEERING (TE)

The general principle of Tissue Engineering (TE) involves a combination of living and viable cells with a synthetic/natural and also biodegradable support (SCAFFOLD) to make a 3D living construct that is FUNCTIONALLY, STRUCTURALLY AND MECHANICALLY equal or better than the tissue it needs to replace.

SCAFFOLD

CELLS



MICROENVIRONMENT

MAIN STEPS OF TISSUE ENGINEERING

- Choice of cell type or types
- Choice of the scaffold and its structure
- Cell seeding into the matrix (scaffold)
- Culture in dynamic conditions: tissue “conditioning”
- Cell replication and differentiation (if needed)
- Secretion by the cell of the extracellular matrix (ECM)
- Remodeling and transformation of ECM materials
- Implantation of the construct in the patient
- Adaptation and assimilation of the system

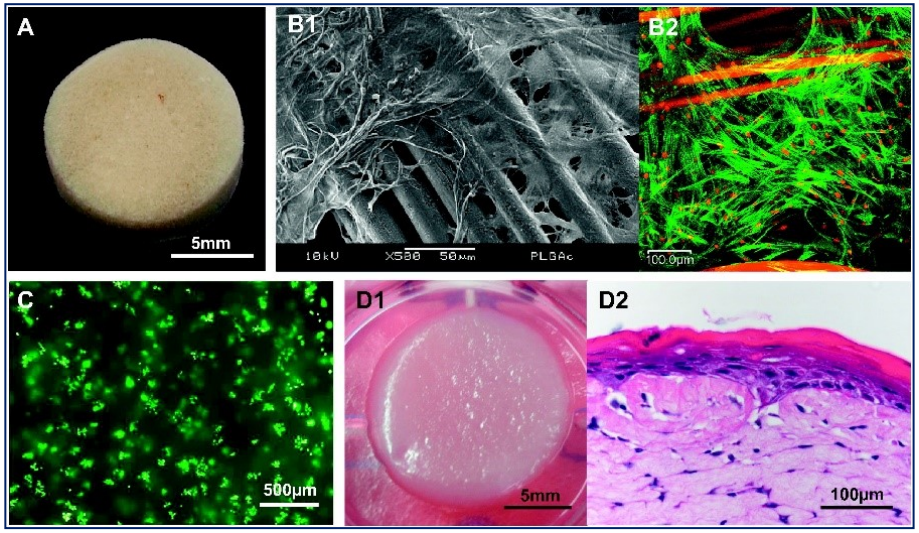
CELLS

Advantages and disadvantages of cell types used in RM.

Cell type	Advantages	Disadvantages
Differentiated endogenous primary cells	No tissue rejection Reduced inflammatory response	Difficult expansion because of in vitro short lifespan Difficulty in getting healthy cells in diseased organs
Adult stem cells (ASCs)	No tissue rejection No ethical problems No tumors Easy isolation In some cases easy access (e.g. apheresis and subcutaneous fat)	Low number in each tissue Difficult in vitro expansion without differentiation
Embryonic stem cells (ESCs)	Unlimited ability to self-renew Potential to differentiate into many specialized cells from all the three germ layers	Ethical and political problems Tumorigenity Need for feeder cell layers (risk of xeno-contamination when mouse fibroblasts are used)

Sampogna, G., Guraya, S. Y., & Forgione, A. (2015). *Journal of Microscopy and Ultrastructure*, *3*(3), 101-107.

SYNTHETIC SCAFFOLDS



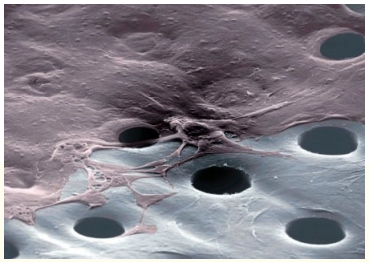
Leong D.T and Ng K.W. (2014) *Adv Drug Del Rev 79: 95-106*.

SCAFFOLD

NATURAL SCAFFOLDS

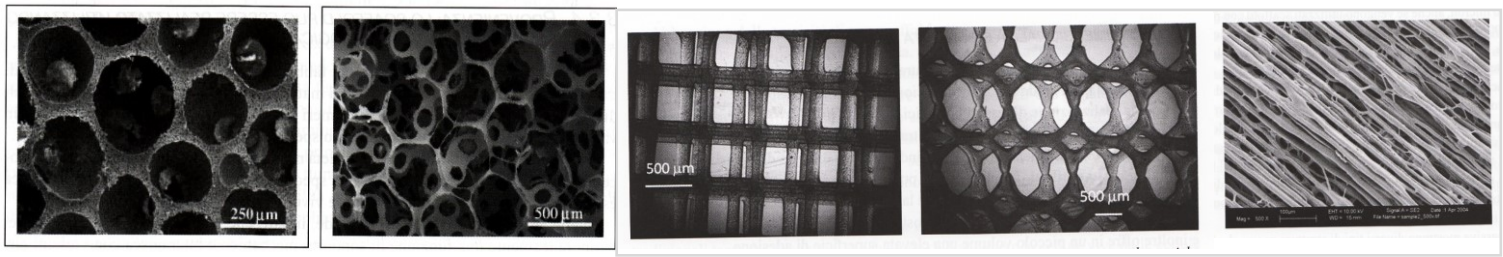
J.E.Arenas- Herrera, Biomed. Mater. 8 (2013)

BIOMATERIALS for SCAFFOLD



Examples of biomaterials used in RM.

Origin	Examples
Natural materials	Collagen, fibrin, chitosan, dextran, alginate, gelatin, cellulose, hyaluronic acid (HA), silk fibroin
Acellular tissue matrix	Bladder acellular matrix (BAM), small intestinal submucosa (SIS), bowel acellular tissue matrix (ATM), bovine pericardium (BPV), human placental membrane (HPM)
Synthetic polymers	Polyglycolic acid (PGA), polylactic acid (PLA), poly(lactic-co-glycolic) acid (PLGA), polycaprolactone (PCL), poly(copralactone-co-ethyl ethylene posphate) (PCLEEP), polydioxane (PDS), polyethylene glycol (PGE), poly-N-(2-hydroxyethyl)metacrylamide (PHEMA), poly-N-(2-hydroxypropyl)methacrylamide (PHPMA)



