Bone Tissue Engineering: Current Trends

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NewGen Training School on "Non Living Materials meet living Biology", May 9-12, 2017, Patras, Greece

Tissue Engineering

- Interdisciplinary field that applies the principles of engineering and life sciences to the development of biological substitutes that restore, maintain or augment tissue function
- An alternative to drug therapy, gene therapy and whole organ transplantation
- “Grow” organs in the lab

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What is Tissue Engineering?

"The science of persuading the body to regenerate or repair tissues that fail to regenerate or heal spontaneously"

C. M. Agrawal

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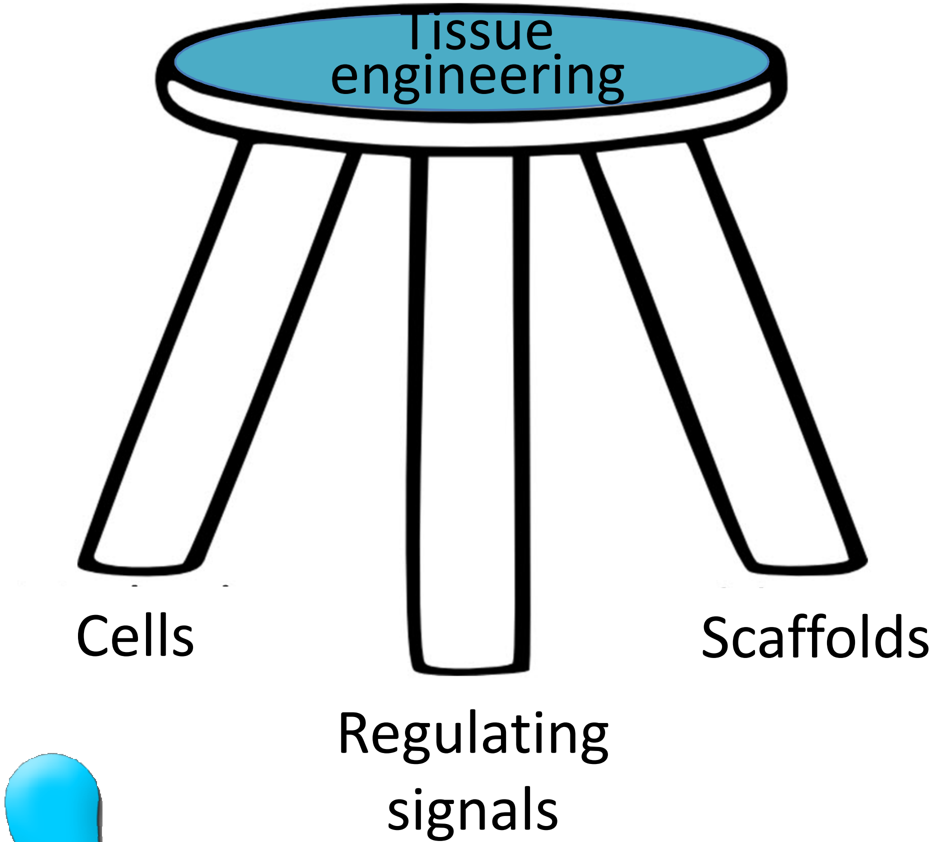
Three legged stool…



1. Progenitor cells for **osteogenesis**,
2. Growth factors and other biochemical cues for **osteo-induction**
3. An **osteo-conductive** scaffold

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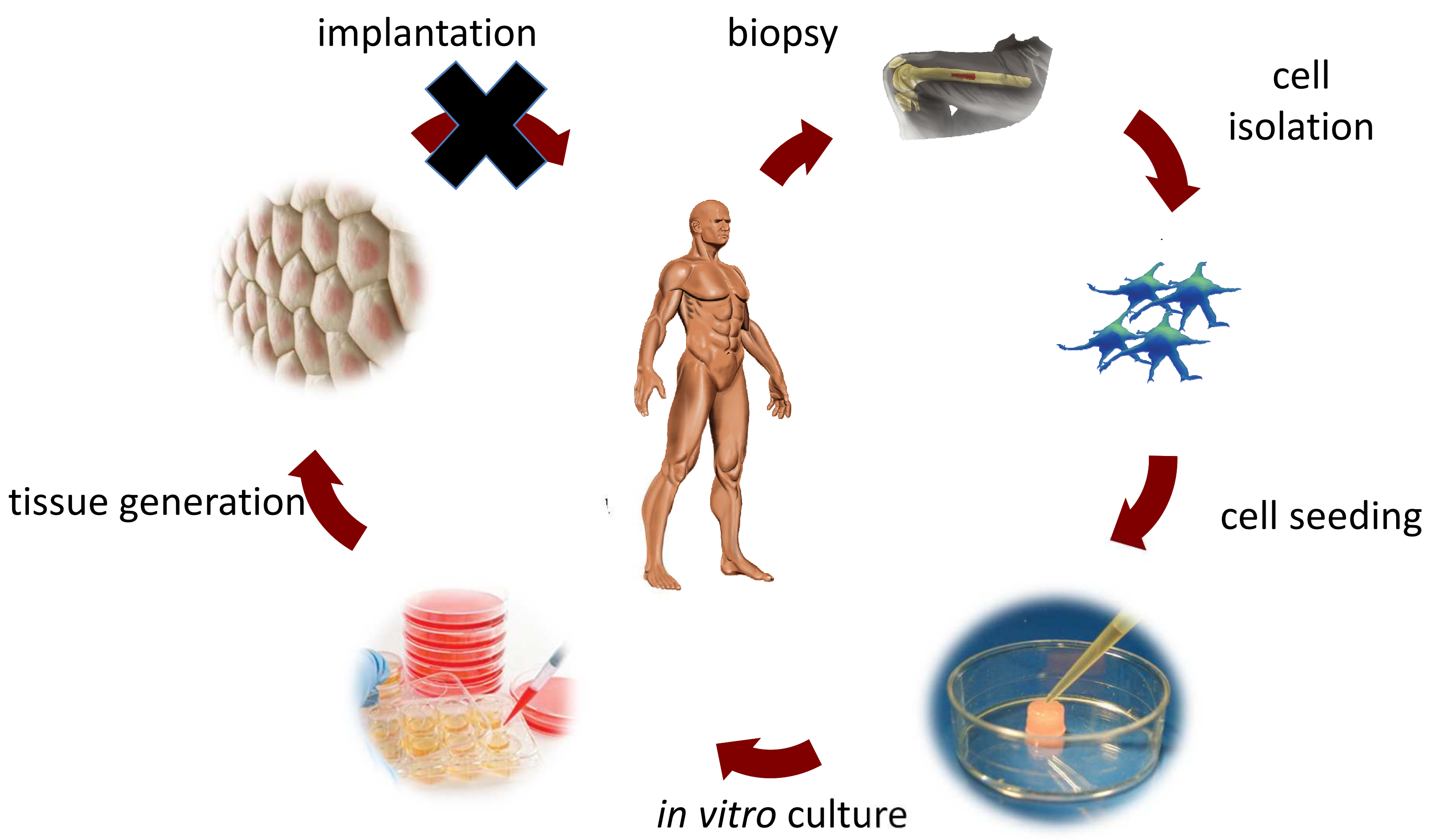
Tissue engineering



"Personalized" medicine by tissue engineered disease models

- Personalized medicine is a medical model that proposes the customization of healthcare - with medical decisions, practices, and/or products being tailored to the individual patient.
- Patient derived stem cells carry the genetic and epigenetic factors specific to the individual, and allow for design of personalized treatment strategies.

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Important Variables

- Delivery
  - Cell Suspensions
  - Tissue-like constructs (scaffolds)
- Chemical properties
  - Growth factors, cytokines
  - ECM surface
  - Degradation particles
- Physical properties
  - Microstructure
  - Topography
  - Rigidity
  - Mechanical Loading
  - Electro-magnetic loading

<u>Modify Cell Behavior</u>

Survival
Organization
Migration
Proliferation
Differentiation

Optimize Cellular Response

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Clinical Questions

- What cell source do you use?
- How should cells be delivered?
- What cells within that pool are beneficial?
- How many cells do you need?
- When should you deliver the cells?
- What type of scaffold should be used?
- What type of stimulants should be used?

The answers all depend on each other !!!

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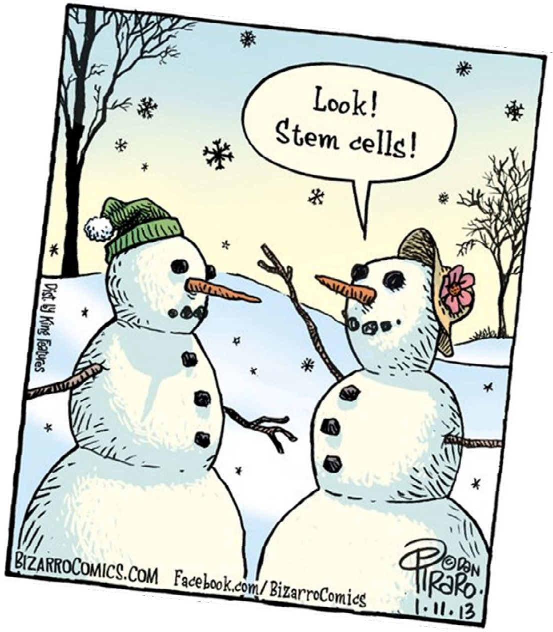
Cells


Look! Stem cells!
Dist. by King Features
BIZARROCOMICS.COM Facebook.com/BizarroComics

Stem and Progenitor Cells

- Long-term self-renewal
- Environment-dependent differentiation
- Classifications
  - Embryonic Stem Cells
  - Fetal Stem Cells
  - Adult Stem Cells
  - Induced Pluripotent Stem (IPS) Cells

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Adult Stem Cells

Strengths

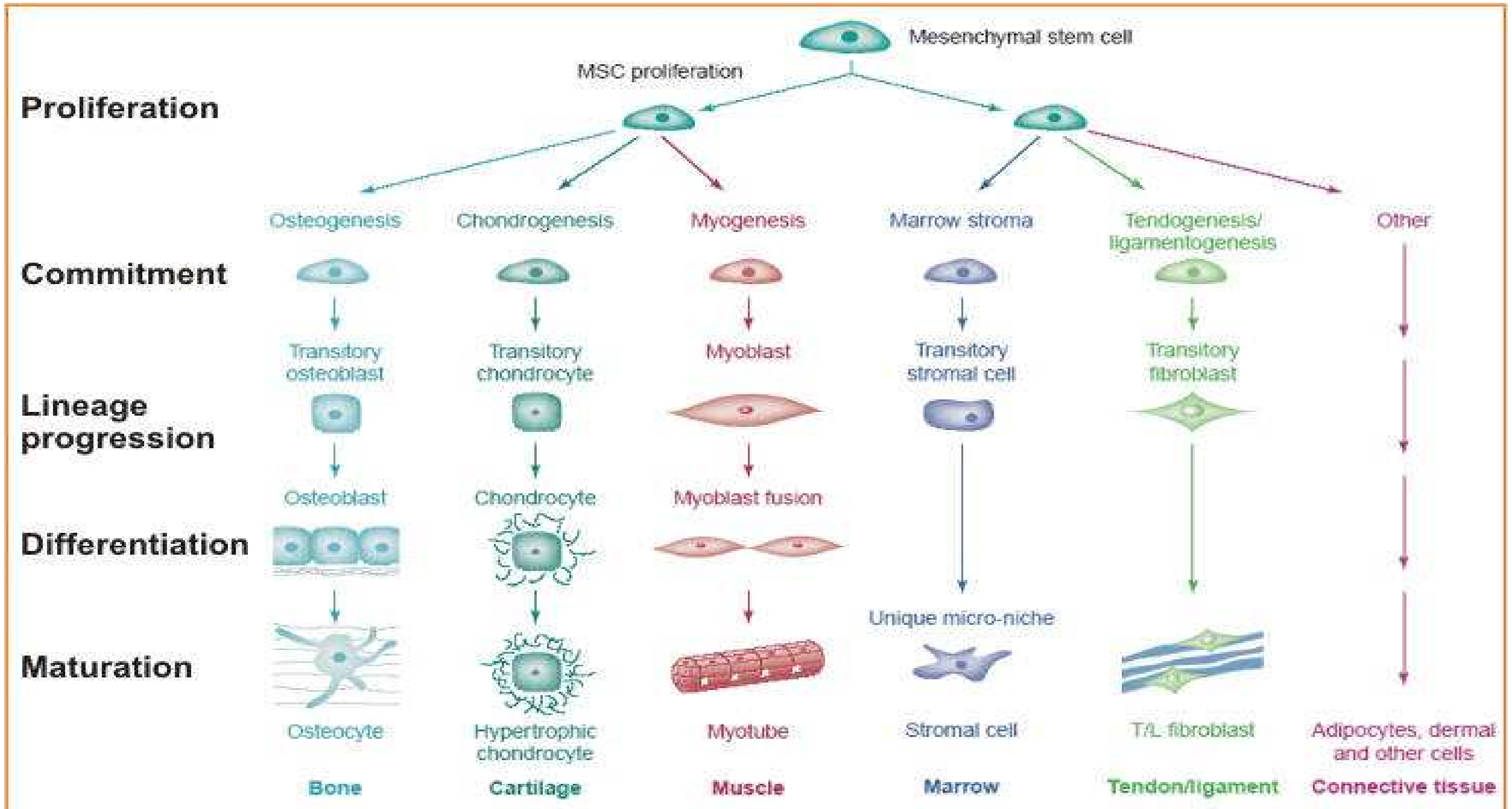
- Ethics, not controversial
- Immune-privileged
  - Allogenic transplantation
- Many sources
  - Most somatic tissues

Limitations

- Differentiation Capacity?
- Self-renewal?
- Rarity among somatic cells

Mesenchymal Stem Cells

- Easy isolation, high expansion, reproducible



From Caplan & Bruder, TIMM 7:259

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Potential Solutions

- Differentiation Capacity
  - Mimic stem cell niche
- Limited Self-renewal
  - Gene therapy
- Limited availability
  - Fluorescence-activated cell sorting
  - Adherence
    - Heterogenous population works better clinically

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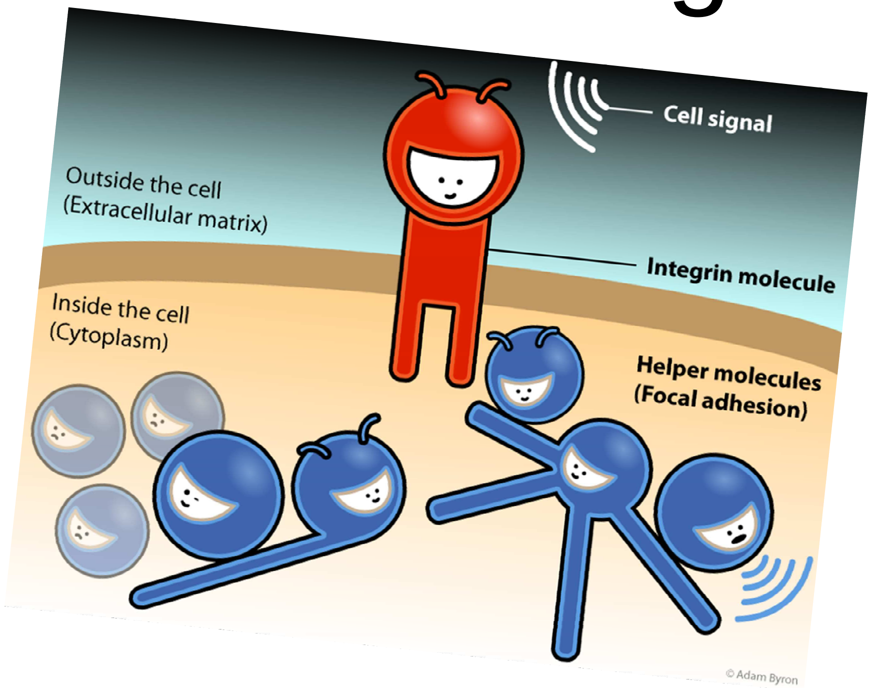
Signals