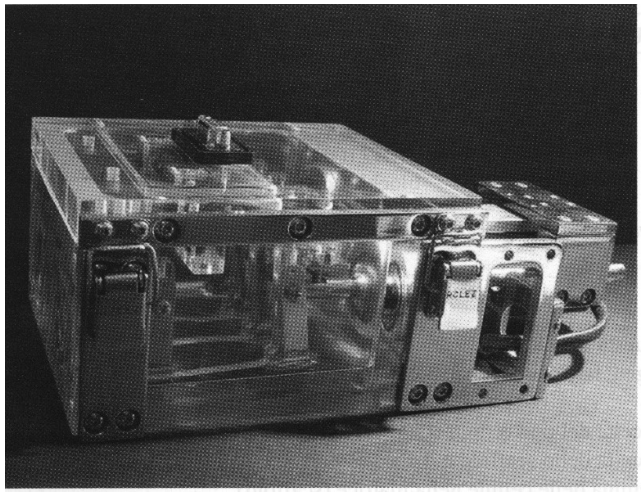
MICROENVIRONMENT& DEVELOPMENTAL BIOLOGY

CHEMICAL AND PHYSICAL SIGNALS

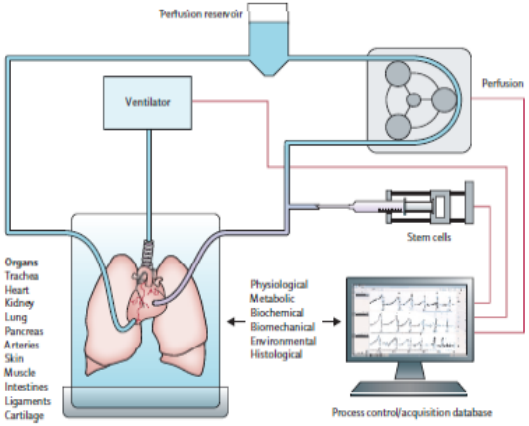
Growth factors

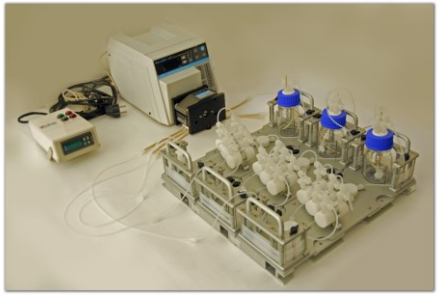
Small molecules

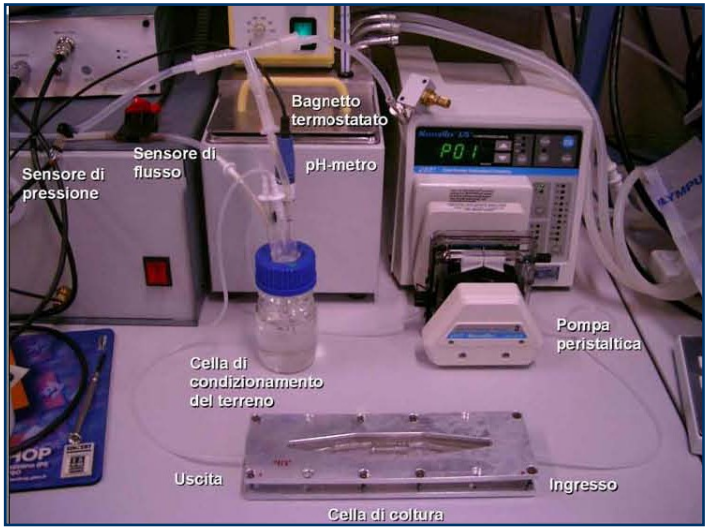
Mechanical forces

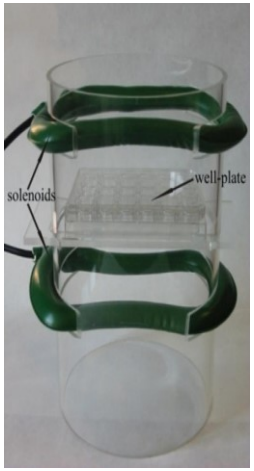


BIOREACTORS







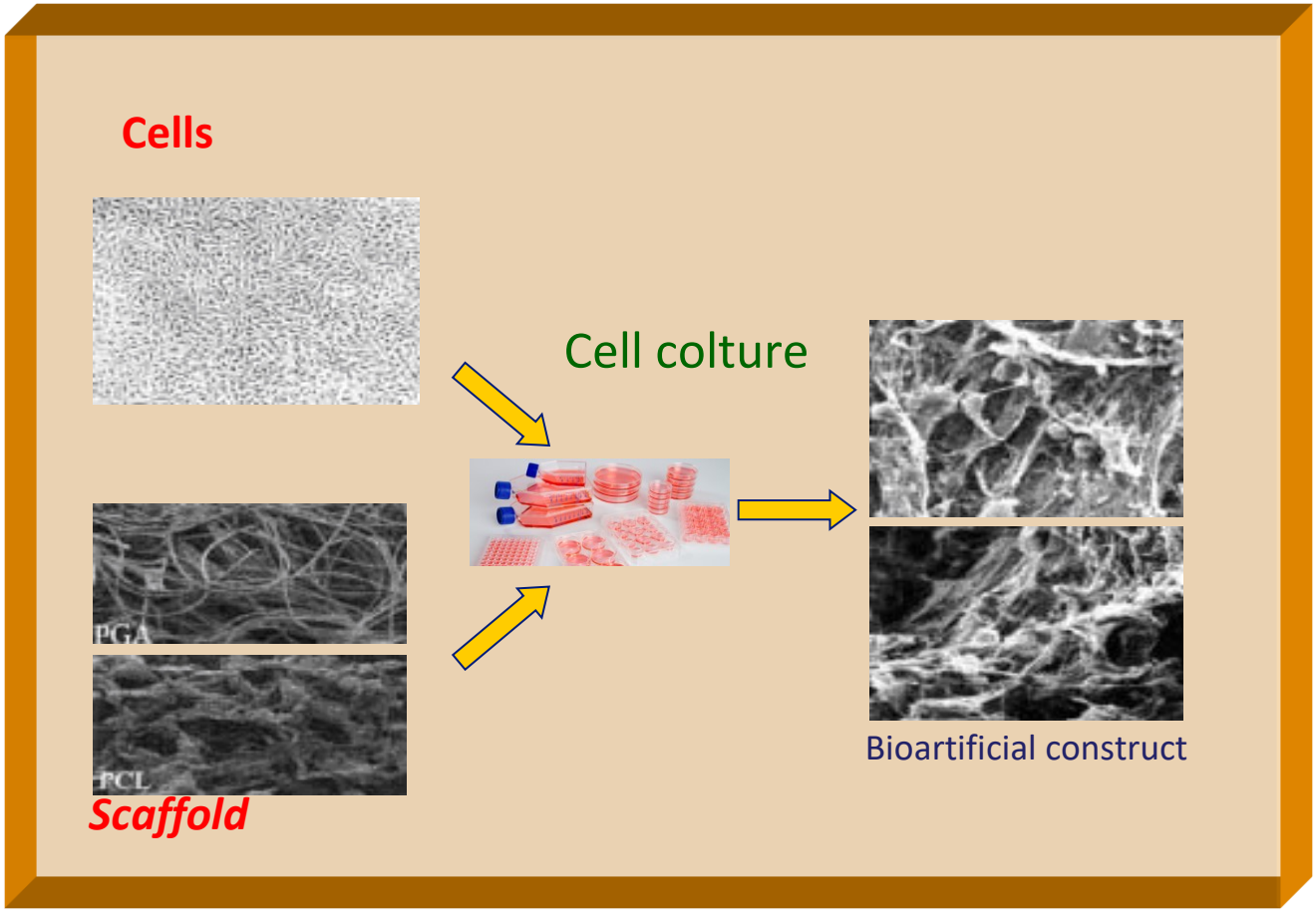


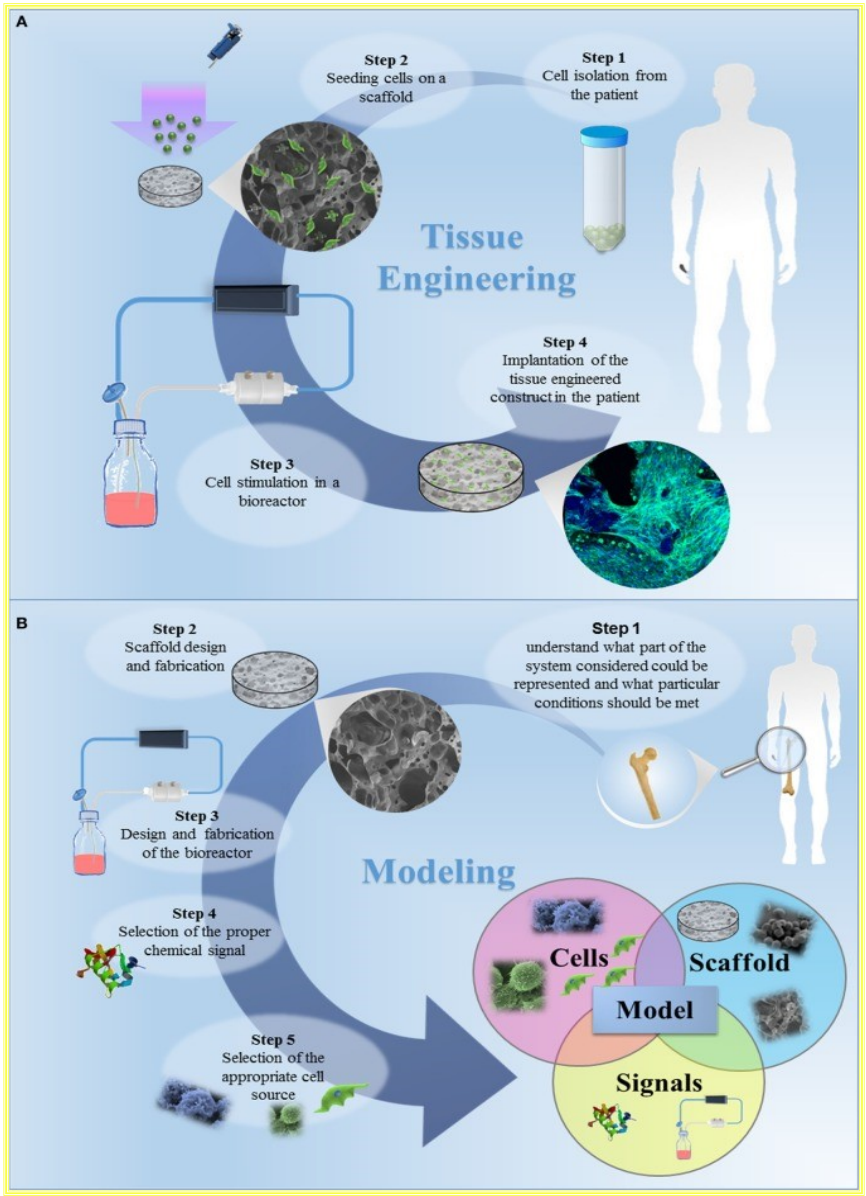
Remember to choose in relation

to the research aim because

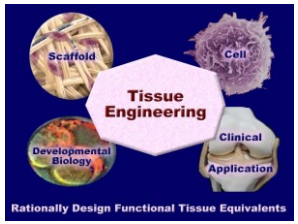
choosing the wrong model may

influence the results


Cells
Cell colture
PGA
PCL
Scaffold
Bioartificial construct



Caddeo S.. et al, *Frontiers In Bioeng Biotech, 2017, Vol. 5, Art. 40*

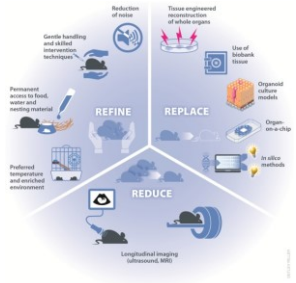


→ CLINICAL APPLICATIONS

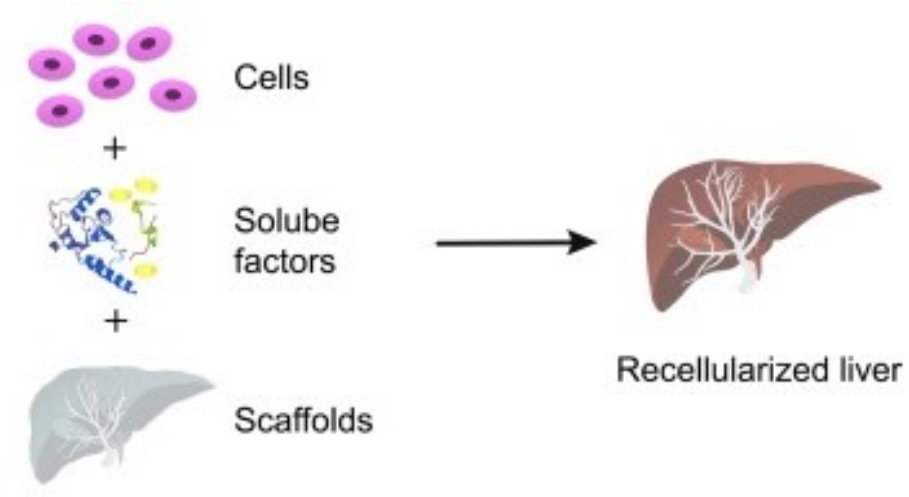
→ EXPERIMENTAL MODEL

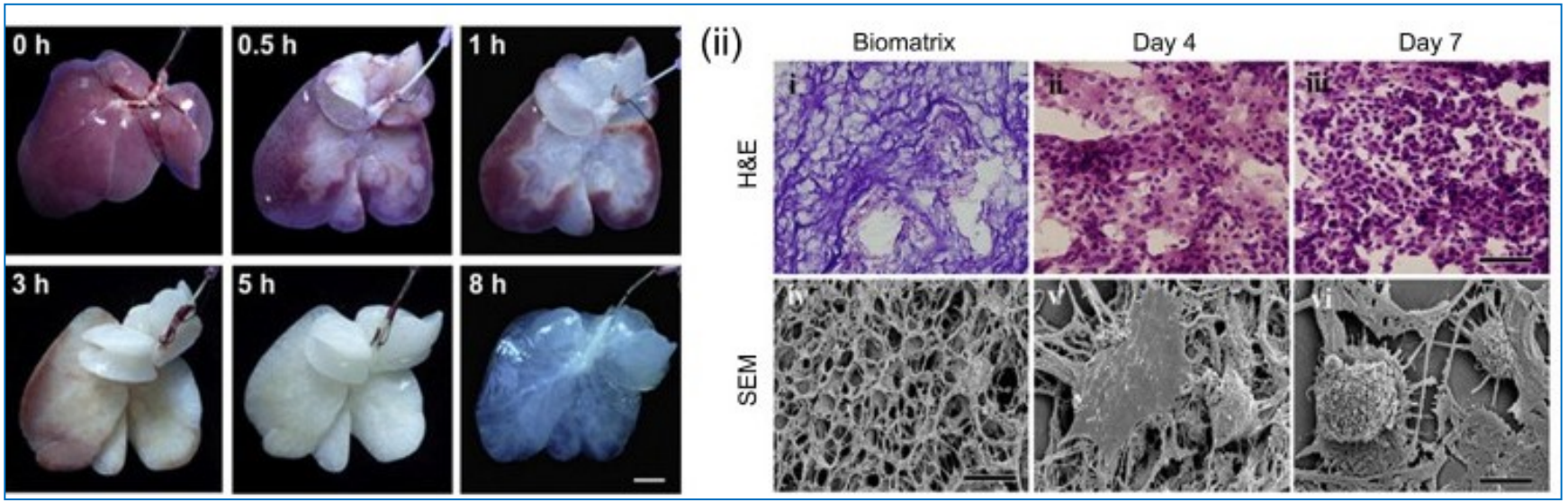
- Biological studies
- Drug tests

[R3 strategy for animal wellness]



TE of liver



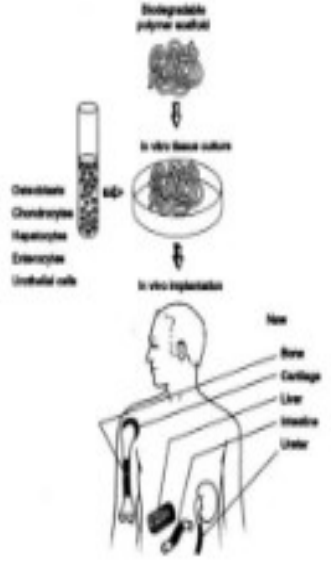


Modified by J.Zhang *et al., Biomaterials* 157 (2018), 161-176.