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Soluble Chemical Factors

- Transduce signals
  - Cell type-dependent
  - Differentiation stage-dependent
    - Timing is critical
  - Dose-dependence
- Adhesion
- Growth
- Survival
- Motility
- Differentiation
- Gene expression
- Secretion and action of other growth factors

Factor	Cell or Tissue of Origin	Selected Target Cells or Tissue
EGF	macrophages, monocytes	epithelium, endothelial cells
FGF	monocytes, macrophages, endothelial cells	endothelium, fibroblasts, keratinocytes
GMCSF	macrophages, fibroblasts, endothelial cells	hematopoietic, inflammatory cells, neutrophils, fibroblasts
HGH	pituitary gland	hepatocytes, bone, fibroblasts
IL-1	lymphocytes, macrophages, keratinocytes	monocytes, neutrophils, fibroblasts, keratinocytes
PDGF	platelets, macrophages, neutrophils, smooth muscle cells	fibroblasts, smooth muscle cells
TGF-β	platelets, bone, most cell types	fibroblasts, endothelial cells, keratinocytes, lymphocytes, monocytes

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Cytokines

- Currently 100+ have been discovered, 20 different families based on structural homology
- Not stored as preformed molecules
- Require proteolytic activation
- May need to bind to ECM for activity and stabilization
- Characterized by short biological half lives (PDGF, 2 minutes in blood for example)

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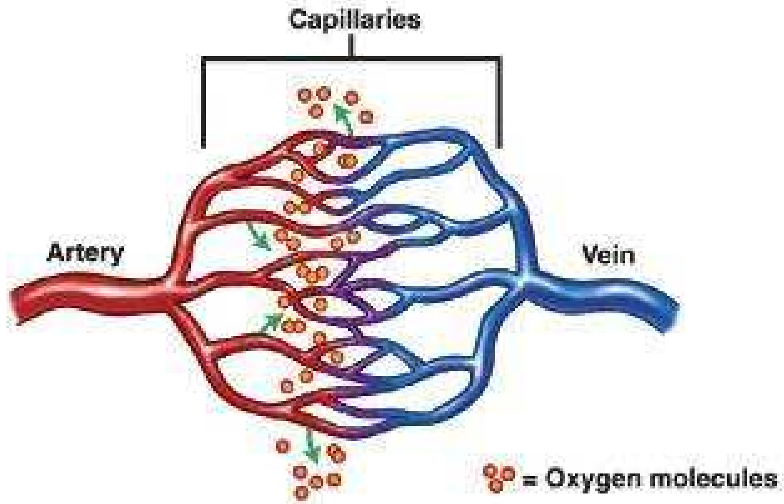
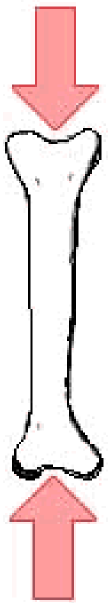
Mechanical Forces

- Flow-induced shear stress
  - Laminar blood flow
  - Rhythmic pulses
- Uniaxial, Equiaxial stretch
  - Magnitude
  - Frequency

<u>Mechanotransduction</u>

Conversion of a mechanical stimulus into a biochemical response





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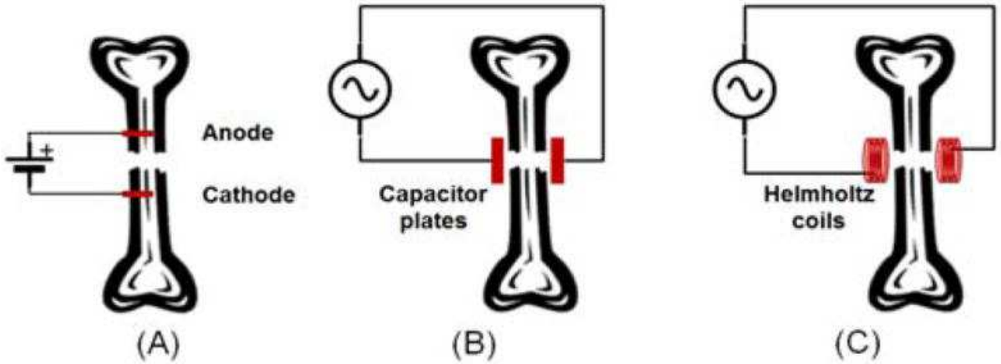
Electro-magnetic Forces

- Our cells communicate (also) with electrical signals
- Neurons, endocrine and muscle cells are called "excitable cells" - they respond to electrical and/or electro (magnetic) fields.



Ramirez-Vick, JSM Biotechnol Biomed Eng. 2013; 1(2): 1014.

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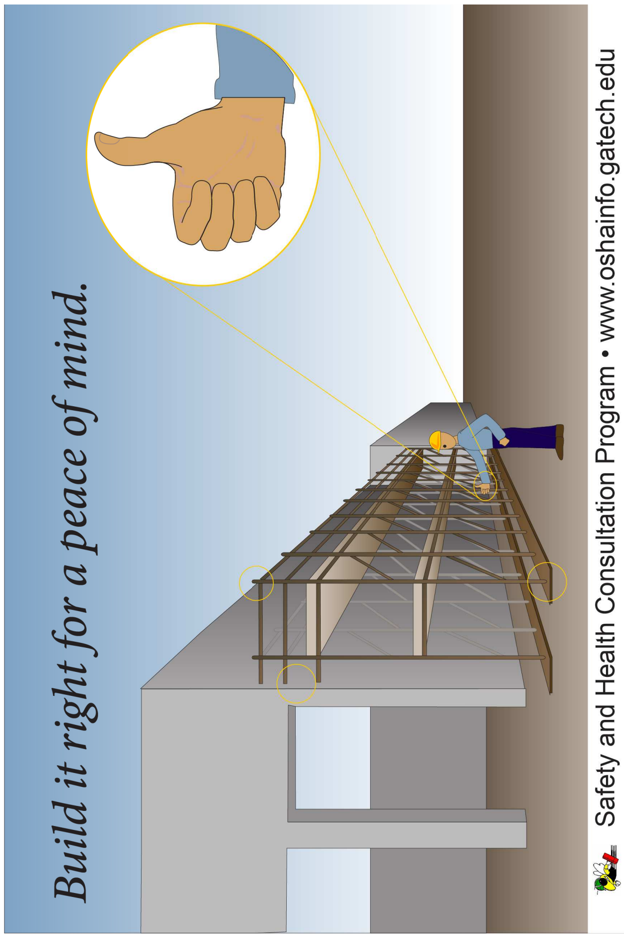
Scaffolds


Build it right for a peace of mind.
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Scaffolds for Tissue Generation

Purpose: replace functions of extracellular matrix (ECM):

- cell anchorage
- cell orientation
- cell growth
- mechanical integrity
- tissue microenvironment
- cell differentiation
- sequester, store & present soluble proteins
- blueprint for tissue organization

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Summary

- Right combination of cells, scaffolds, and factors depends on the clinical problem

- EXTENSIVE PHYSICIAN/SCIENTIST/ENGINEER COLLABORATION IS VITAL FOR SUCCESS

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Bone Tissue Engineering

Clinical Motivation:

- Engineered tissues can restore lost bone without the need for autografts or allografts (traumatic, degenerative, cancer, or congenital malformation).
- More than two million bone graft procedures Are performed annually worldwide

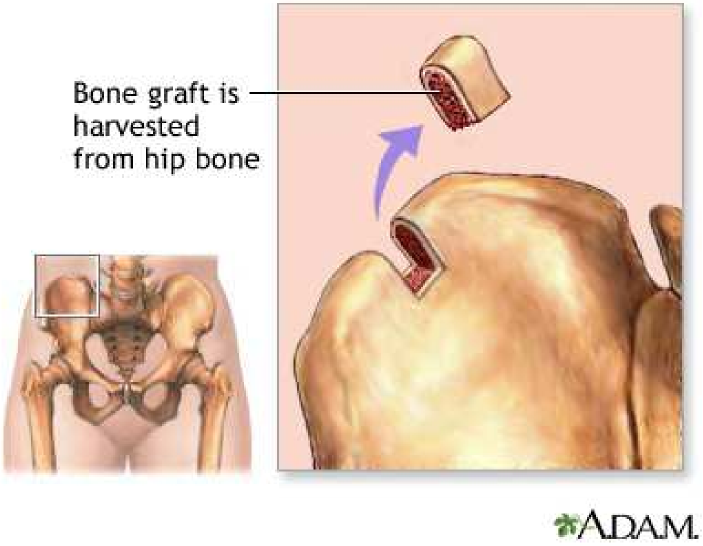

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Table 1. List of some of the most successful commercially available bone tissue-engineered products along with their description.

No.	Company	Product	Product description
1	Medtronic Inc., Minneapolis, MN, USA	Infuse® bone graft	Solution containing rhBMP-2 and an ACS used to fill the LT-CAGE® lumbar tapered fusion device
2	Baxter International Inc., Deerfield, IL, USA	Actifuse	Silicate substituted calcium phosphate
3	Orthovita Inc. Malvern, PA, USA	Vitoss	Bioactive glass and calcium phosphate
4	Olympus Biotech, Hopkinton, MA, USA	OP-1® Putty	rhBMP-7, Type I bovine collagen matrix and putty additive carboxymethylcellulose sodium
		OP-1 Implant	Osteoinductive and osteoconductive bone graft material consisting of collagen matrix, 1 g of Type I bovine collagen, 3.3 mg of rhBMP-7 and 2–3 cc of saline
5	DePuy Orthopaedics, Inc., Warsaw, IN, USA	Vertigraft® Allograft	Variety of structural allografts and an array of osteobiological properties, including, but not limited to, VG1 ALIF, VG2 PLIF, VG2 RAMP and VG2 TLIF
		Symphony® I/C Graft Chambers	Osteoinductive and osteoconductive product comprised of a mixture of allograft cancellous chips and DBM. Also contains naturally occurring cascade of BMP’s and growth factors.
		Restore® Orthobiologic Soft Tissue Implant	Resorbable scaffold to reinforce weakened or damaged soft tissue repair including rotator cuff, patellar, Achilles, biceps, quadriceps and other tendons
		Optium DBM® System	Includes both Optium DBM® Gel and Putty. Both contain osteoinductive and osteoconductive properties
		Healos® Fx Injectable Bone Graft Replacement	An osteoconductive and easy to use bone graft replacement which provides a scaffold. It has potentially osteogenic properties when autologous bone marrow is added.
		Healos Bone Graft Replacement	Osteoconductive matrix comprised of cross-linked collagen fibers that are fully coated with hydroxyapatite. When combined with BMA, product provides an environment for osteoprogenitor cell attachment, proliferation, and differentiation
		Conduit® TCP Granules	Osteoconductive synthetic porous ceramic graft material made of tricalcium phosphate which provides a scaffold for bone cell attachment

ACS: Absorbable collagen sponge; BMA: Bone marrow aspirate; DBM: Demineralized bone matrix: allograft bone particle.

Mishra, Regen. Med. (2016) 11(6), 571–587

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- The most common form of BTE products used clinically are mixes of polymer and ceramic ingredients to form either moldable putties or injectable pastes employed to fill small, subcritical size bone defects or voids.
- Almost all of these biomaterials are intended for defects which will not immediately incur significant loads (e.g., dental extraction fillers).

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Why has BTE not fulfilled its potential, yet?

- Bone is an inherently complex and dynamic form of mineralized collagenous tissue that remodels throughout life to adapt to mechanical stress.
- The biggest challenge of BTE has been:
  - Lack of hierarchical microstructure that can adapt to the dynamic *in vivo* environment,
  - The vascular supply that is required for the high demand of O_2 and nutrients through this adaptation.

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Current trends to accelerate utilisation of BTE products in clinical environments :

1. Bioinspired scaffold fabrication techniques;
2. Injectable polymeric or peptide hydrogel systems;
3. Pre-vascularisation strategies with co-culture systems;
4. Bioreactor systems that mimic the mechano-environment of bone;
5. Utilisation of induced pluripotent stem (IPS) cells and other gene therapy approaches.

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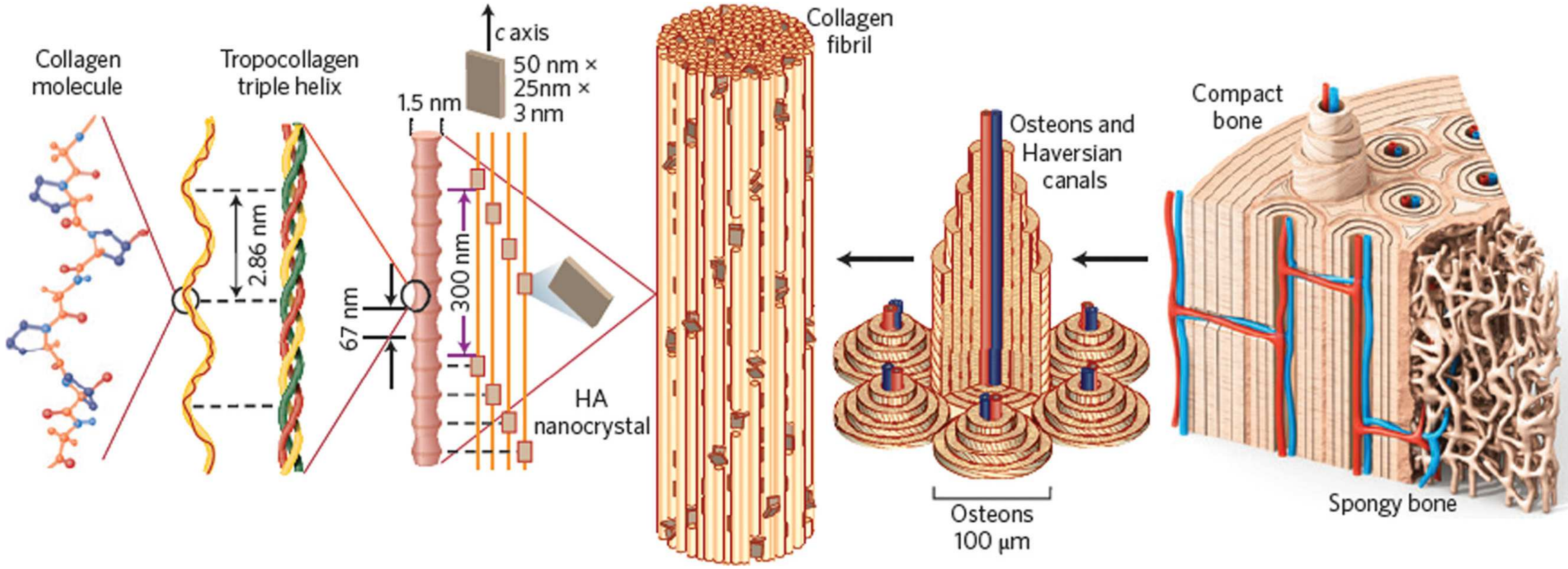
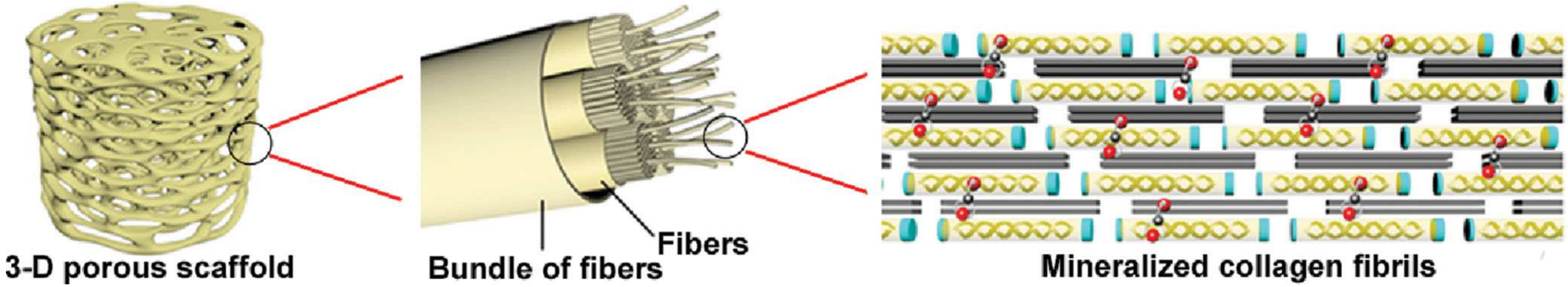
Hierarchical bone structure

- Hierarchical arrangement in bone produces nanomechanical heterogeneities, which enable a mechanism for high energy dissipation and resistance to fracture.