TE of cartilage: ear-shaped cartilage structure

The Beginning.....

Joseph Vacanti* & Robert Langer** (1993)

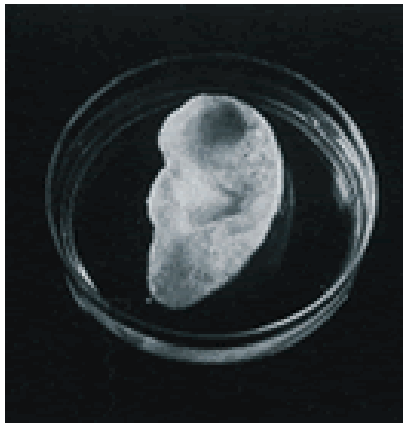


Langer R1, Vacanti JP., Tissue engineering, Science, 1993

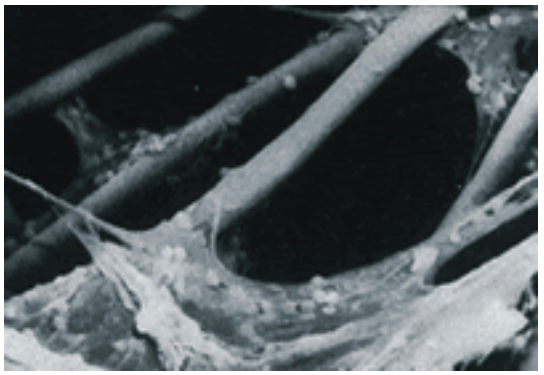
* Harvard Stem Cell Institute

** Massachusetts Institute of Technology (MIT)

PGA scaffold

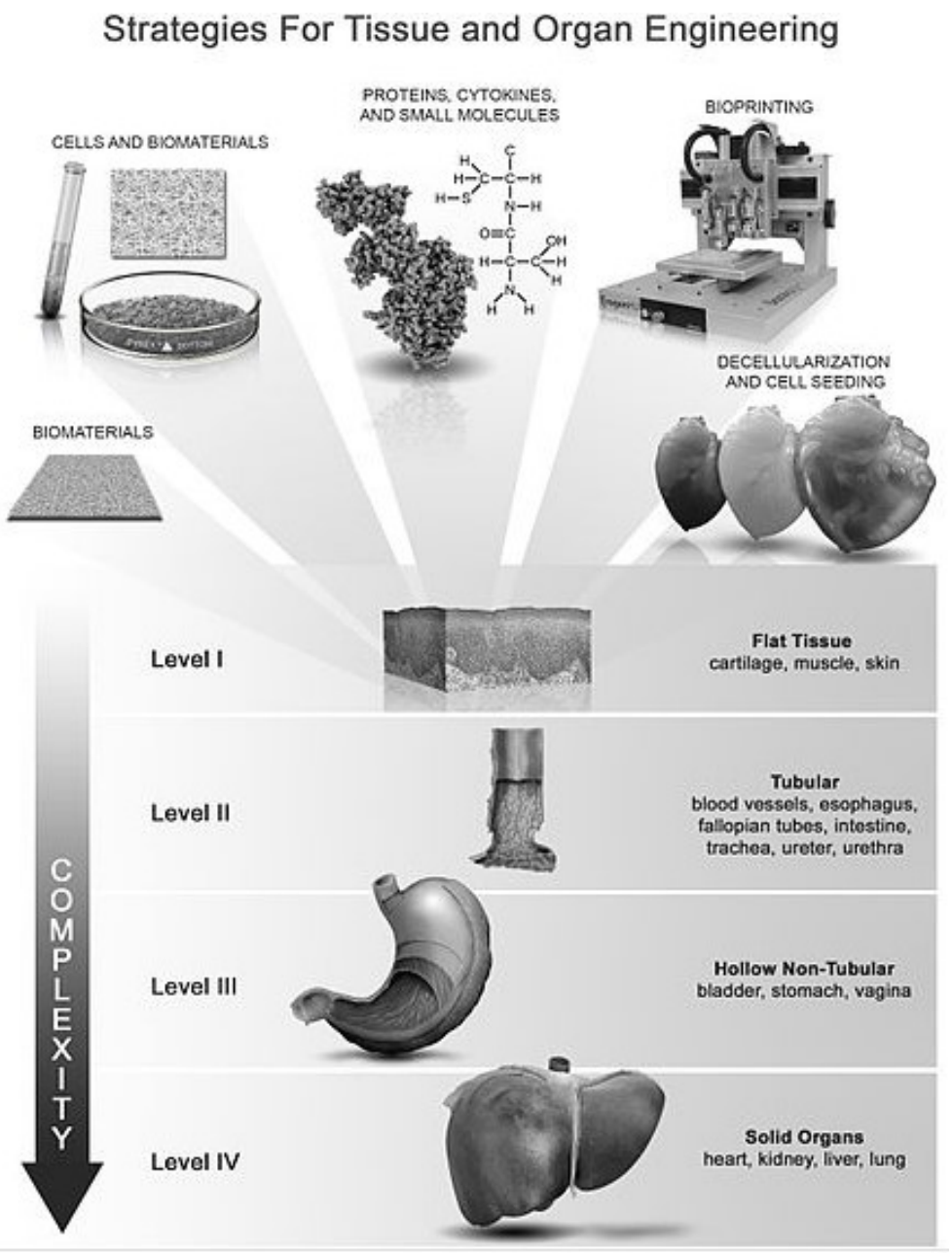


PGA +cells at S.E.M.

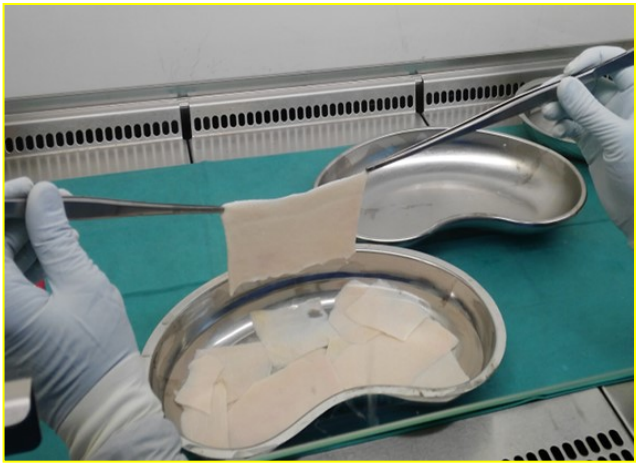


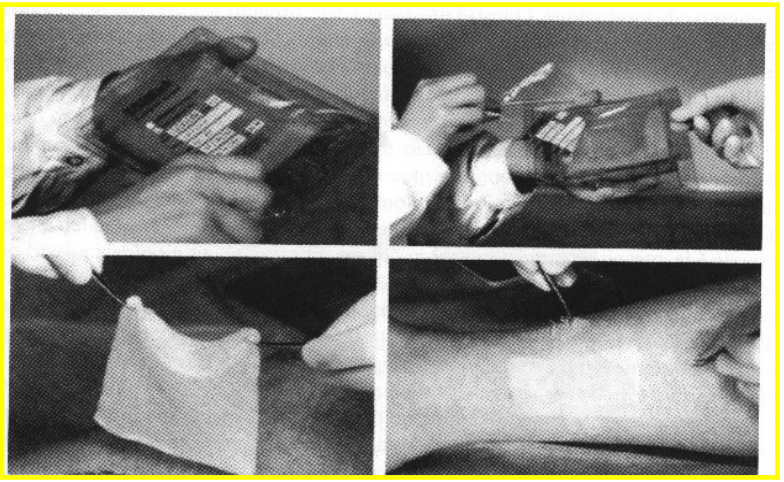
Ear-shaped construct implanted in mouse (12w)

University of Boston, USA, 2013

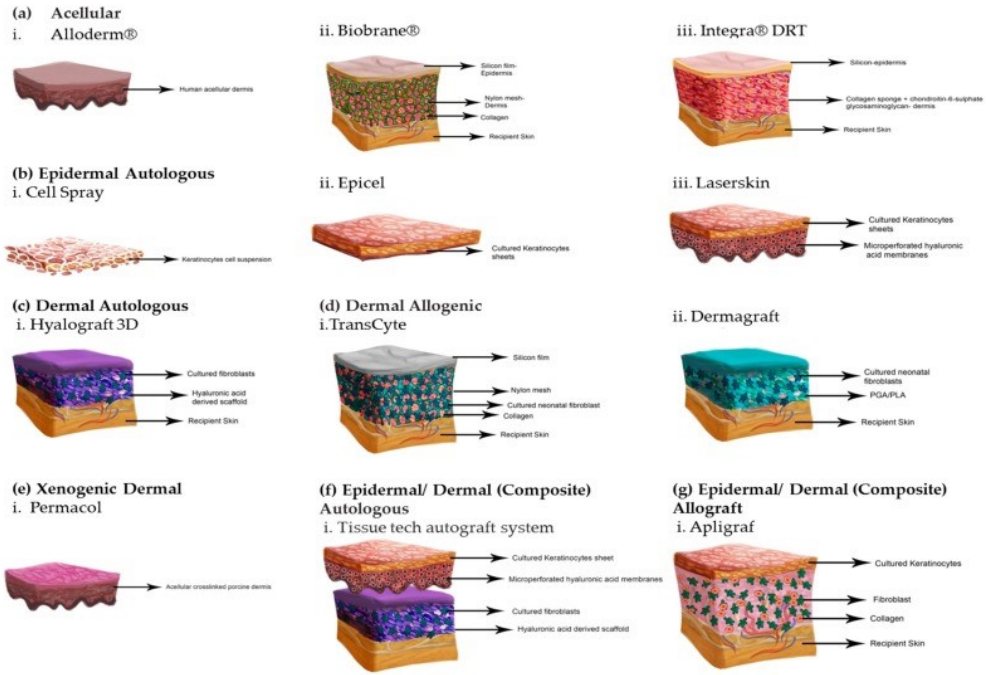

Strategies For Tissue and Organ Engineering
CELLS AND BIOMATERIALS
PROTEINS, CYTOKINES, AND SMALL MOLECULES
BIOPRINTING
DECELLULARIZATION AND CELL SEEDING
BIOMATERIALS
COMPLEXITY
Level I
Flat Tissue
cartilage, muscle, skin
Level II
Tubular
blood vessels, esophagus, fallopian tubes, intestine, trachea, ureter, urethra
Level III
Hollow Non-Tubular
bladder, stomach, vagina
Level IV
Solid Organs
heart, kidney, liver, lung