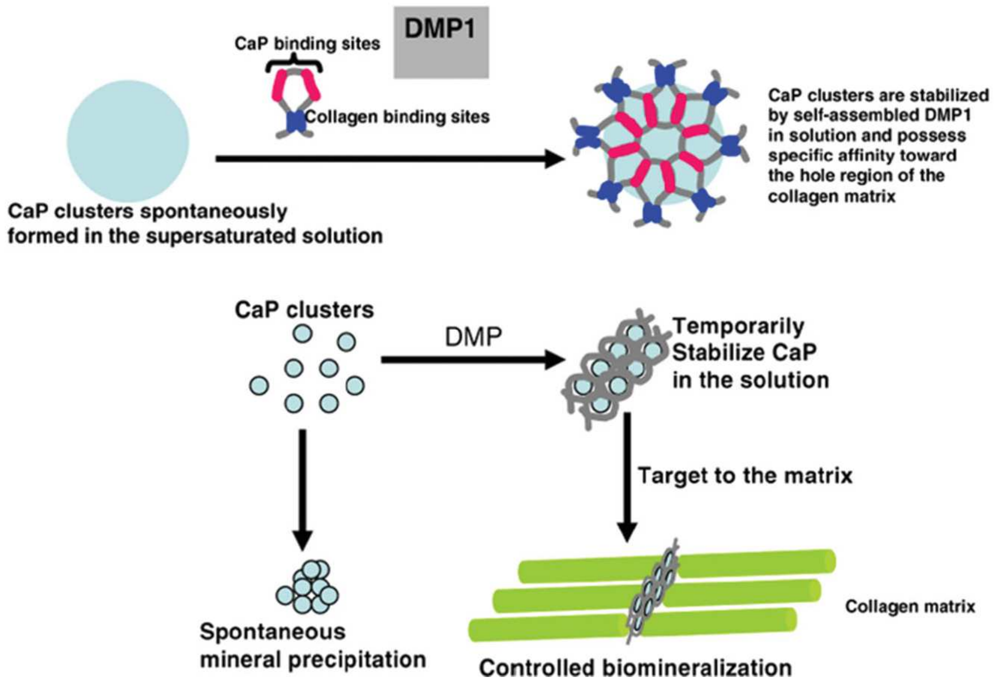
Wegst , Nat. Mater. 2015 , 14 , 23 .

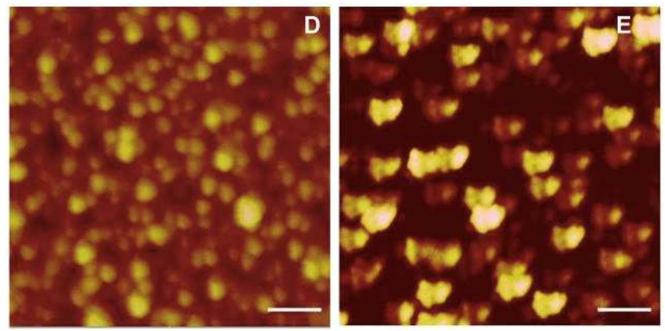
Bioinspired scaffold fabrication

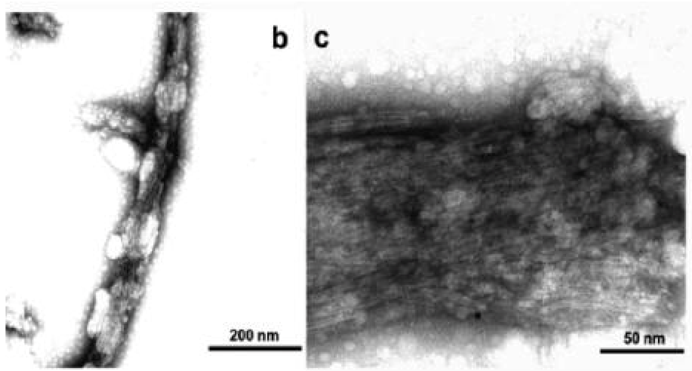


Liu, et.al., Small 2016, 12, No. 34, 4611–4632

Bioinspired scaffold fabrication

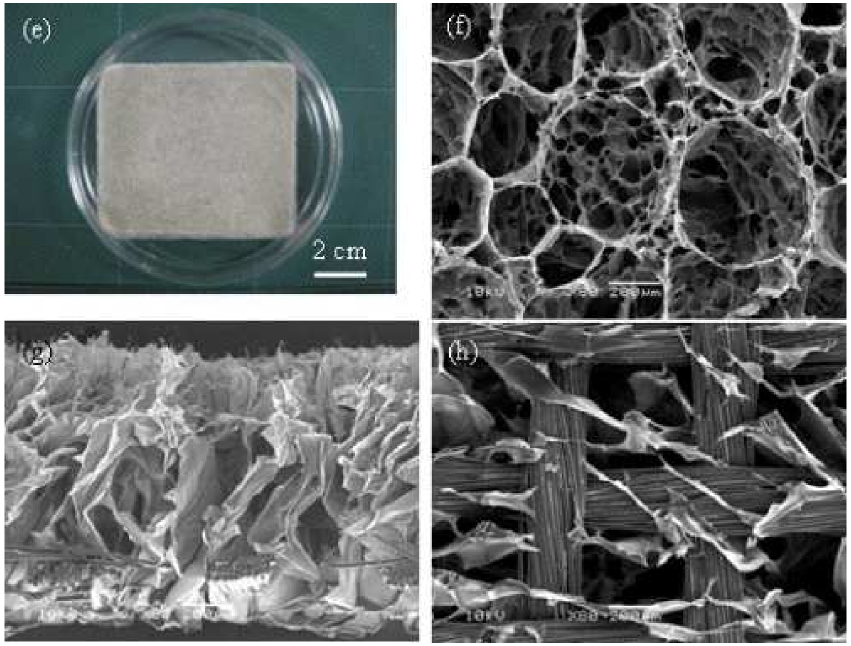




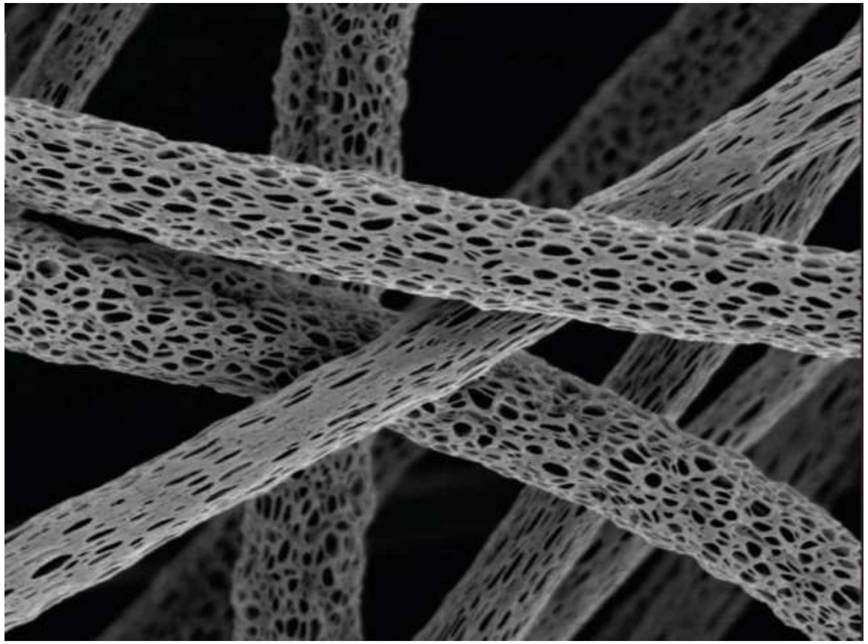


He, G.; Biochemistry 2005, 44, 16140.

Bioinspired scaffold fabrication



Lu, et.al. 2012, Science and Technology of Advanced Materials, 13.



Z. Rezvani et al. Nanomedicine: Nanotechnology, Biology, and Medicine 12 (2016) 2181–2200

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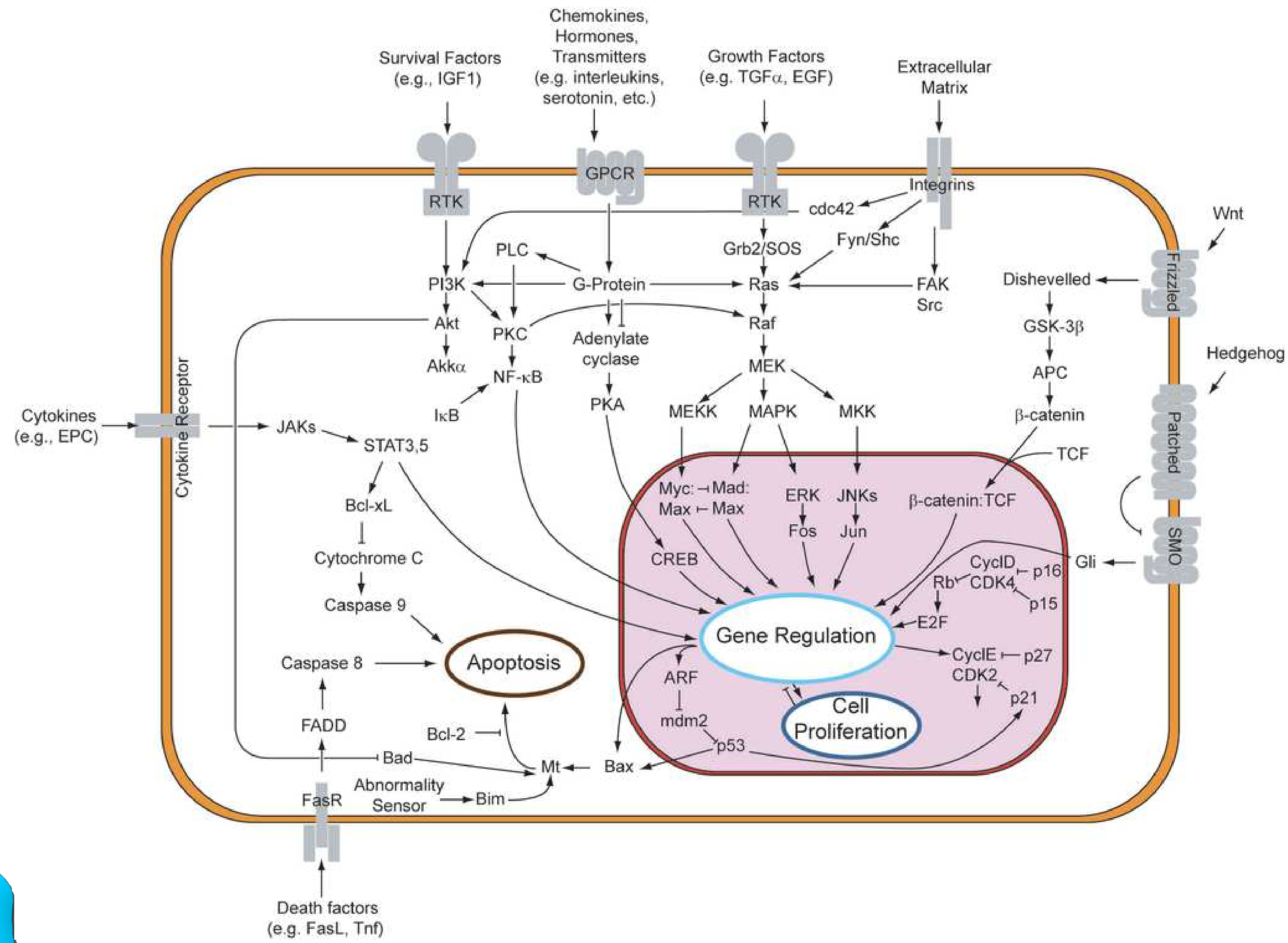
Peptides for BTE

- Molecular signals in the form of growth factors are the main modulators of cell behavior.
- However, the use of growth factors in tissue engineering has several drawbacks, including:
  - their costs,
  - difficult production,
  - Immunogenicity
  - short half-life



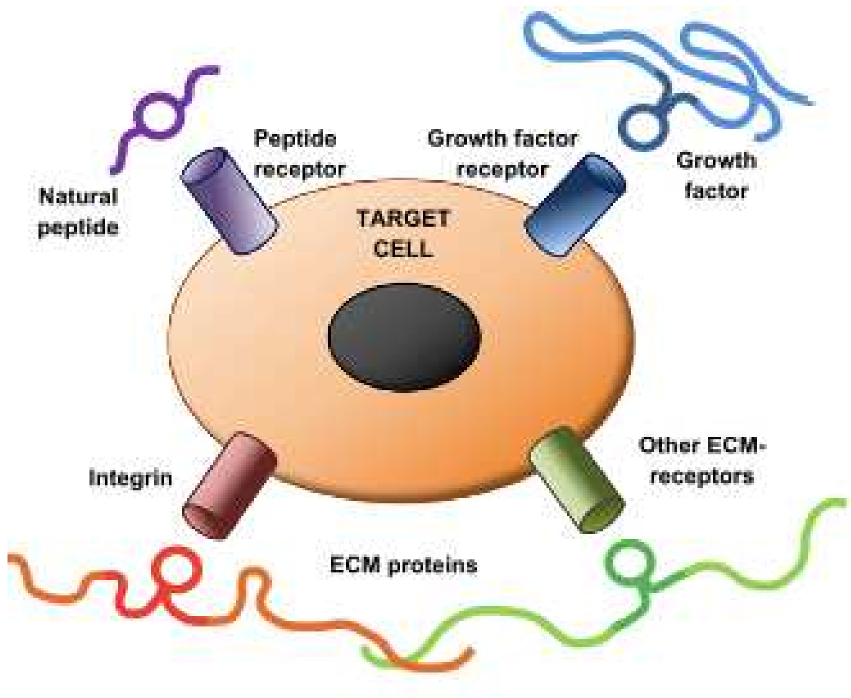
http://en.wikipedia.org/wiki/Paracrine_signalling

Peptides for BTE

- Biomimetic peptides are sequences derived from the active domains of soluble or extracellular matrix proteins;
- They can be used to functionalize the scaffolds;
- These short peptides can be easily designed and cost-effectively synthesized *in vitro*.