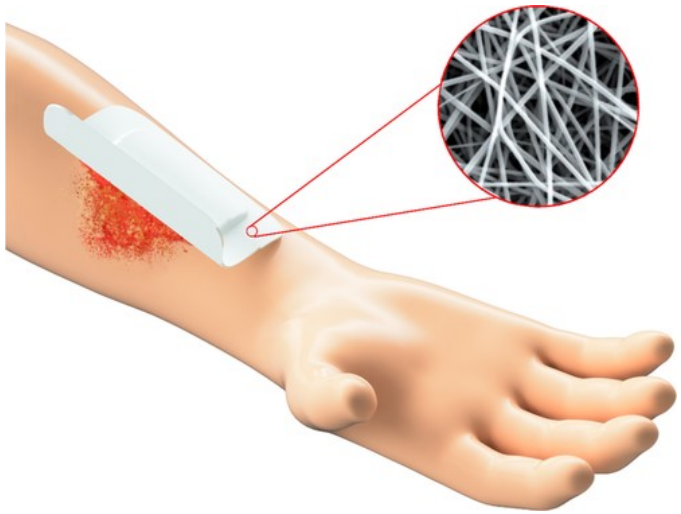
TE of skin





Tissue engineered skin substitutes





Keirouz A, Chung M, Kwon J, Fortunato G, Radacsi N. 2D and 3D electrospinning technologies for the fabrication of nanofibrous scaffolds for skin tissue engineering. Rev Nanomed Nanobiotechnol. **2020** Mar 12

TE of bladder

JOURNAL OF TISSUE ENGINEERING AND REGENERATIVE MEDICINE
J Tissue Eng Regen Med 2007; 1: 83–96.
Published online in Wiley InterScience (www.interscience.wiley.com) DOI: 10.1002/term.18

REVIEW ARTICLE

Engineering tissues, organs and cells

Anthony Atala*
Wake Forest Institute for Regenerative Medicine, Wake Forest University School of Medicine, Winston-Salem, NC, USA

Abstract

Patients suffering from diseased and injured organs may be treated with transplanted organs; however, there is a severe shortage of donor organs that is worsening yearly, given the ageing population. In the field of regenerative medicine and tissue engineering, scientists apply the principles of cell transplantation, materials science and bioengineering to construct biological substitutes that will restore and maintain normal function in diseased and injured tissues. Therapeutic cloning, where the nucleus from a donor cell is transferred into an enucleated oocyte in order to extract pluripotent embryonic stem cells, offers a potentially limitless source of cells for tissue engineering applications. The stem cell field is also advancing rapidly, opening new options for therapy, including the use of amniotic and placental fetal stem cells. This review covers recent advances that have occurred in regenerative medicine and describes applications of these technologies using chemical compounds that may offer novel therapies for patients with end-stage organ failure. Copyright © 2007 John Wiley & Sons, Ltd.

Received 27 February 2007; Accepted 28 February 2007

Keywords tissue engineering; regenerative medicine; stem cells; biomaterials; bladder; urethra; kidney; genital tissues

1. Introduction

Tissue engineering, a major component of regenerative medicine, follows the principles of cell transplantation, materials science and engineering to develop biological substitutes that can restore and maintain normal function. Tissue engineering strategies generally fall into two categories: the use of acellular matrices, depending on the body's natural ability to regenerate for proper orientation and direction of new tissue growth, and the use of matrices with cells. Acellular tissue matrices can be prepared by manufacturing artificial scaffolds or by removing cellular components from tissues by mechanical and chemical manipulation to produce collagen-rich matrices (Chen *et al.*, 1999; Dahms *et al.*, 1998; Yoo *et al.*, 1998). These matrices slowly degrade on implantation and are generally replaced by the extracellular matrix (ECM) proteins secreted by the ingrowing cells. Cells can also be used for therapy via injection, either with carriers, such as hydrogels, or alone. Tissue engineering, stem cells and cloning are three areas technology encompassed by the field of regenerati medicine.

When cells are used for tissue engineering, a sm piece of donor tissue is dissociated into individual cel These cells are either implanted directly into the host are expanded in culture, attached to a support matr and then reimplanted into the host after expansion. T source of donor tissue can be heterologous (such bovine), allogeneic (same species, different individual) autologous (from the host). Ideally, both structural a functional tissue replacement will occur with minim complications. The preferred cells to use are autologo cells, where a biopsy of tissue is obtained, the cells a dissociated and expanded in culture and the expand cells are implanted into the same host (Atala *et al.*, 199 1999; Atala and Lanza, 2001; Fauza *et al.*, 1998; Godb and Atala, 2002; Machluf and Atala, 1998; Oberpenni *et al.*, 1999; Yoo and Atala, 1997; Yoo *et al.*, 1998, 199 Although it could cause an inflammatory response, the u of autologous cells avoids rejection and the deleterio side effects of immunosuppressive medications can avoided.

Current strategies for tissue engineering primar depend upon a sample of autologous cells from t

*Correspondence to: Anthony Atala, Wake Forest Institute for Regenerative Medicine, Wake Forest University School of Medicine, Medical Center Boulevard, Winston-Salem, NC 27157, USA. E-mail: aatala@wfubmc.edu

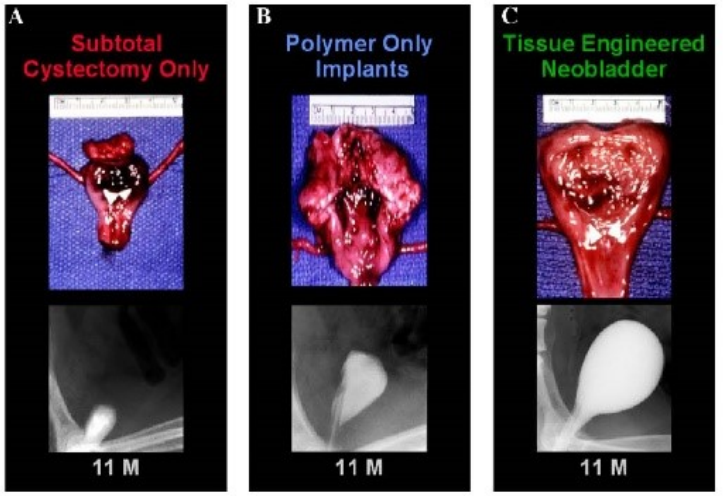


Figure 4. Comparison of tissue-engineered neo-bladders in dogs. Gross specimens and cystograms at 11 months of (A) cystectomy only, (B) non-seeded controls and (C) cell-seeded tissue-engineered bladder replacements. The cell-seeded tissue-engineered bladder replacements achieved an average bladder capacity of 95% of the original precystectomy volume, and the compliance showed almost no difference from preoperative values. The others showed considerable loss of capacity and compliance

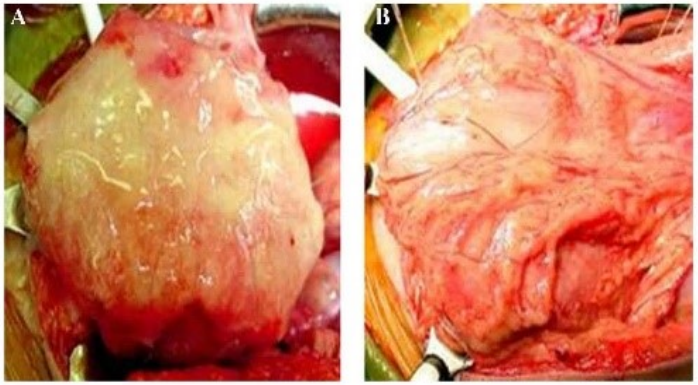
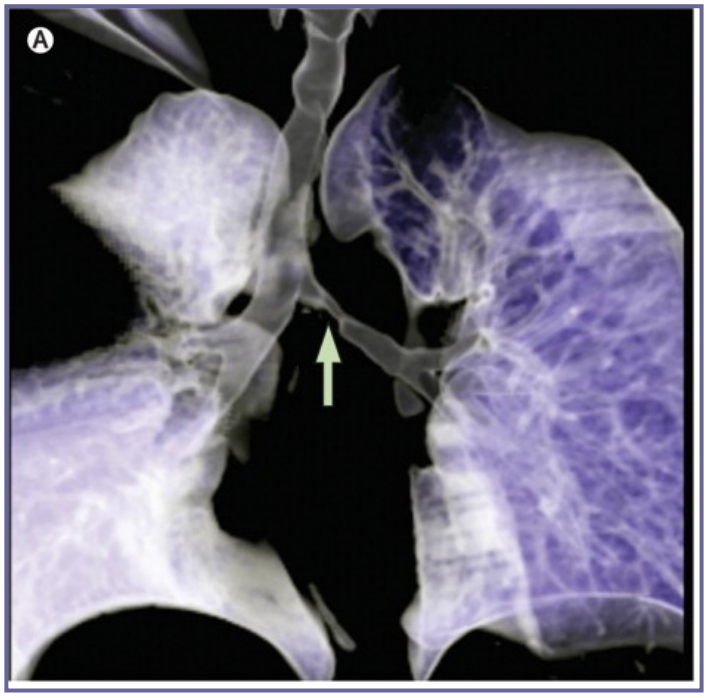
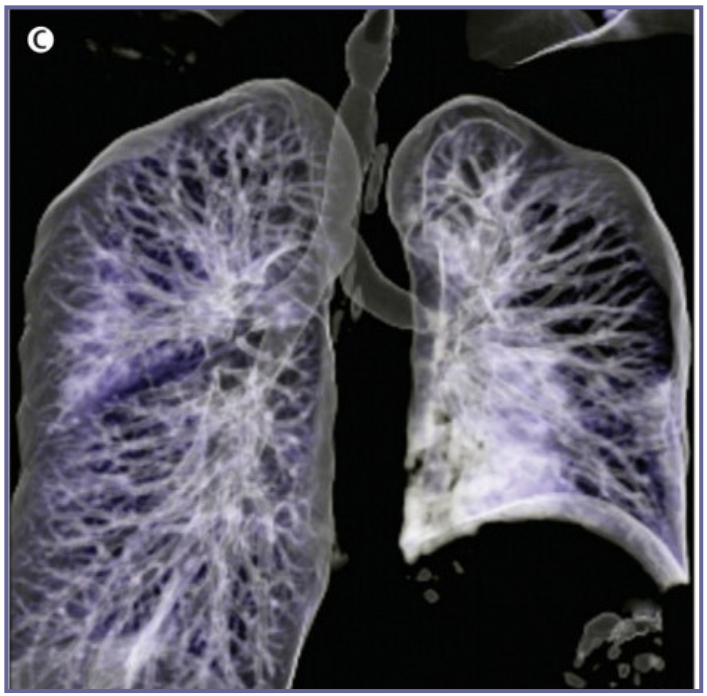


Figure 5. Construction of engineered bladder. (A) Engineered bladder anastamosed to native bladder with running 4-0 polyglycolic sutures. (B) Implant covered with fibrin glue and omentum

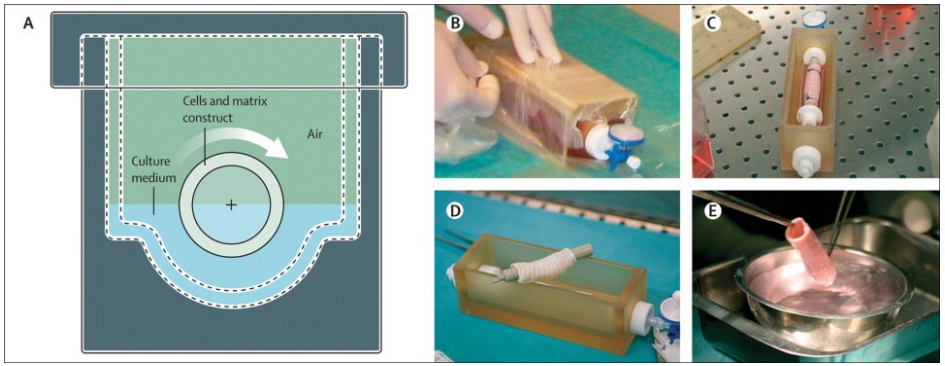
Atala *Tissue Eng Reg Med* 2007.

TE TRACHEA





Construct ø 12-10 mm; l 6,5 cm

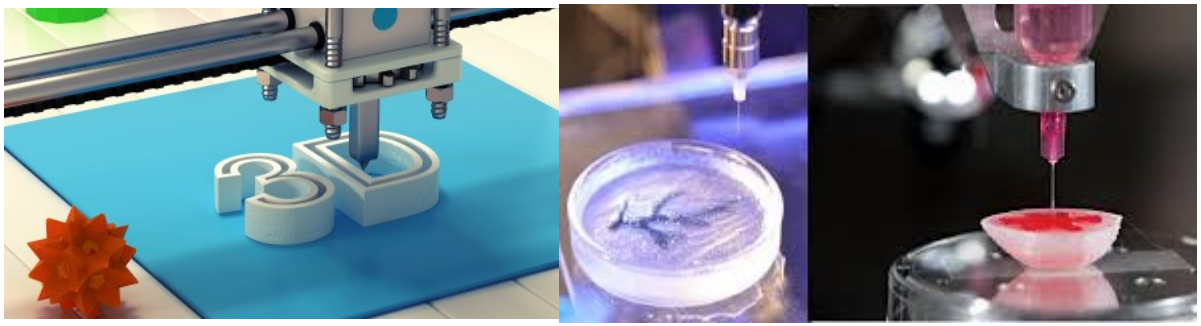


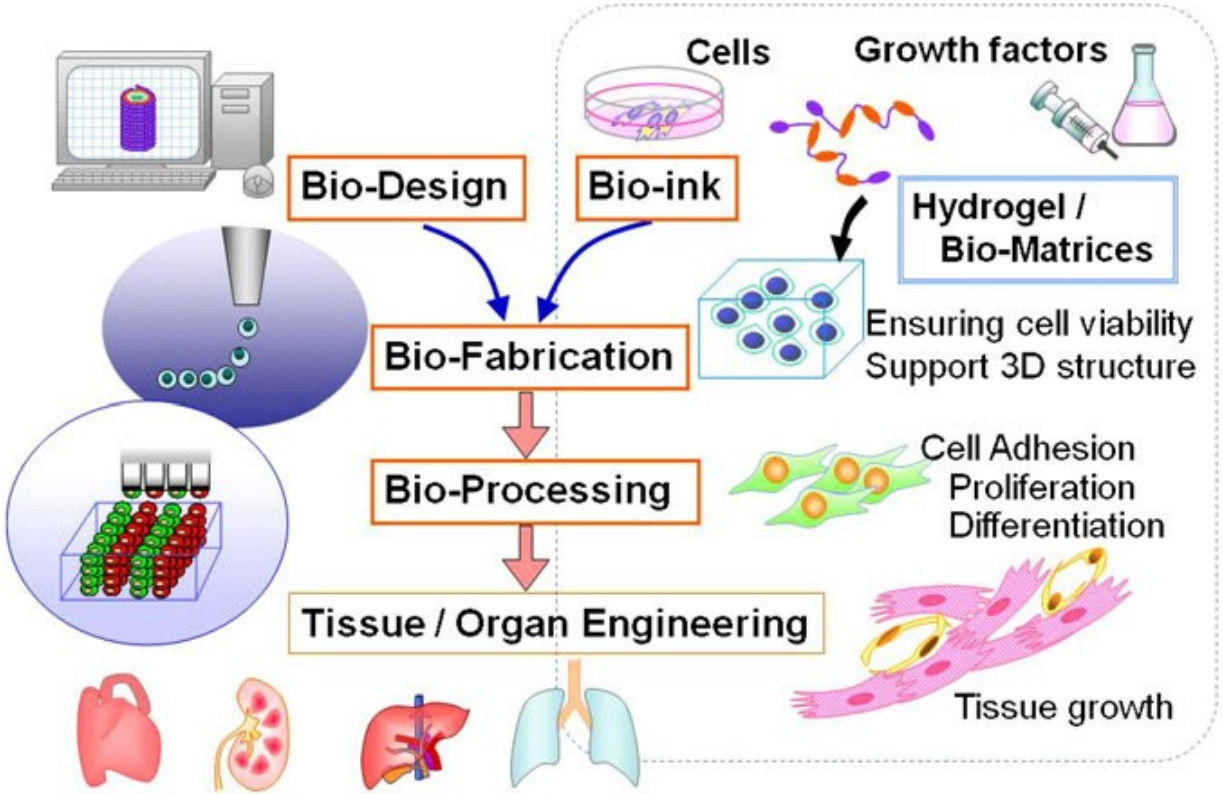
Macchiarini et al., *The Lancet*, (2008), 372:2023-2030.

Nanotechnology and Microfluidics in Tissue Engineering

- Advances in fabrication technologies have brought a new dimension to the field of tissue engineering.
- Fabricate tissue engineering scaffolds with complex 3-D architectures and customized chemistries that mimic the in vivo tissue environment.

3D BIOPRINTING





EXPERIMENTAL MODELS *in vitro*

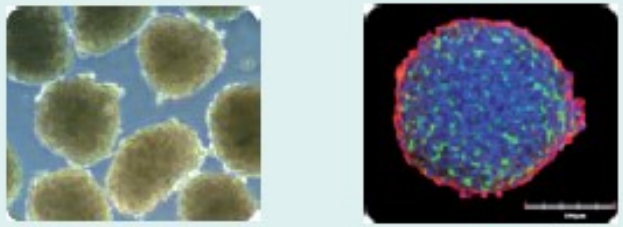
Potential of Regenerative Medicine

Chip Technology

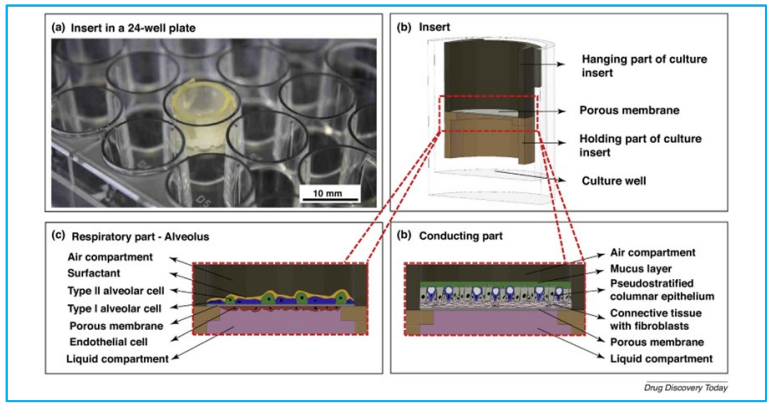
[Geraldine Hamilton, Body parts on a chip, TEDx Boston, June 2013: https://youtu.be/CpkXmtJOH84]

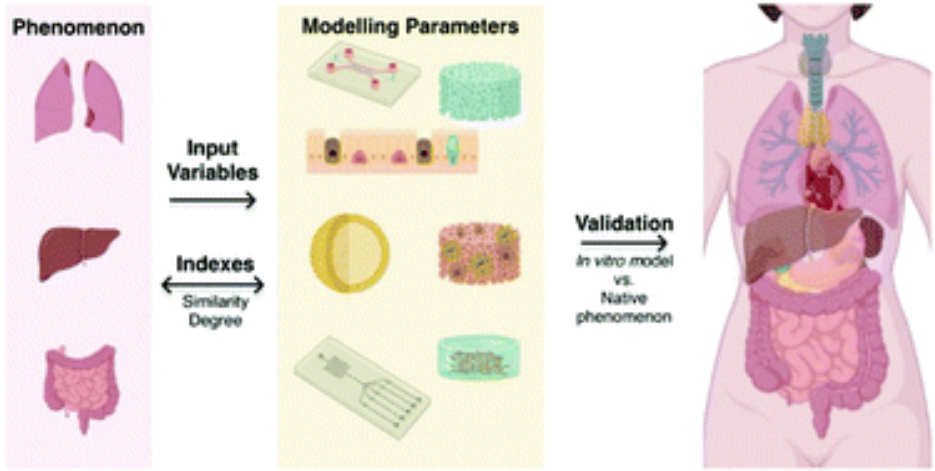
- Reduces Need for Animal Testing
- 3-D Printed Organs on Chips Used to Test Vaccines