Peptides for BTE

- Peptides involved in cell adhesion
- Osteoinductive peptides
- Angiogenic peptides
- Cathelicidins



Visser, at.al., Journal of Controlled Release 244 (2016) 122–135

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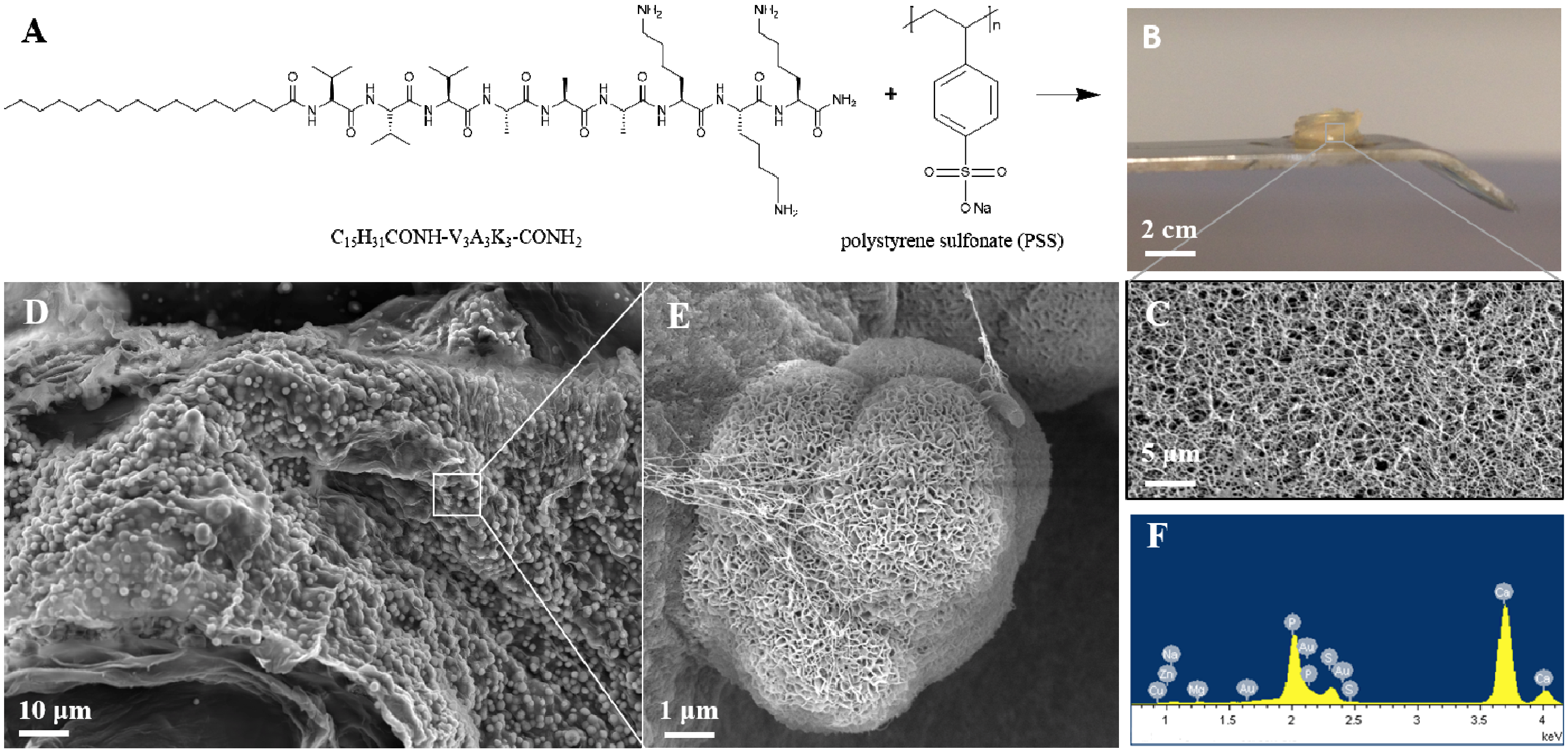
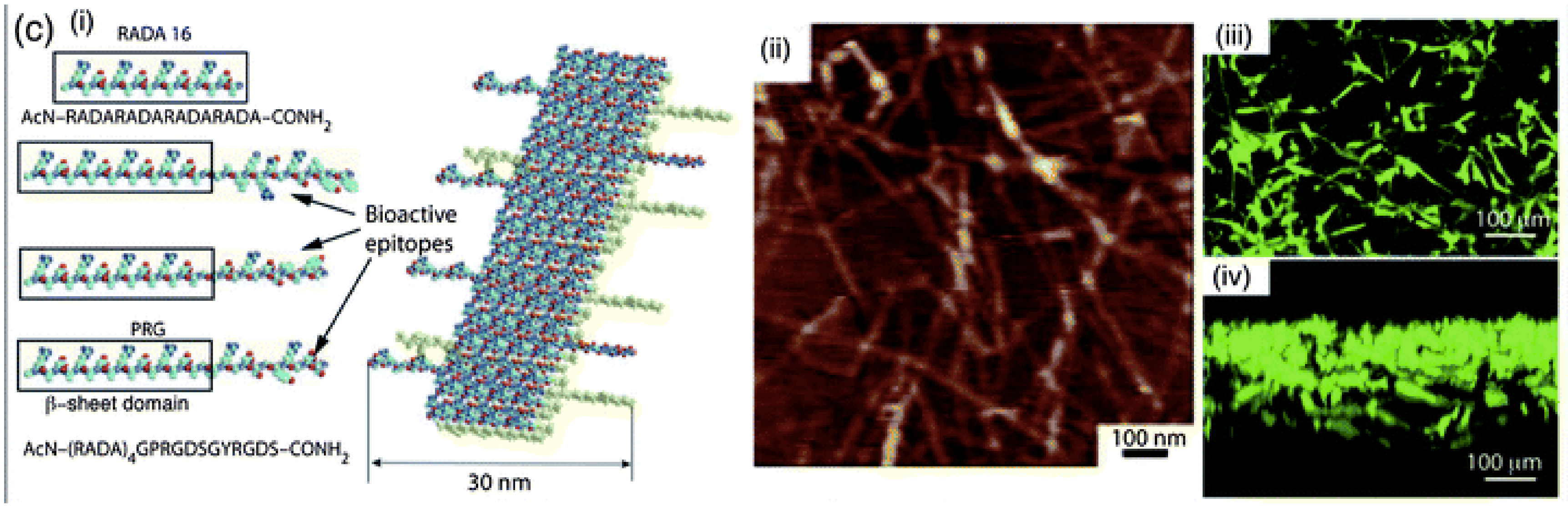
Injectable peptide hydrogels



Radvar, et.al. (2016). Front. Bioeng. Biotechnol. doi: 10.3389/conf.FBIOE.2016.01.02610

Injectable self-assembled peptide hydrogels

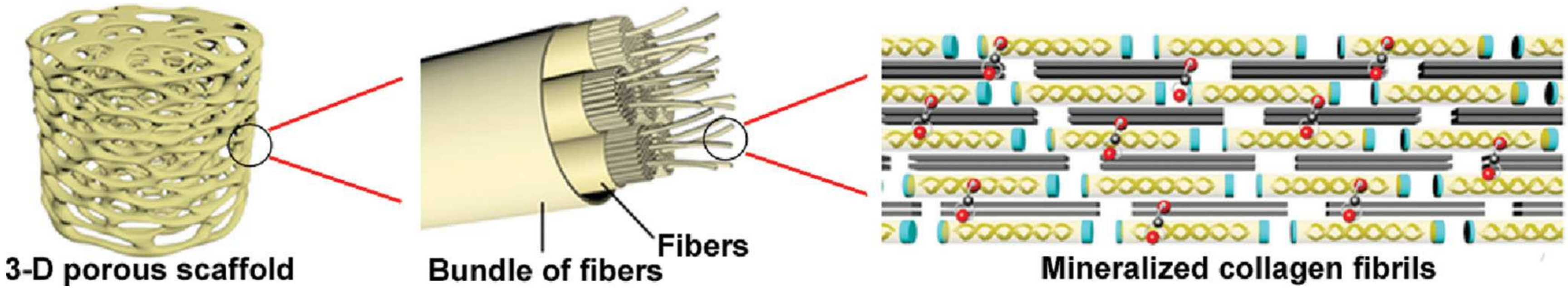
Injectable self-assembled peptide hydrogels



A. Horii,et.al., PLoS One, 2007, 2, e190

Combined with Bioprinting or 3D printing?

- Complex pore geometries can be fabricated and rendered in ways that no other technique can easily or affordably replicate



Liu, et.al., Small 2016, 12, No. 34, 4611–4632

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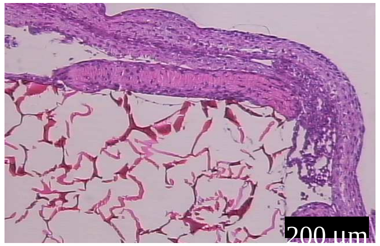
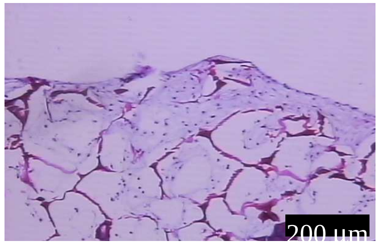
Pre-vascularization strategies

Challenge:

- Due to lack of vasculature, cells within the scaffolds either die or migrate toward the periphery of the scaffolds during *in vitro* culture period.
- Cells must be located within 150 – 200μm from a blood supply *in vivo* in order to survive.





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Current Approaches for Vascularization in BTE:

- Delivery of angiogenic growth factors

- Transplantation of mature or precursor endothelial cells with or without a vascularized pattern

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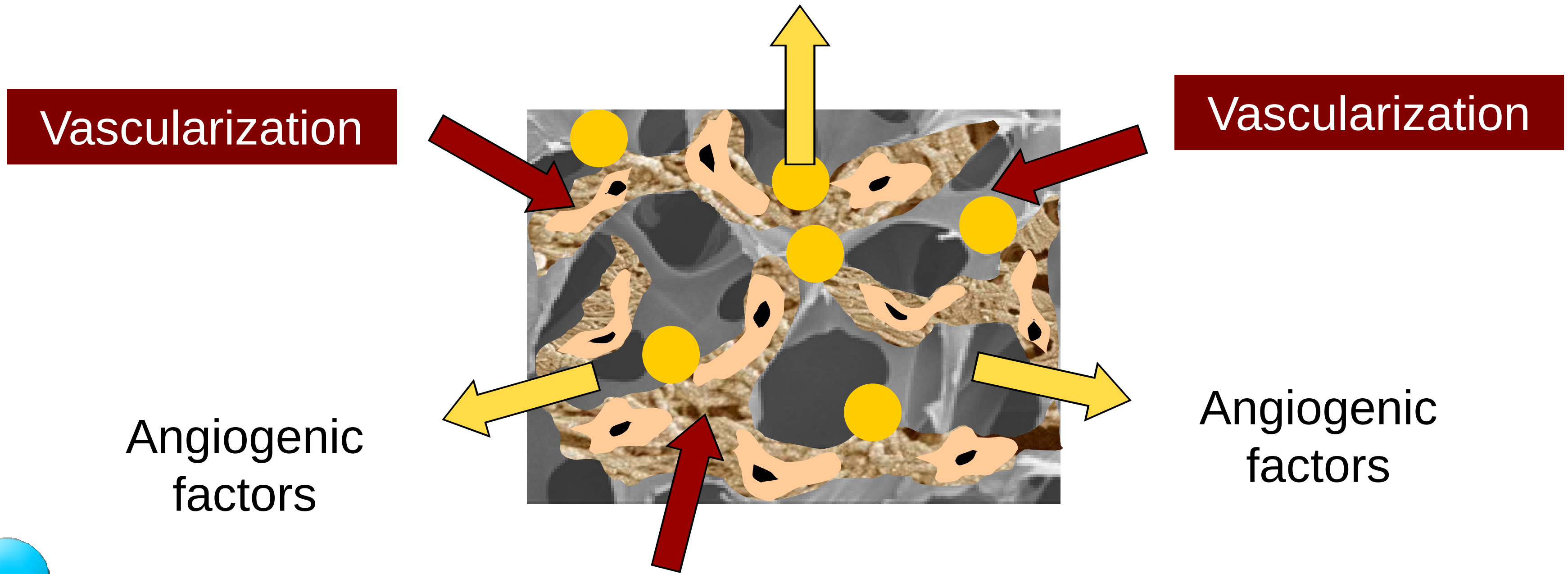
Previous experience:

Delivery of osteoblastic cells and matrix together with an angiogenic factors



Major drawbacks of direct growth factor delivery approaches:

- Short half-life
- Toxicity of growth factors
- Limited control over distribution of factors can lead to neovascularization at undesired sites, rheumatoid arthritis and tumor growth!
- An alternative strategy for the delivery of angiogenic growth factors is *ex-vivo* gene therapy

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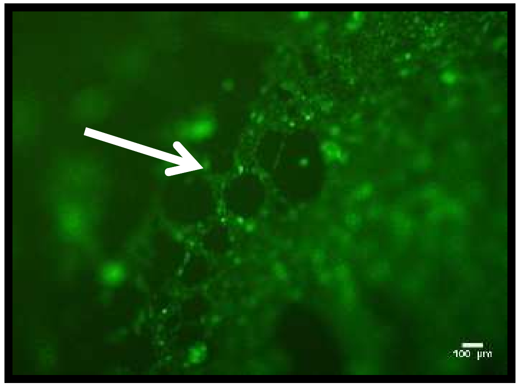
Pre-vascularized Tissue Engineering:

- Co-culture of osteoblastic cells (and others) with mature or progenitor endothelial cells (and smooth muscle cells) into scaffolds to serve as precursors of a vascular network



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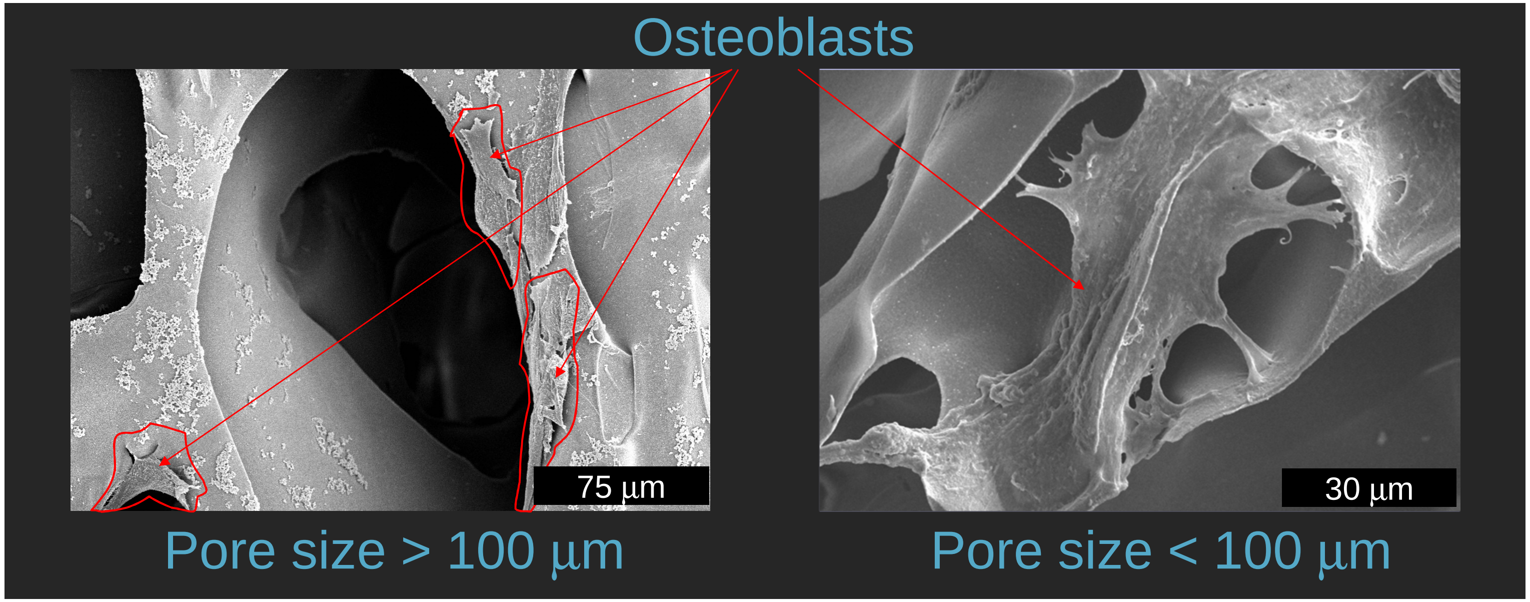
Advantages of Cell-based Strategies:

- Release of multiple factors controlled by environmental conditions,
- No requirement for large reservoirs of agents that could rupture prematurely
- The ability of the tissue engineered capillaries to integrate with the host vasculature and become functional perfused blood vessels

Effects of pore size for Pre-vascularized Tissue Engineering:

- Cell migration is essential for vascularization
- Sufficient pore size in the scaffold is required for cell migration and spreading



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Which type of endothelial cells?

- Classical choice: human umbilical vein endothelial cells (HUVECs)
  - phenotypically and functionally heterogeneous
  - cannot be isolated from adults
- Endothelial Progenitor Cells (EPCs)
  - More proliferative than HUVECs
  - Can be isolated from peripheral blood

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Co-culture techniques

- Mixed culture
- Patterned culture (bioprinting)
- Mixed-directed co-culture

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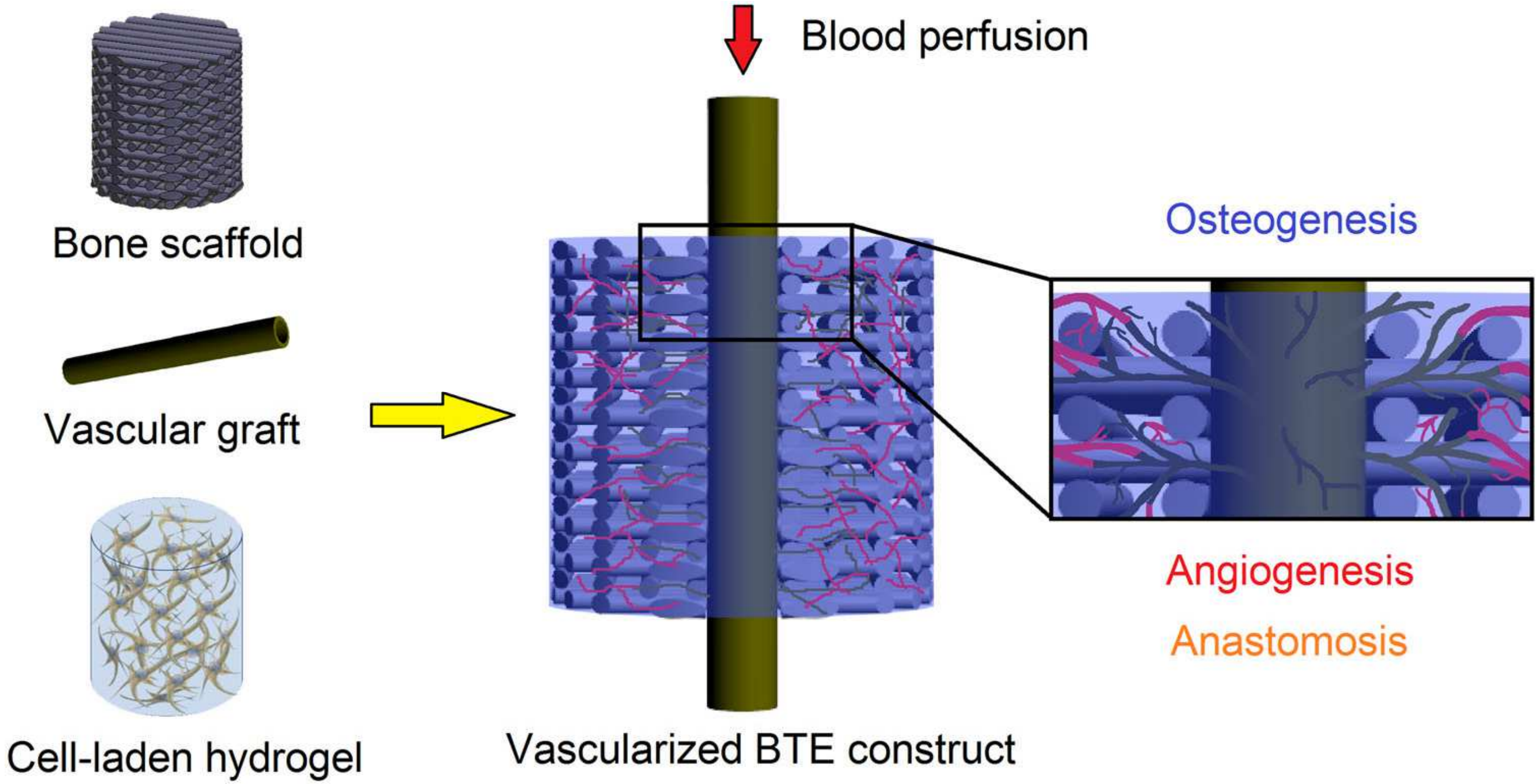
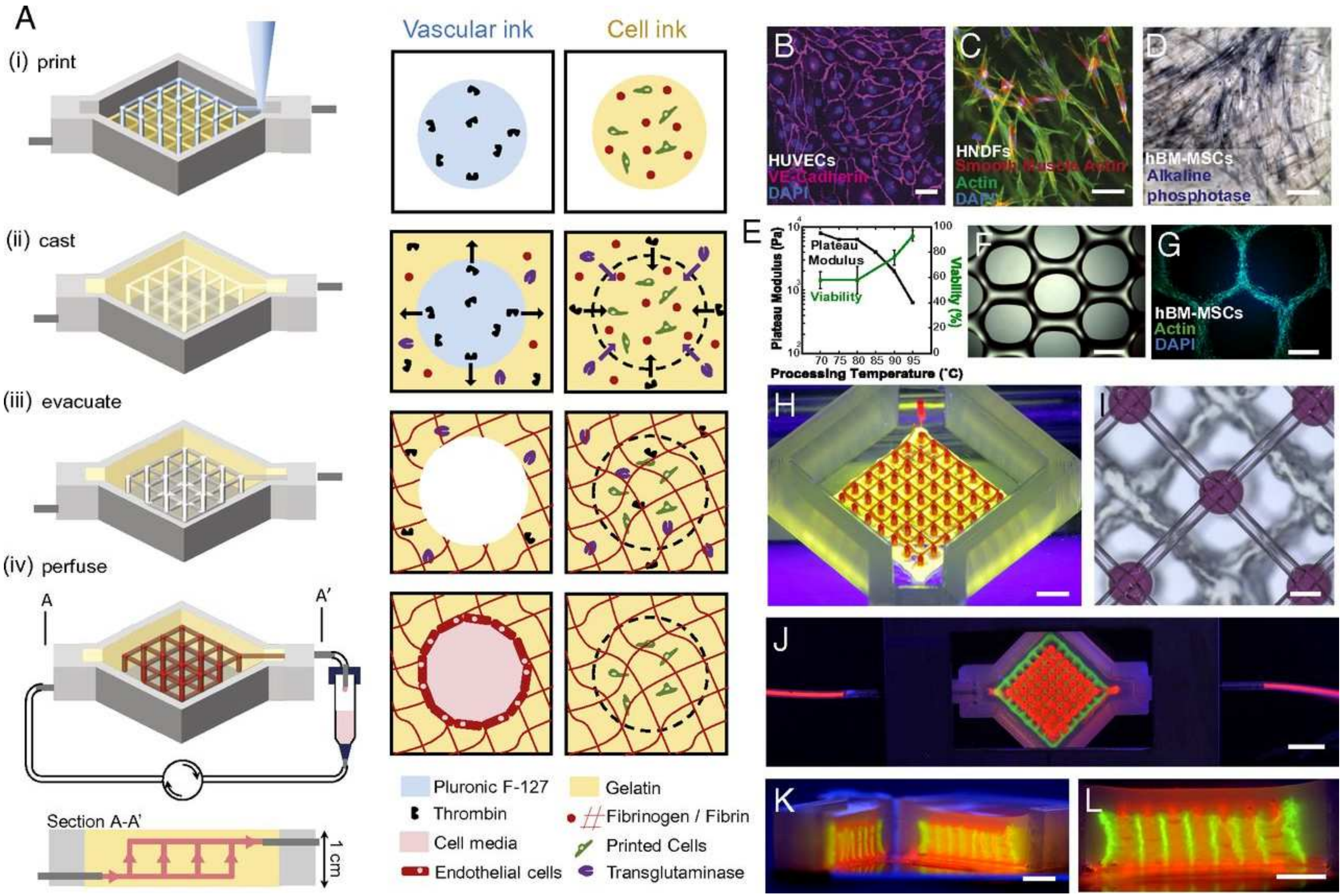


Mercado-Pagan, et.al. Annals of Biomedical Engineering, Vol. 43, No. 3, March 2015, pp. 718–729

Three-dimensional vascularized tissue fabrication.



David B. Kolesky et al. PNAS 2016;113:3179-3184

BTE Bioreactors

- The term “bioreactor” refers to a system in which conditions are closely controlled to permit or induce certain behaviour in living cells or tissues.
- Bioreactors can be used to aid in the *in vitro* development of new tissue by providing biochemical and physical regulatory signals to cells and encouraging them to undergo differentiation and/or to produce extracellular matrix prior to *in vivo* implantation

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Engineering Parameters in TE Bioreactor Design

Generally providing biochemical environment which is controlled by nutrient transfer e.g. glucose and dissolved oxygen to the cells along with metabolism products from the cells and biomechanical environment is of a bioreactor responsibility

- ***Mass Transfer through Bioreactors***
- ***Mechanical Stimulation***

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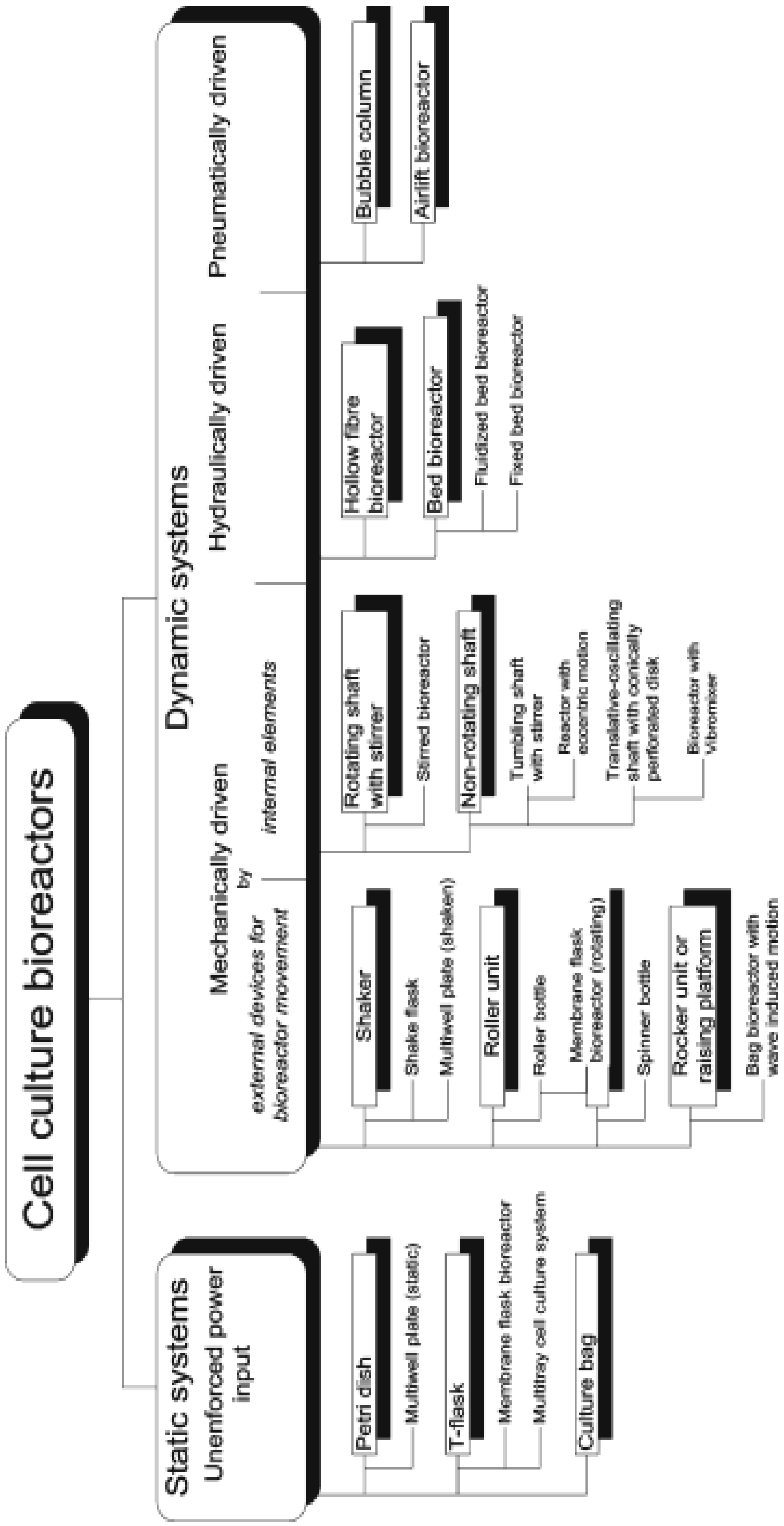
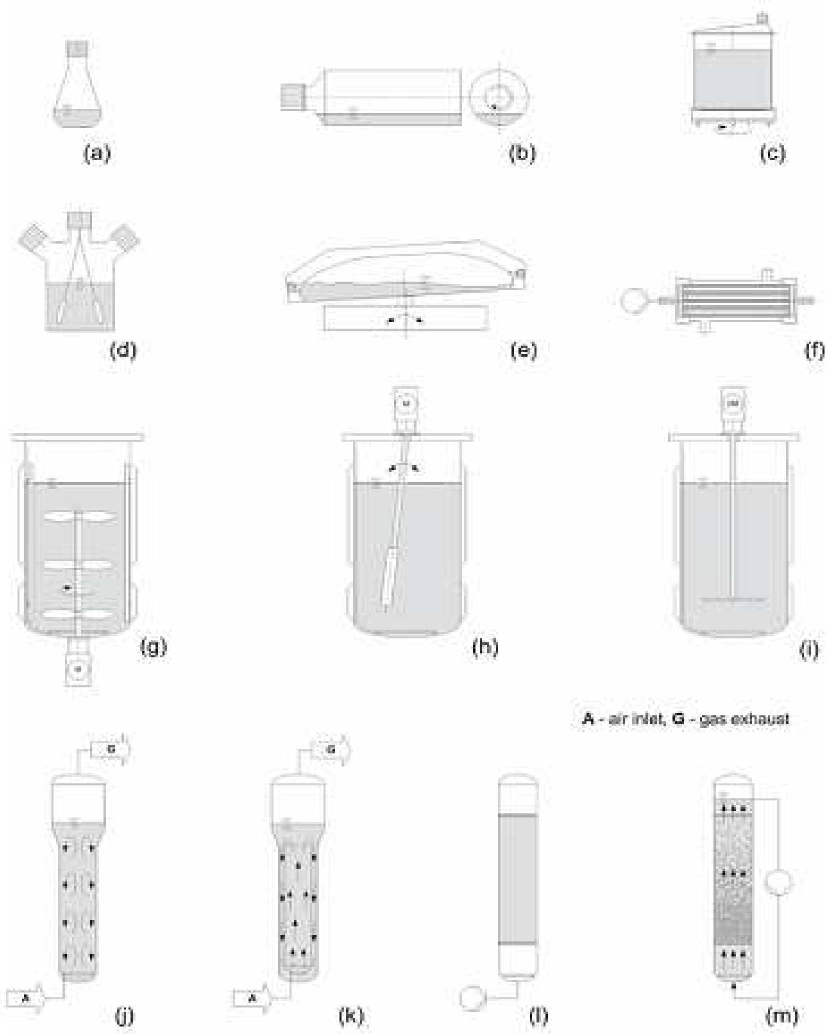
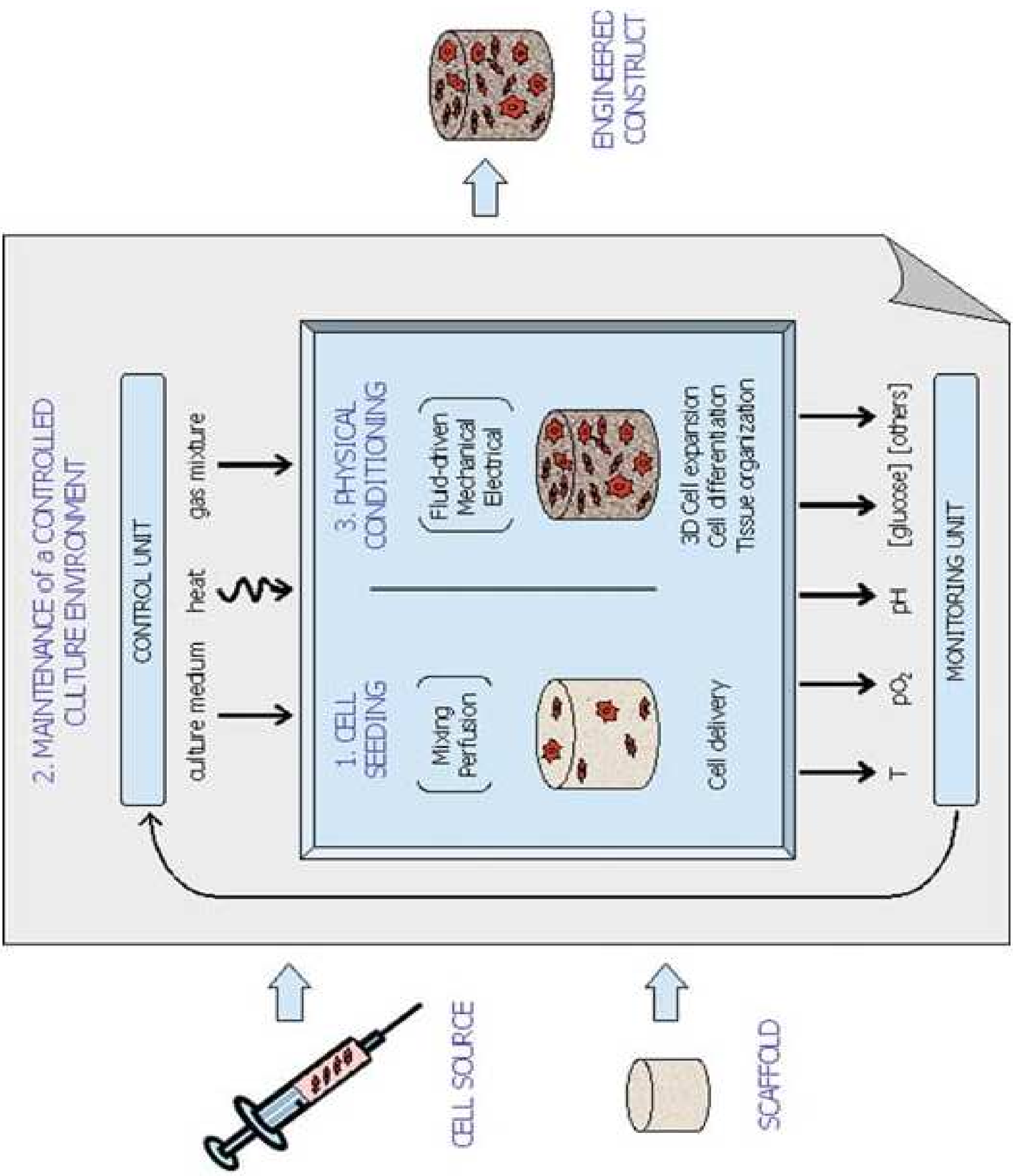