ORGANOIDS and SPHEROIDS

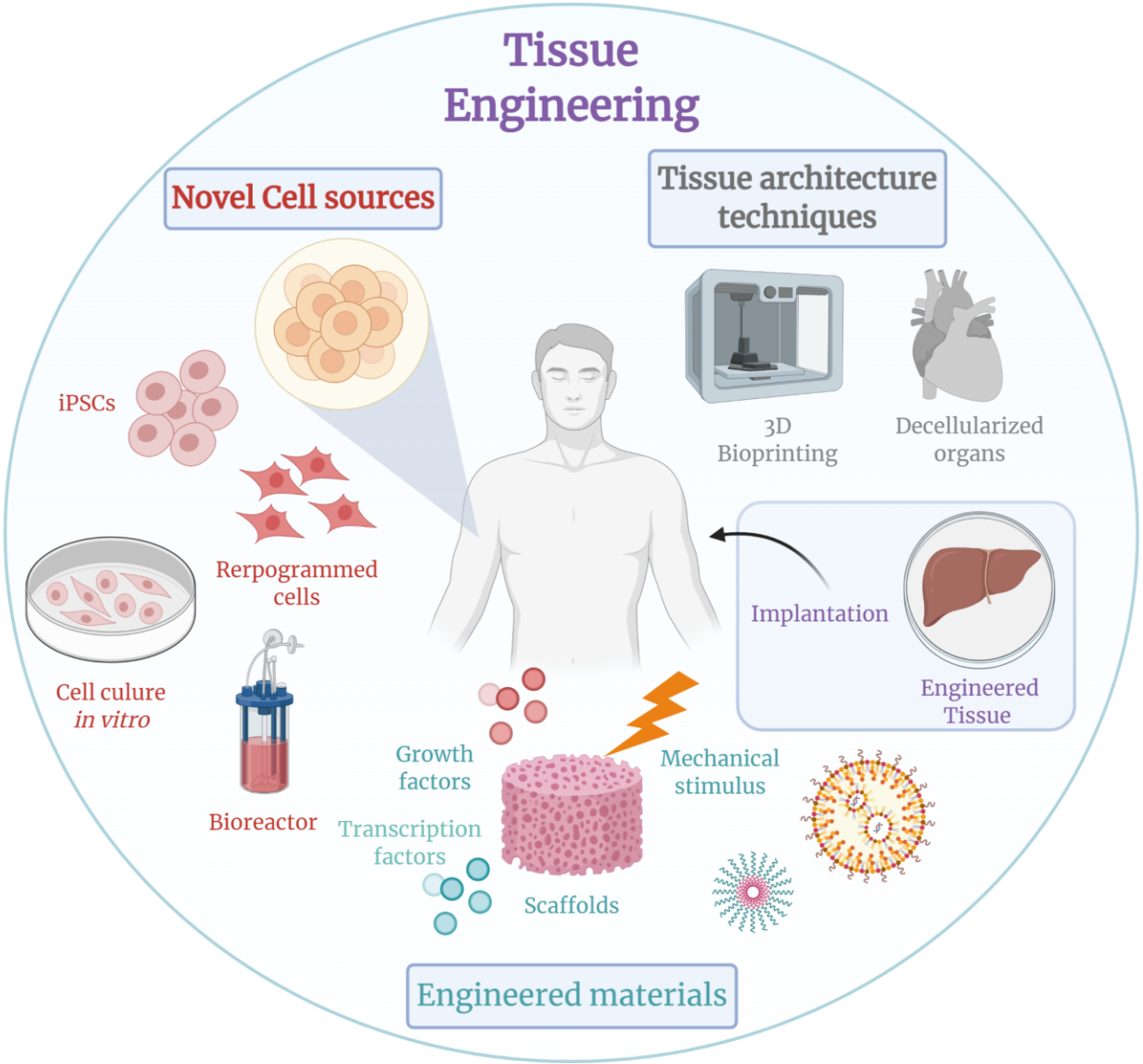


TRANSWELL- INSERT SYSTEM





Biomater Sci, . 2021 Jan 5;9(1):70-83

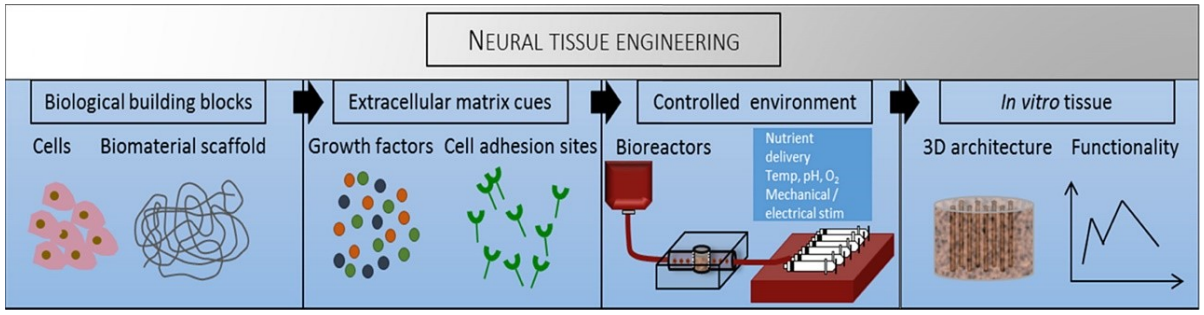

Tissue Engineering
Novel Cell sources
Tissue architecture techniques
iPSCs
Rerpogrammed cells
Cell culure in vitro
Bioreactor
3D Bioprinting
Decellularized organs
Implantation
Engineered Tissue
Growth factors
Transcription factors
Scaffolds
Mechanical stimulus
Engineered materials

Major challanges

Parkinson's disease

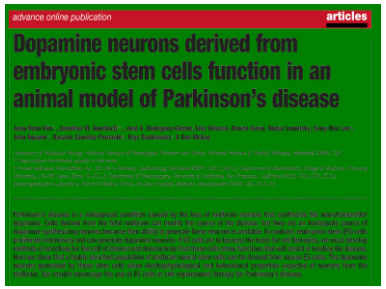
- first noted by James Parkinson, 200 years ago, in London
- progressive movement disorder
- onset in middle to late life
- 1% of population, 65 years & older

• "pill-rolling" slow movements, particularly when starting, short, rapid steps;
• no intellectual impairments

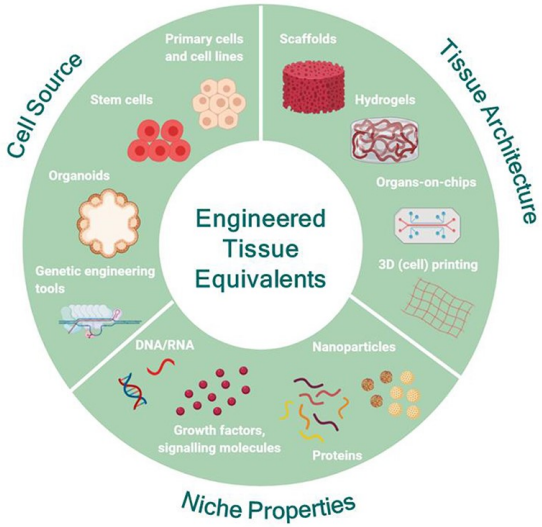


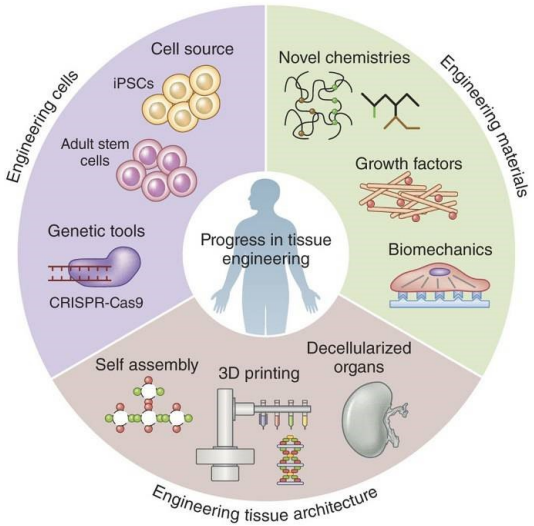
Michael J. Fox born 1961

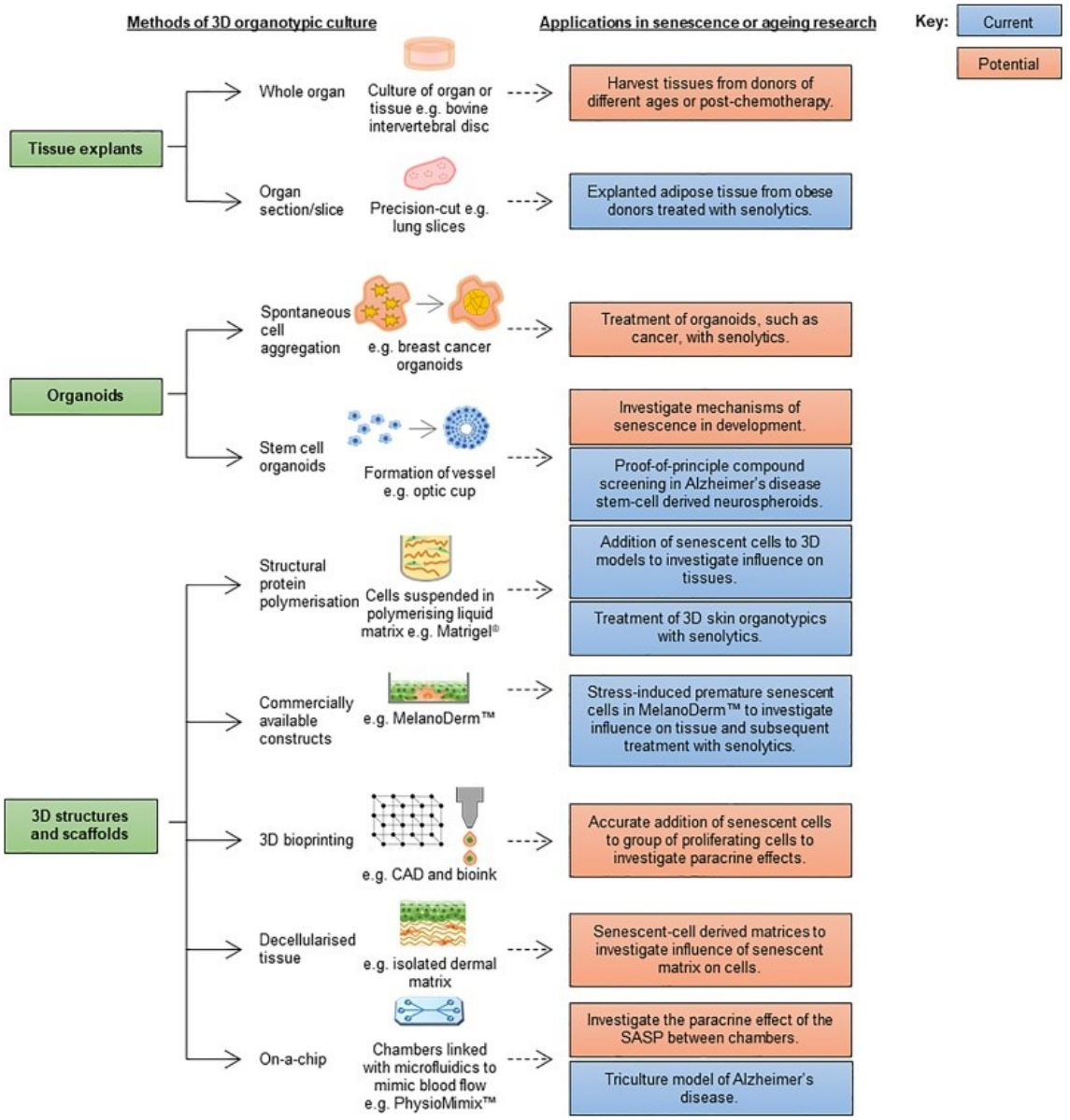
diagnosed with young-onset Parkinson's disease in 1991