Cell culture bioreactors
Static systems
Unenforced power input
Petri dish
Multiwell plate (static)
T-flask
Membrane flask bioreactor
Multitray cell culture system
Culture bag
Dynamic systems
Mechanically driven
by
external devices for bioreactor movement
internal elements
Shaker
Shake flask
Multiwell plate (shaken)
Roller unit
Roller bottle
Membrane flask bioreactor (rotating)
Spinner bottle
Rocker unit or raising platform
Bag bioreactor with wave induced motion
Rotating shaft with stirrer
Stirred bioreactor
Non-rotating shaft
Tumbling shaft with stirrer
Reactor with eccentric motion
Translative-oscillating shaft with conically perforated disk
Bioreactor with Vibromixer
Hydraulically driven
Hollow fibre bioreactor
Bed bioreactor
Fluidized bed bioreactor
Fixed bed bioreactor
Pneumatically driven
Bubble column
Airlift bioreactor

Dynamic Bioreactors

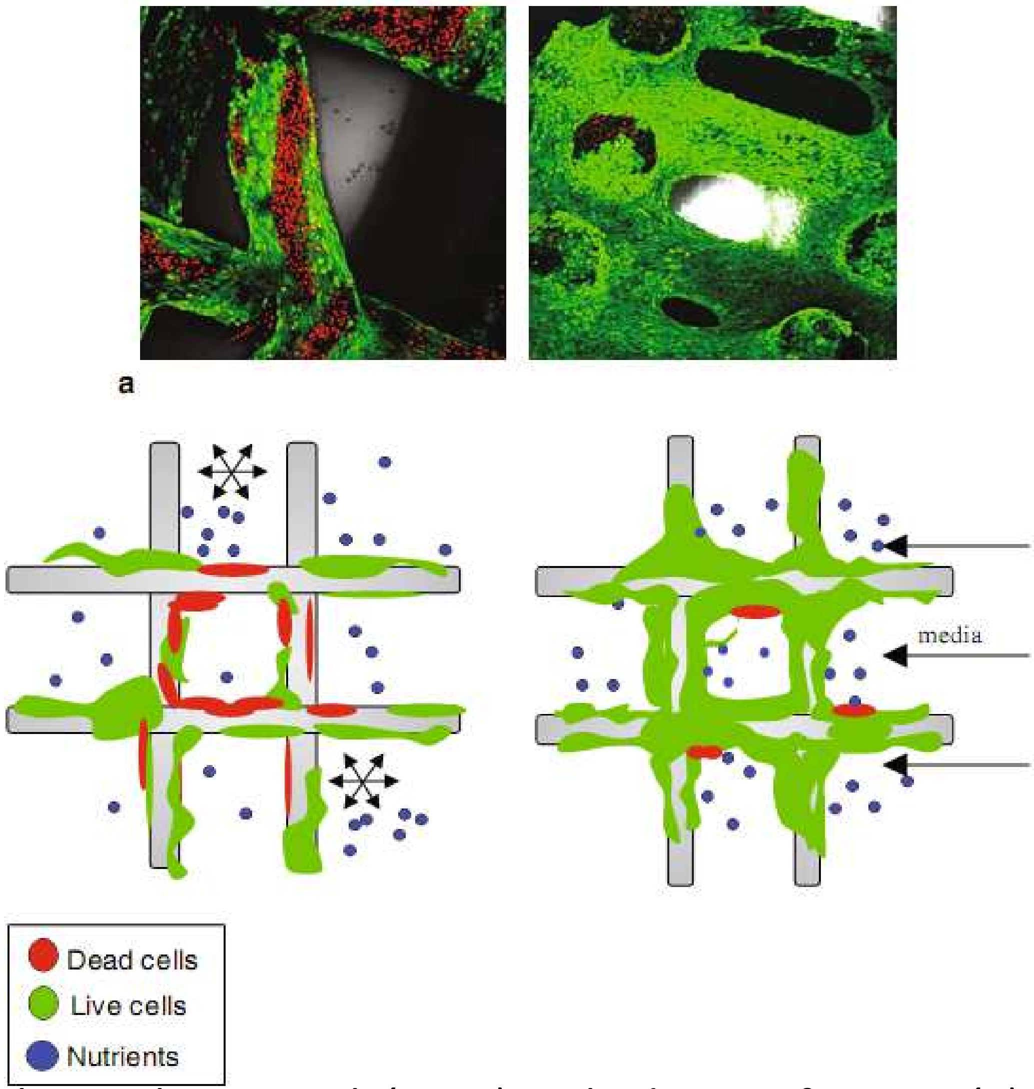
Basic scheme of dynamic cell culture bioreactors: (a) Shake flask, (b) Roller bottle, (c) Rotating membrane flask bioreactor (MiniPerm), (d) Spinner flask, (e) Rocking bag bioreactor with wave induced motion, (f) Hollow fiber bioreactor, (g) Stirred bioreactor, (h) Bioreactor with eccentric motion stirrer, (i) Bioreactor with Vibromixer, (j) Bubble column, (k) Airlift bioreactor, (l) Fixed bed bioreactor, (m) Fluidized bed bioreactor



Bioreactors Mechanically Driven by Internal Elements (g)
Hydraulically Driven Systems (f)
Pneumatically Driven Systems (j, k)


CELL SOURCE
SCAFFOLD
2. MAINTENANCE of a CONTROLLED CULTURE ENVIRONMENT
CONTROL UNIT
culture medium
heat
gas mixture
1. CELL SEEDING
Mixing
Perfusion
Cell delivery
3. PHYSICAL CONDITIONING
Fluid-driven
Mechanical
Electrical
3D Cell expansion
Cell differentiation
Tissue organization
T
pO2
pH
[glucose]
[others]
MONITORING UNIT
ENGINEERED CONSTRUCT

Dynamic Culturing



Vunjak-Novakovic, et.al. (2005) Orthod Craniofac Res 8(3):209–218

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Physical Conditioning of Developing Tissues

- physical forces
  - Hydrodynamic/hydrostatic
    - establish shear stress acting directly on cells: cartilage, bone, cardiac tissue
    - create a differential pressure
  - Mechanical
    - Direct tension
    - Compression
    - Bending
  - Electrical / Electro-magnetic
    - skeletal muscle
    - cardiac constructs

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Mechanical Stimulation

- A continuous flow rate of 0.3–3.0 mL/min was shown to increase both the calcium matrix deposition and the rate of late osteoblastic differentiation

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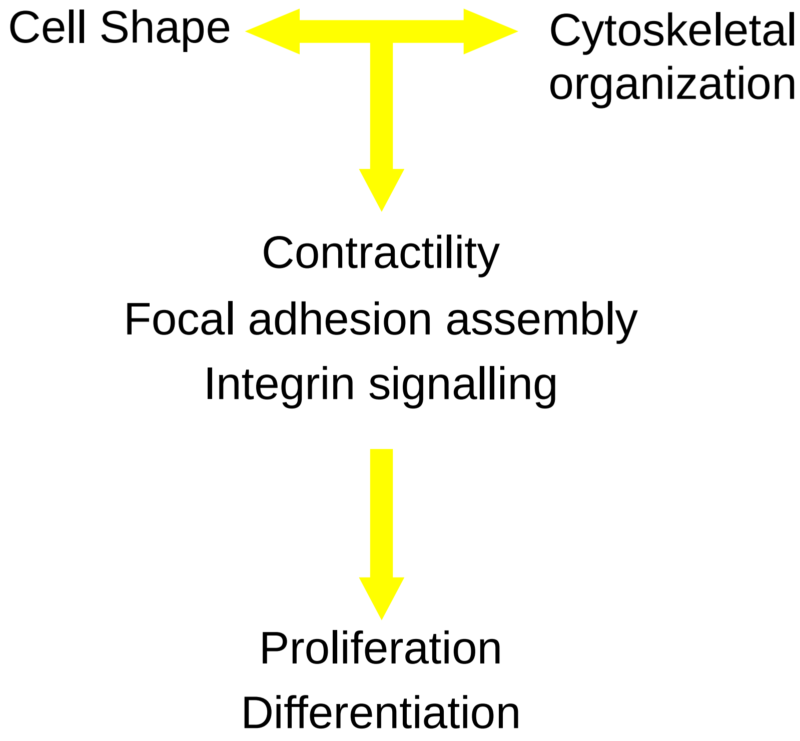
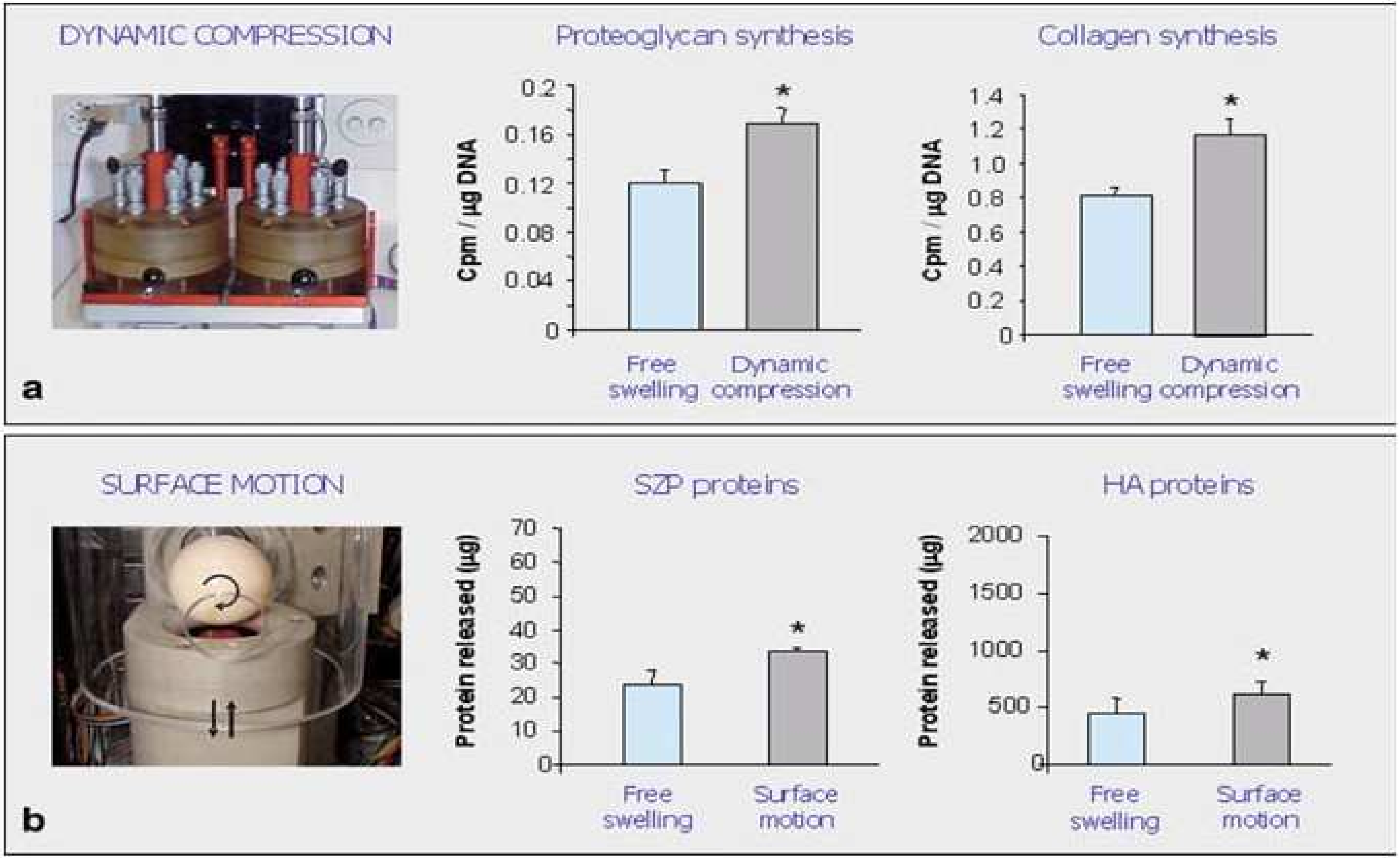
Mechanotransduction

- The process by which the cells transduce mechanical stresses into biochemical signals to regulate their function.
- Physical stimuli such as shear-stress, fluid-flow, compression and tension, not only alter the organization and distribution of structural elements and organelles within cells, but also become transduced into biochemical inputs that modulate signalling networks within and between cells.



"Mechanoepigenetics"

Front. Cell Dev. Biol., 2016
https://doi.org/10.3389/fcell.2016.00113



Candrian, et.al. (2007) Arthritis Rheum 58:197

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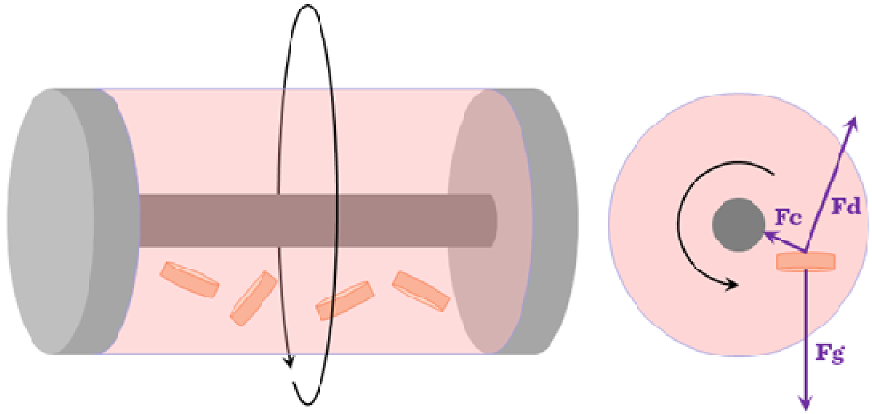
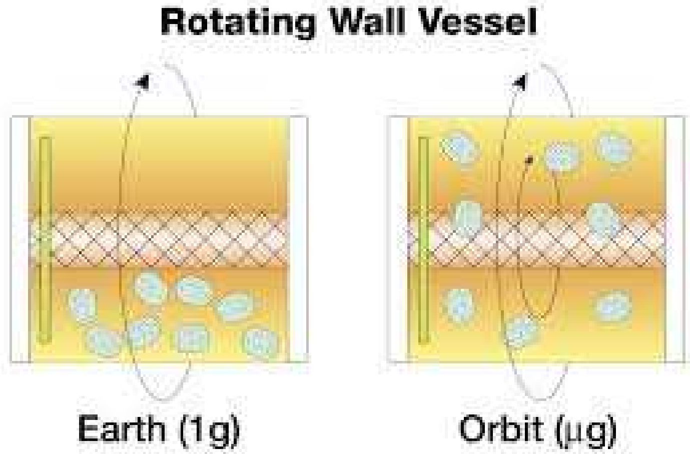
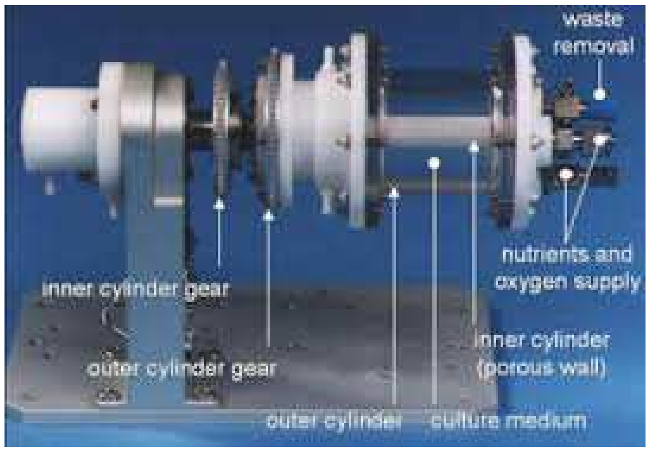
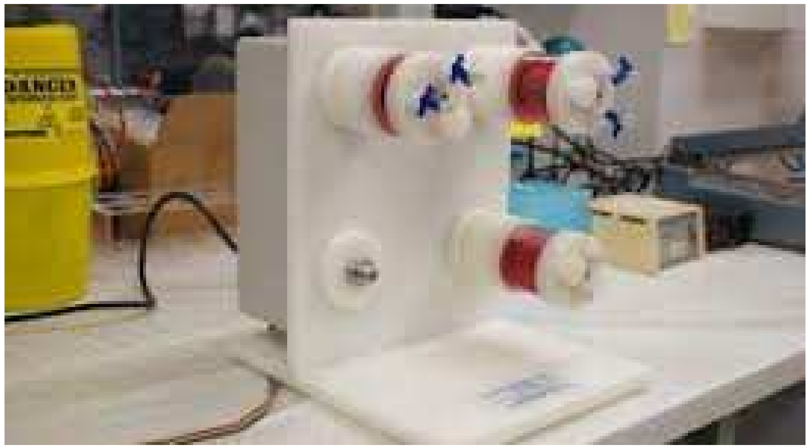
Rotating wall bioreactors









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Perfusion bioreactors

– force culture medium through the scaffold pores
– enhancing nutrient transport and providing mechanical stimuli to the cells
– The flow rate can be optimized wrt nutrient, and chiefly oxygen due to its low solubility in culture medium
– If the medium perfused directly through the pores of the scaffold, not only mass transfer rate is enhanced at the construct periphery, but also within the internal pores.
– easily monitor the metabolite consumption of the cells e.g. oxygen, glucose and cellular proliferation using online biosensors

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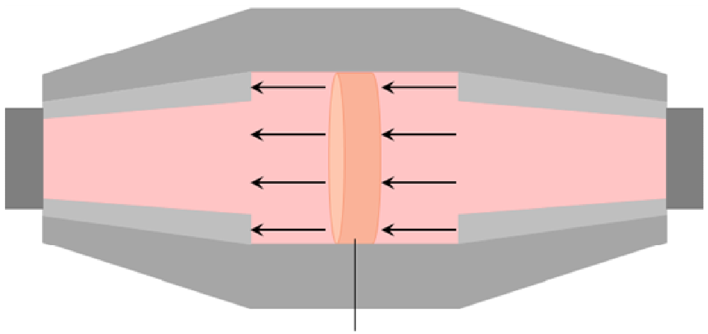
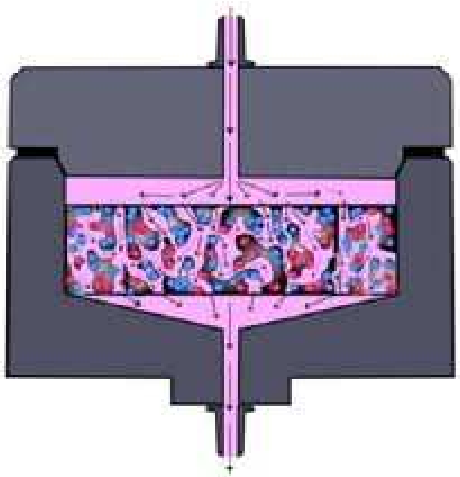
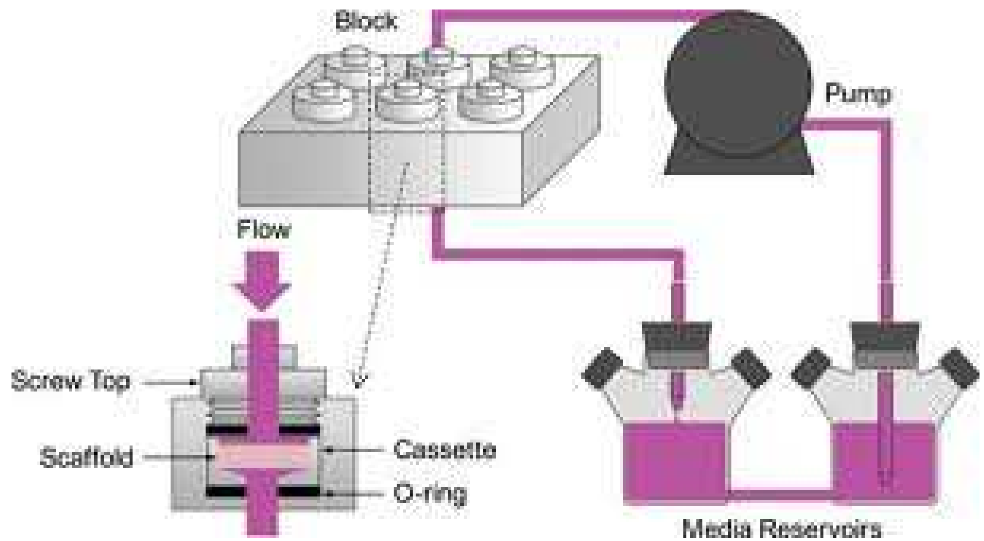
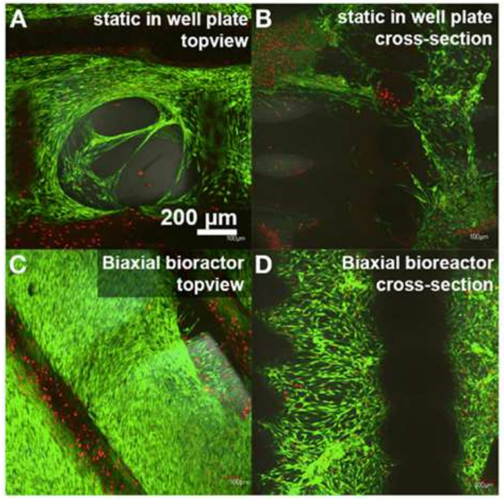
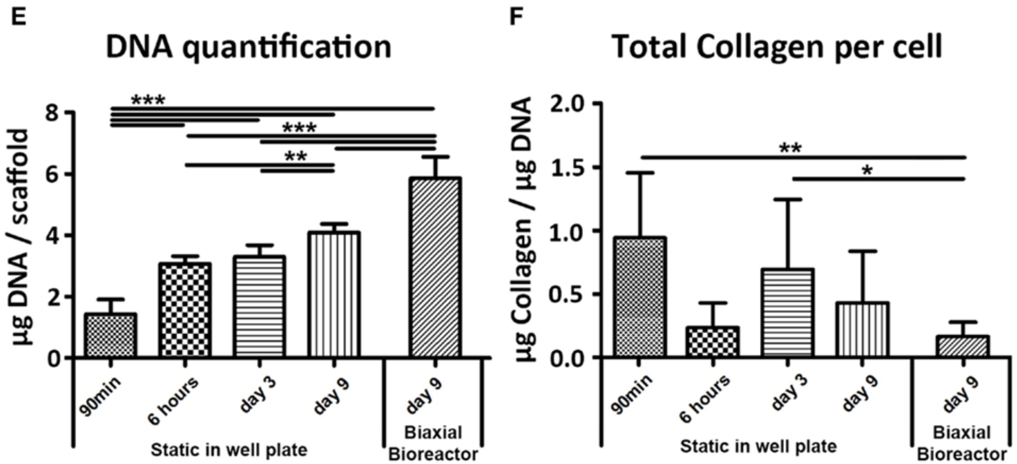
Perfusion bioreactors

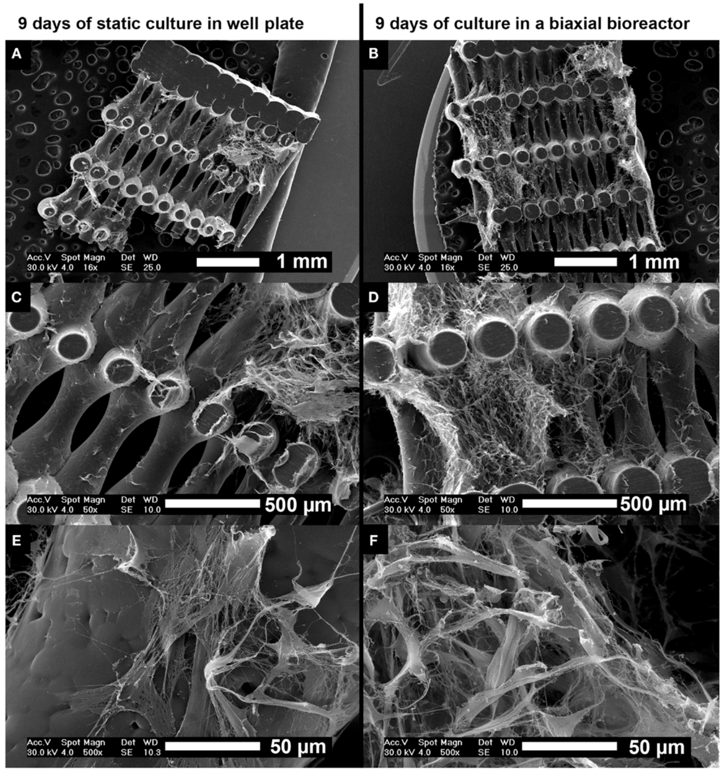








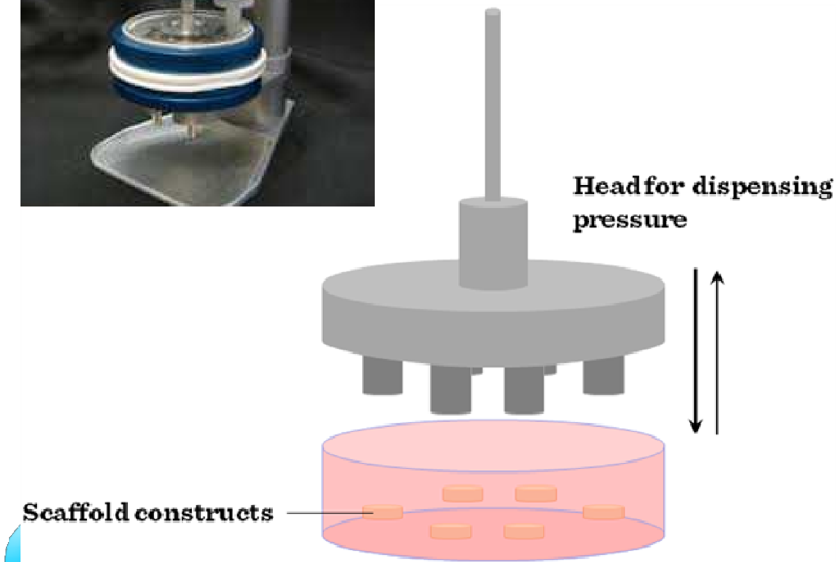


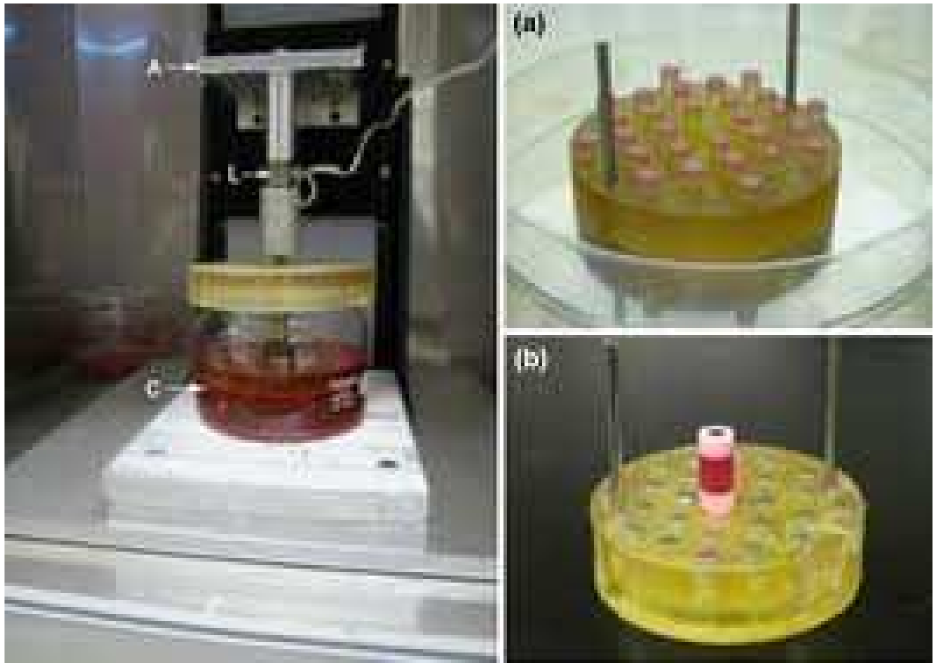


Leferink, et.al., Front. Bioeng. Biotechnol.,2015 | https://doi.org/10.3389/fbioe.2015.00169