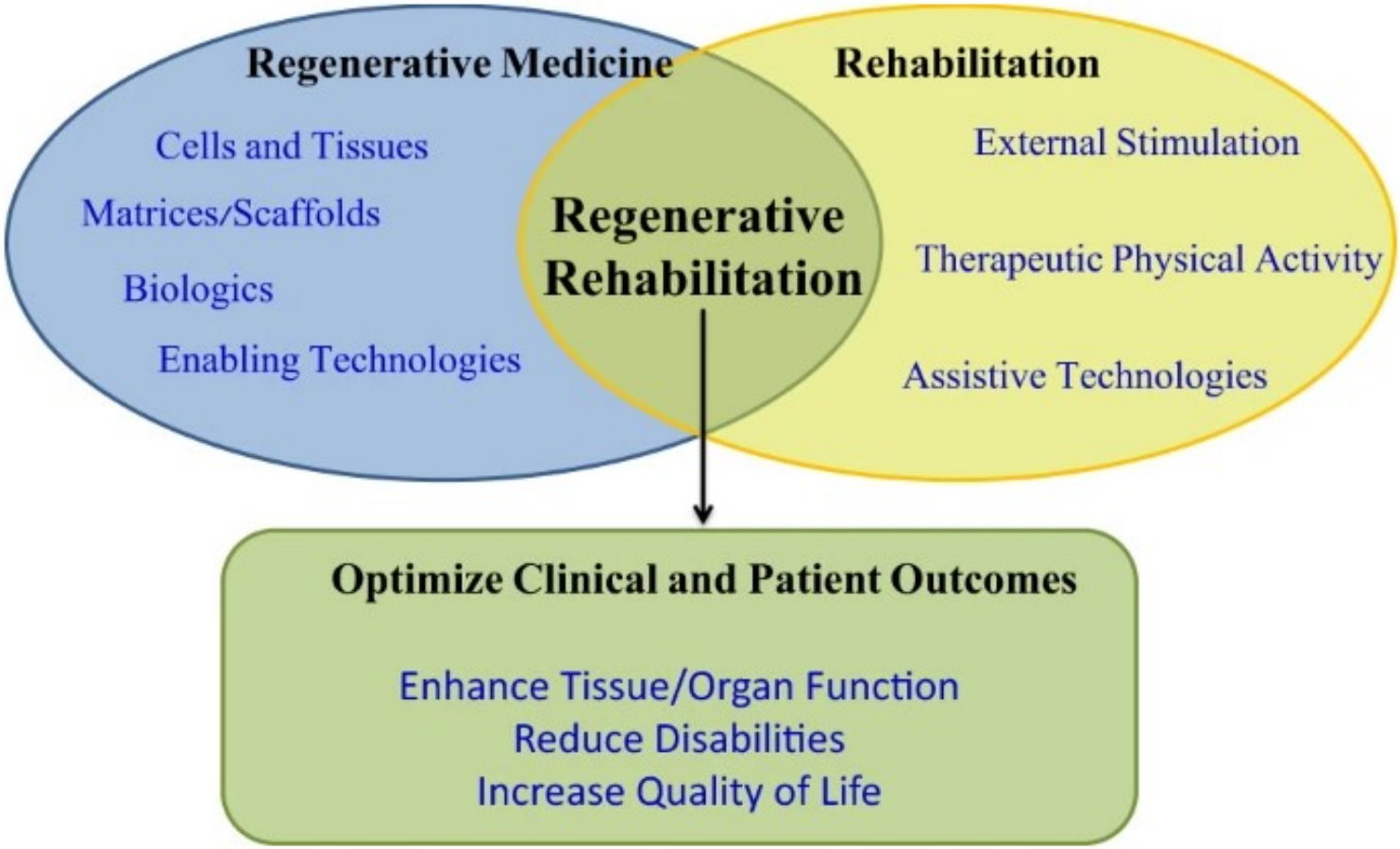

advance online publication articles

Dopamine neurons derived from embryonic stem cells function in an animal model of Parkinson's disease


Cell Source
Tissue Architecture
Niche Properties
Primary cells and cell lines
Stem cells
Scaffolds
Hydrogels
Organoids
Organs-on-chips
Genetic engineering tools
3D (cell) printing
DNA/RNA
Nanoparticles
Growth factors, signalling molecules
Proteins
Engineered Tissue Equivalents


Engineering cells
Engineering materials
Engineering tissue architecture
Cell source
iPSCs
Adult stem cells
Genetic tools
CRISPR-Cas9
Novel chemistries
Growth factors
Biomechanics
Decellularized organs
Self assembly
3D printing
Progress in tissue engineering

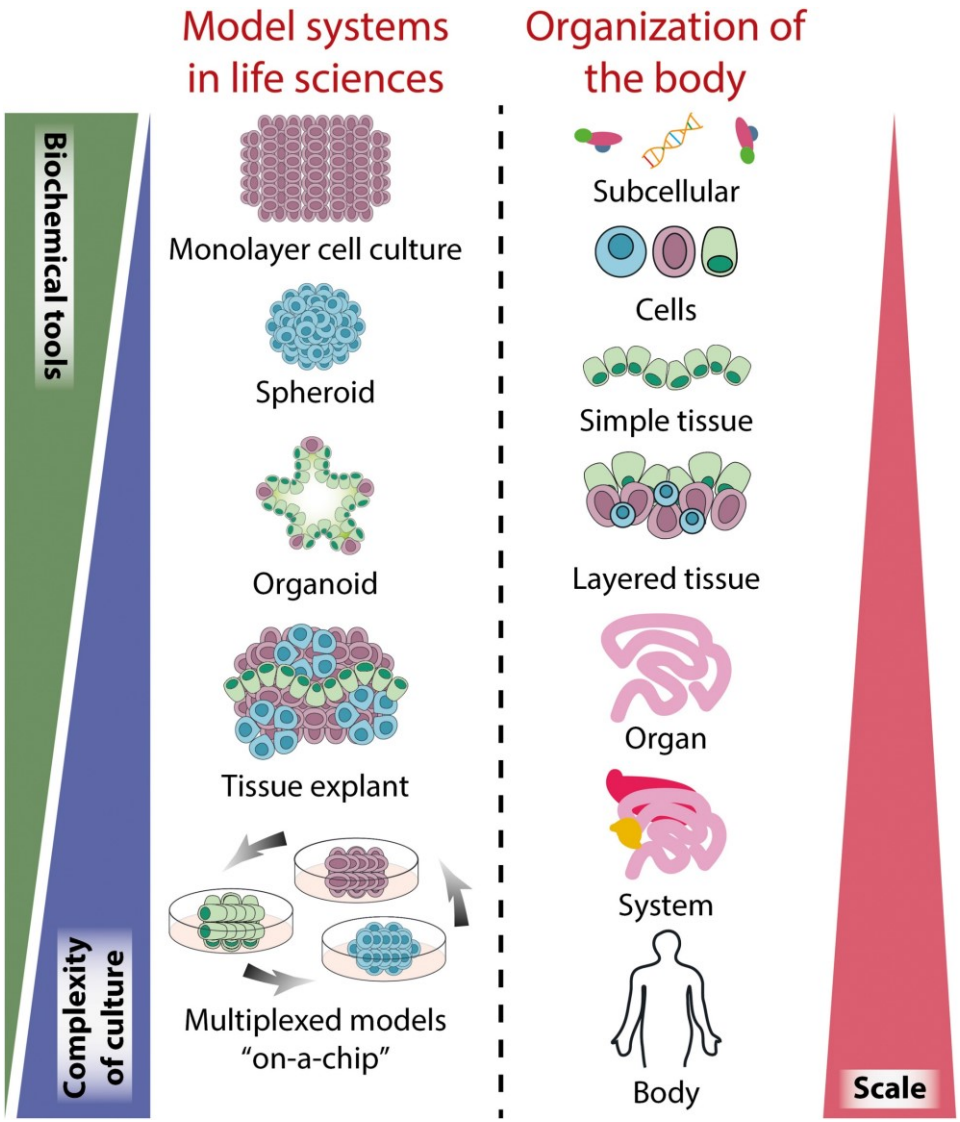

Methods of 3D organotypic culture
Applications in senescence or ageing research
Key:
Current
Potential
Tissue explants
Whole organ
Culture of organ or tissue e.g. bovine intervertebral disc
Harvest tissues from donors of different ages or post-chemotherapy.
Organ section/slice
Precision-cut e.g. lung slices
Explanted adipose tissue from obese donors treated with senolytics.
Organoids
Spontaneous cell aggregation
e.g. breast cancer organoids
Treatment of organoids, such as cancer, with senolytics.
Stem cell organoids
Formation of vessel e.g. optic cup
Investigate mechanisms of senescence in development.
Proof-of-principle compound screening in Alzheimer's disease stem-cell derived neurospheroids.
3D structures and scaffolds
Structural protein polymerisation
Cells suspended in polymerising liquid matrix e.g. Matrigel®
Addition of senescent cells to 3D models to investigate influence on tissues.
Treatment of 3D skin organotypics with senolytics.
Commercially available constructs
e.g. MelanoDerm™
Stress-induced premature senescent cells in MelanoDerm™ to investigate influence on tissue and subsequent treatment with senolytics.
3D bioprinting
e.g. CAD and bioink
Accurate addition of senescent cells to group of proliferating cells to investigate paracrine effects.
Decellularised tissue
e.g. isolated dermal matrix
Senescent-cell derived matrices to investigate influence of senescent matrix on cells.
On-a-chip
Chambers linked with microfluidics to mimic blood flow e.g. PhysioMimix™
Investigate the paracrine effect of the SASP between chambers.
Triculture model of Alzheimer's disease.



The goals of the regenerative rehabilitation approach are to synergize regenerative medicine

LF Rose et al. The convergence of regenerative medicine and rehabilitation, npj Regenerative Medicine (2018) 19

Model Systems in the Life Sciences

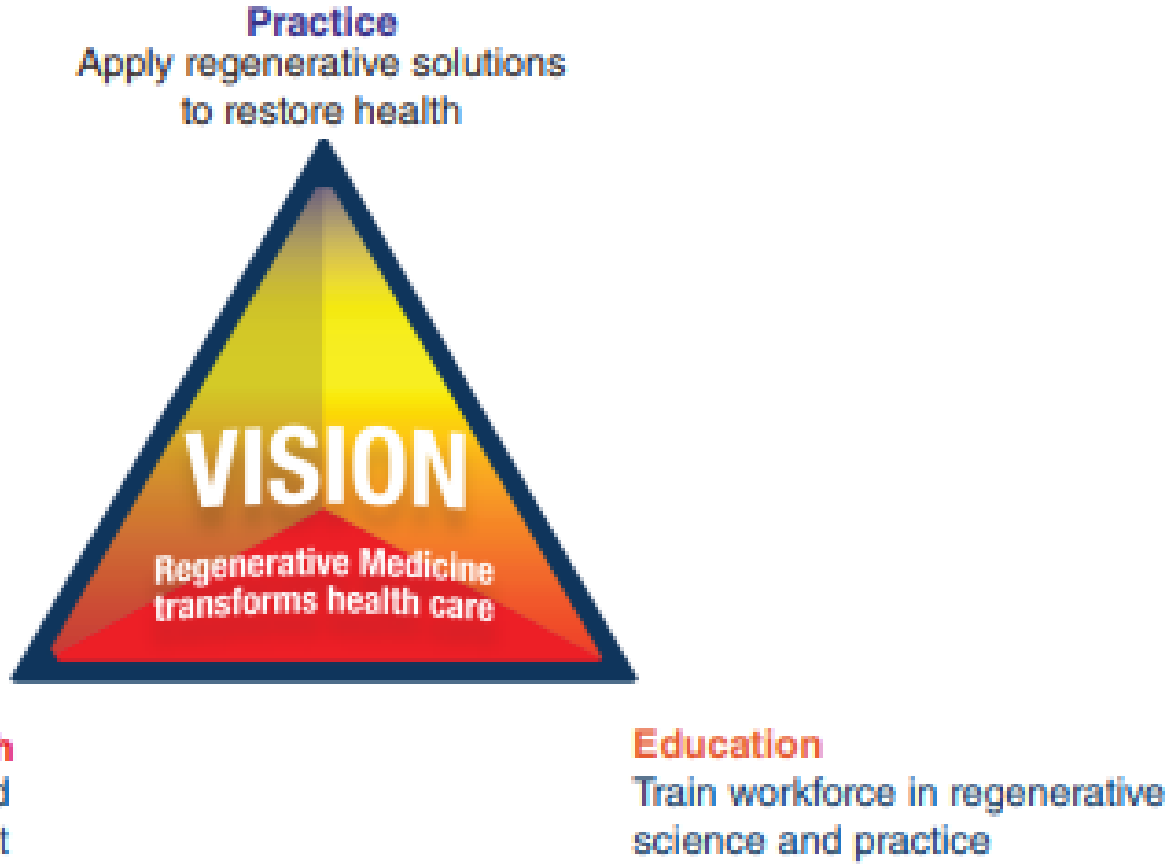


Cell PRESS

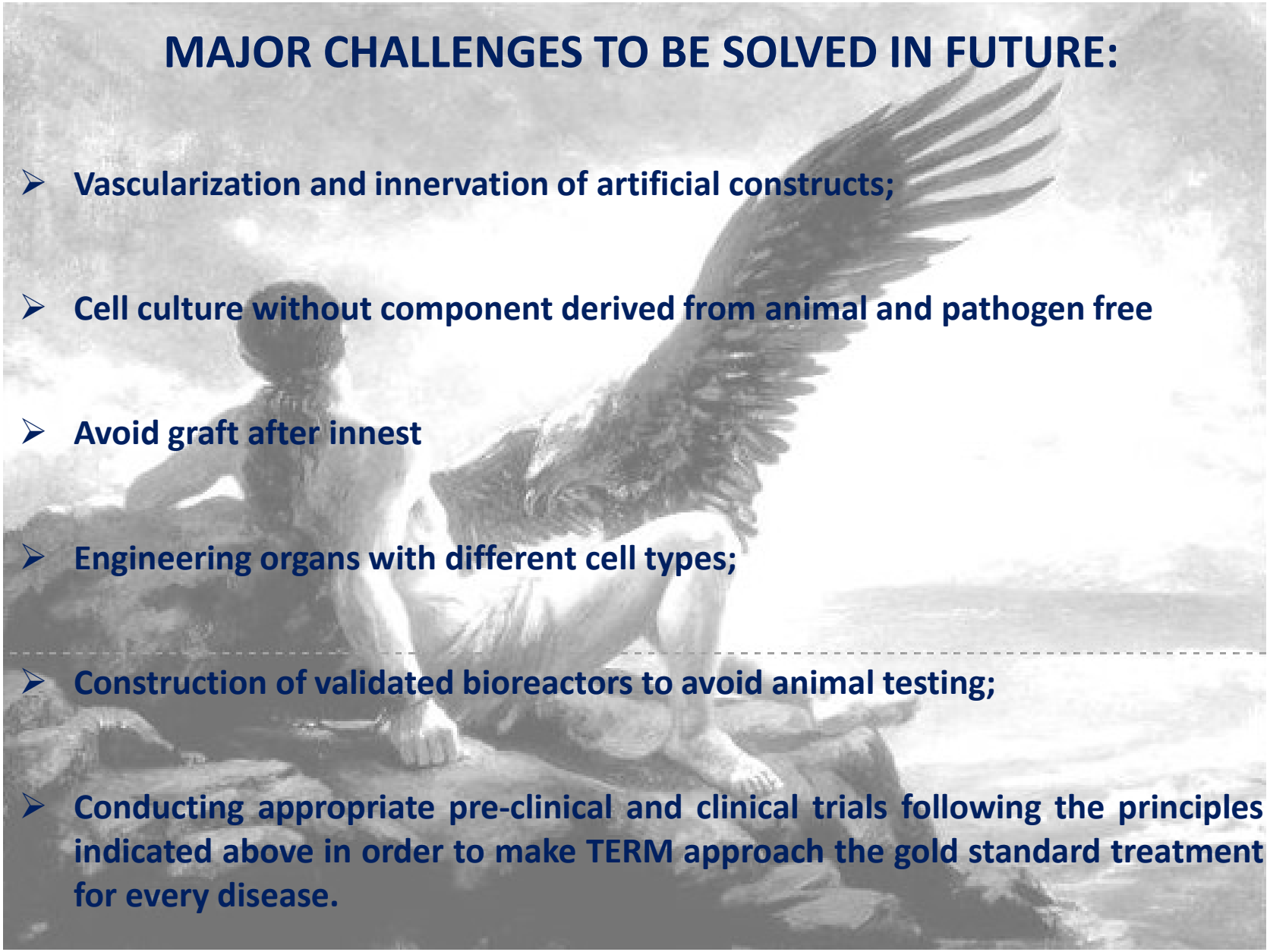
Cell Stem Cell 2016 18, 25-38DOI: (10.1016/j.stem.2015.12.005)

Take home messages…

Regenerative Medicine Perspectives

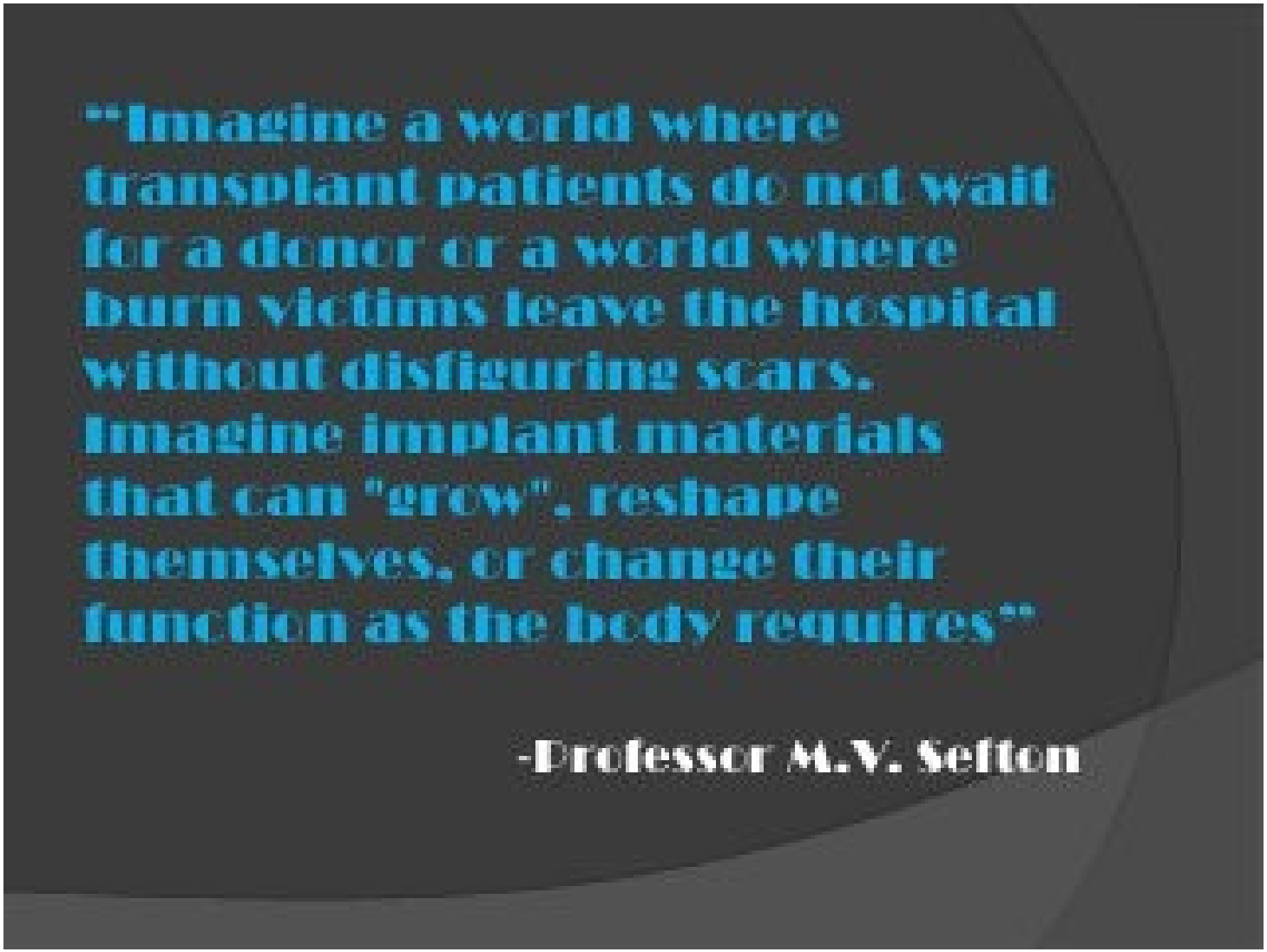


Regen. Med. (2021) 16(3)

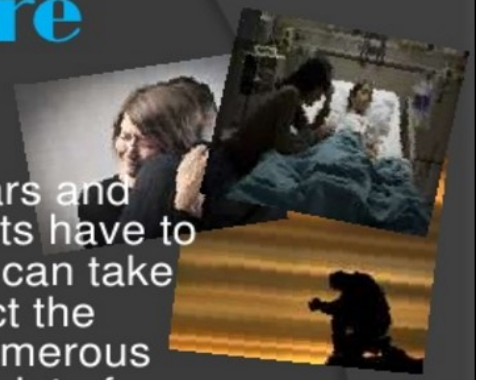


MAJOR CHALLENGES TO BE SOLVED IN FUTURE:

- Vascularization and innervation of artificial constructs;
- Cell culture without component derived from animal and pathogen free
- Avoid graft after innest
- Engineering organs with different cell types;
- Construction of validated bioreactors to avoid animal testing;
- Conducting appropriate pre-clinical and clinical trials following the principles indicated above in order to make TERM approach the gold standard treatment for every disease.


"Imagine a world where transplant patients do not wait for a donor or a world where burn victims leave the hospital without disfiguring scars. Imagine implant materials that can "grow", reshape themselves, or change their function as the body requires"
-Professor M.V. Sefton

(Before and After Tissue Engineering) What the Future will look like

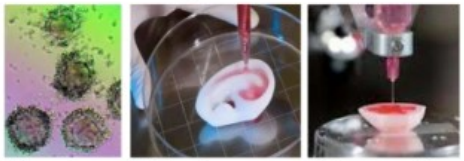


Before: Victims of burns and severe injuries have permanent scars and disfiguration. People with organ defects, for example heart defects have to wait until someone dies and can provide a heart transplant. This can take years and years and there is a chance that their body could reject the transplant. People with these defects may have to go through numerous surgeries even before having a transplant which can cost them a lot of money. Many lives are lost while waiting for an organ donor and from rejection of the transplant. Tons of money is spent for research on tissue engineering and researchers are continuing to find a way to create more complex organs. Tissues and organs, illnesses are hard to treat.

After: People will not have to wait long periods of time before their organ or tissue transplants because they will not need to rely on organs from others. They will not have to worry about their body rejecting their new organ because it will be created using their own tissue cells. Patients will only have to undergo one surgery. Their organ or tissue will have a permanent function. Many lives will be saved and improved by this technology. Burned victims will be easily treated and their skin able to recover. Bones, cartilage you name it can all improved. Common problems like arthritis will all be treated. Researchers will be able to continue their research from these discoveries and perhaps discover more. People will be able to buy lab created organs and tissues.

Futuristic!

Stem Cells + Organ Scaffold + 3D Printer



= Libraries of Replacement Organs?

The elixir of life may still be evading us but the human "spare parts industry" is waiting in the wings to sustain man's desire for eternity.

"THE BEST WAY TO BE BORING IS TO LEAVE NOTHING OUT"

Voltaire