Compression bioreactors

- Used in cartilage and bone engineering





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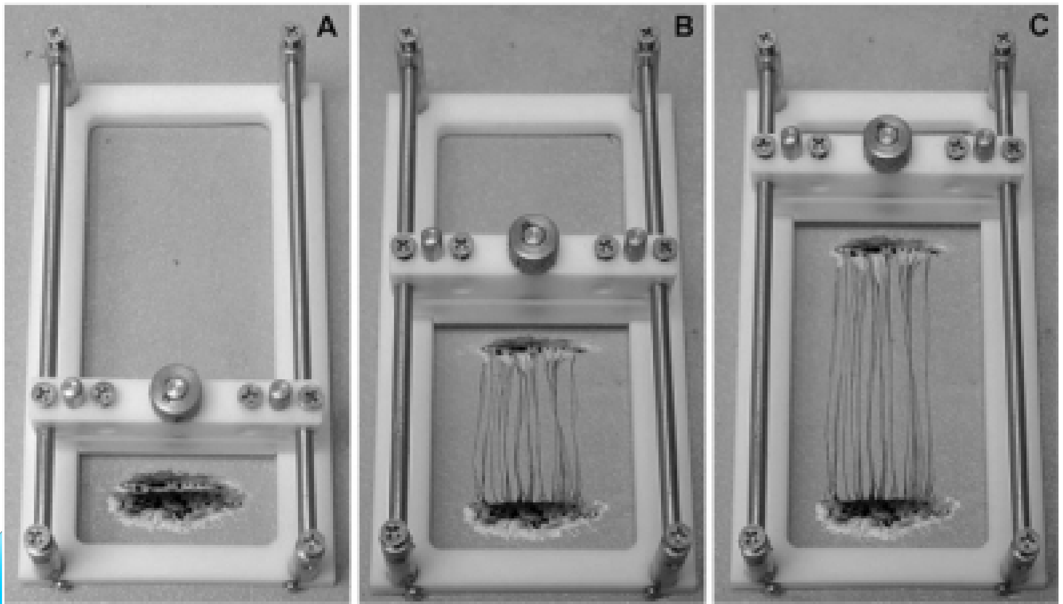
Strain bioreactors

- tendon, ligament, bone, cartilage, nerve and cardiovascular tissue.



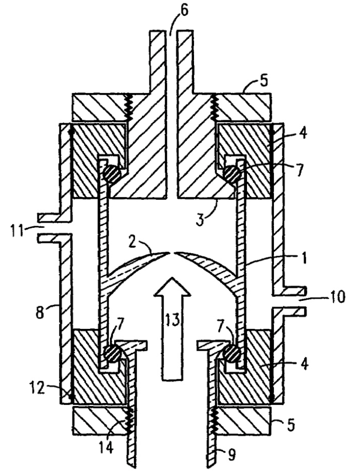
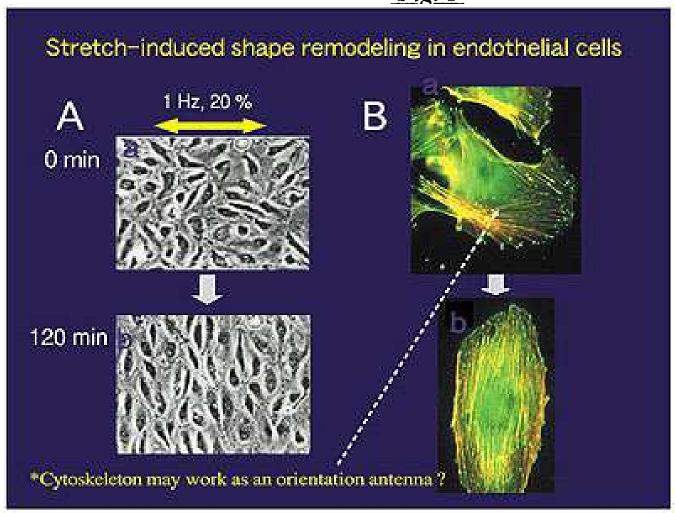
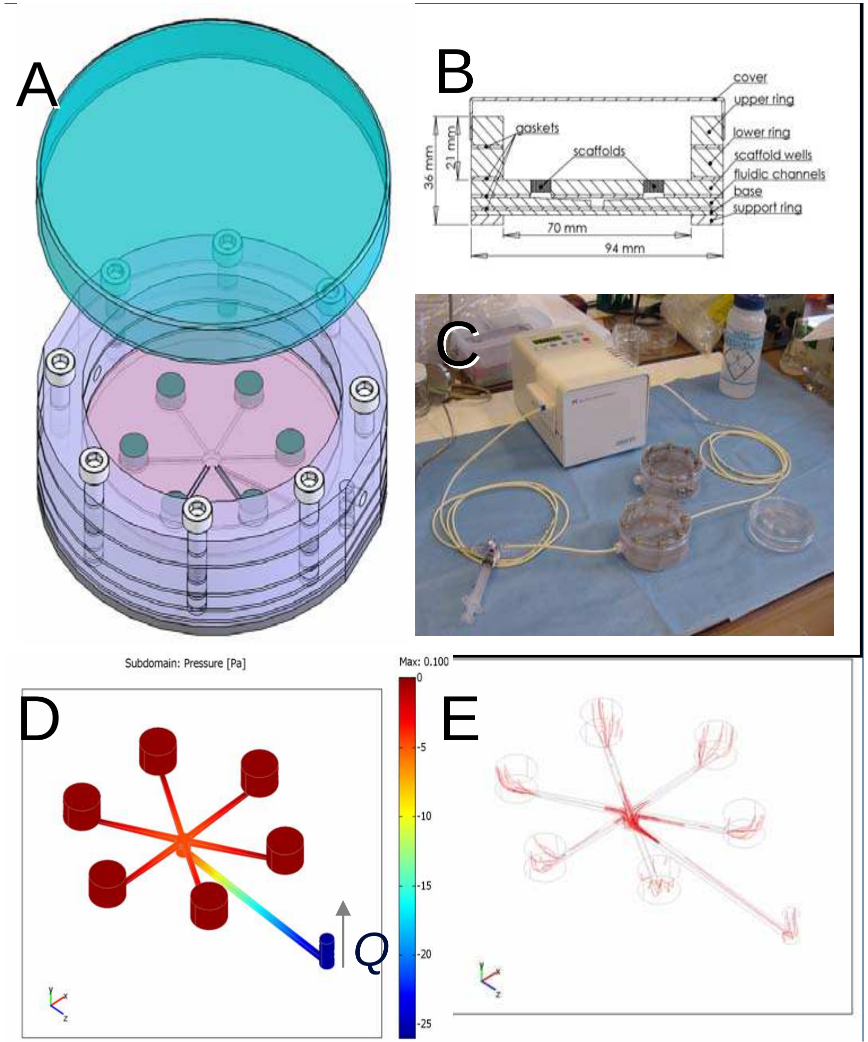


Fig. 1



Round perfusion bioreactor

- Essentially a functionalized Petri dish
- Medium reservoir serves as both gas exchanger and bubble trap
- Imaging compatible:
- – Light and fluorescent microscopy



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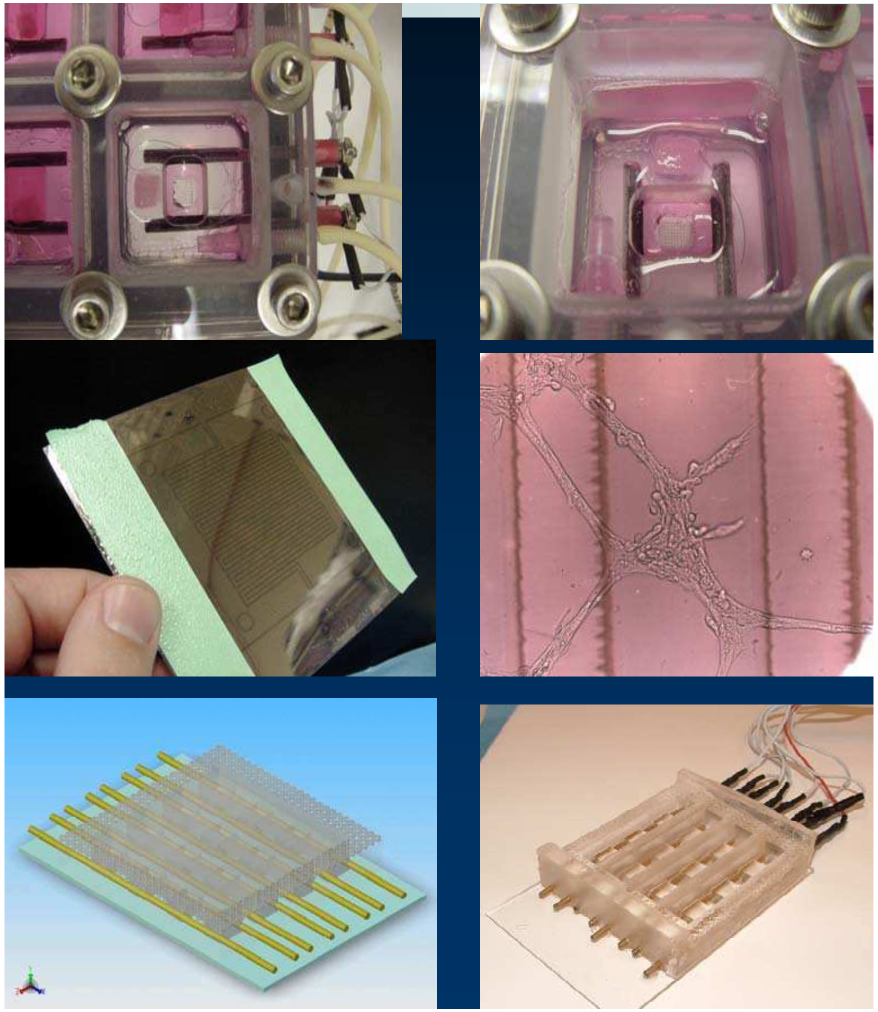
Bioreactors for application of electrical fields

- Functional assembly of engineered myocardium with combined electrical stimulation and perfusion
- Stem cell differentation and cardiac cell pacing using clear conductive electrodes



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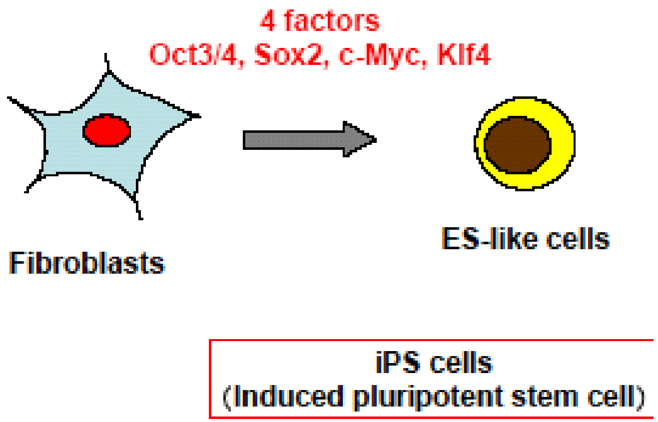
Induced Pluripotent Stem (IPS) Cells

Strengths

- Patient DNA match
- Similar to embryonic stem cells?

Limitations

- Same genetic pre-dispositions
- Viral gene delivery mechanism



Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors

Kazutoshi Takahashi,[1] Koji Tanabe,[1] Mari Ohnuki,[1] Megumi Narita,[1,2] Tomoko Ichisaka,[1,2] Kiichiro Tomoda,[3] and Shinya Yamanaka[1,2,3,4,*]

Cell 131, 1–12, November 30, 2007

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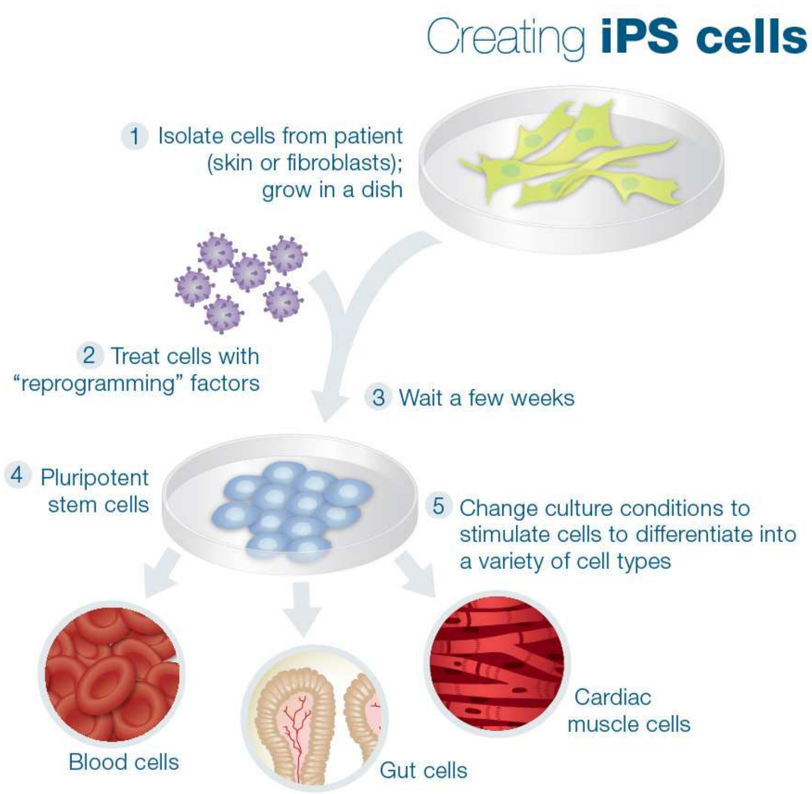
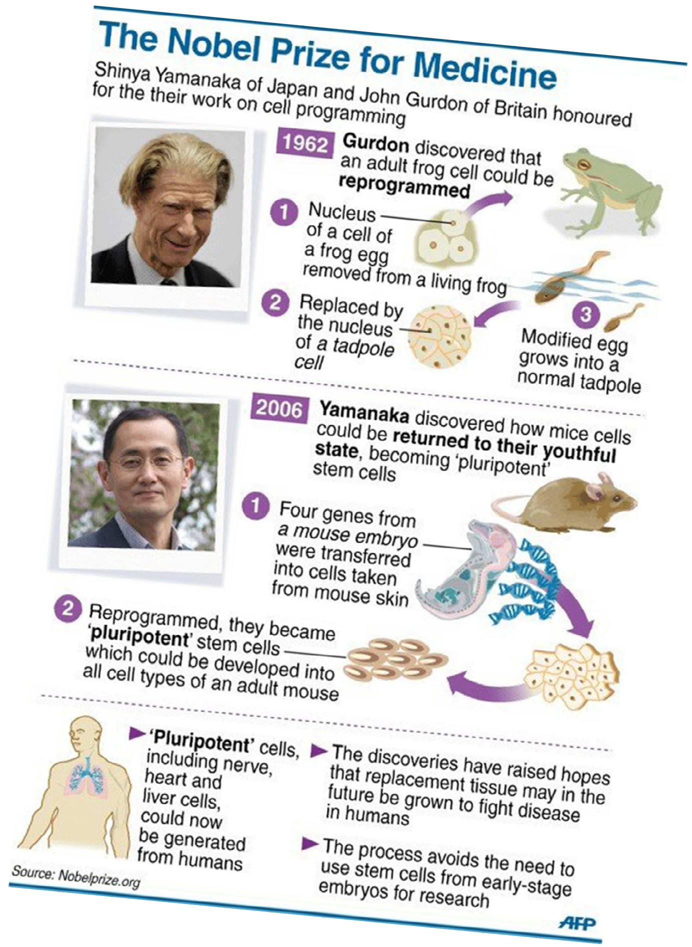
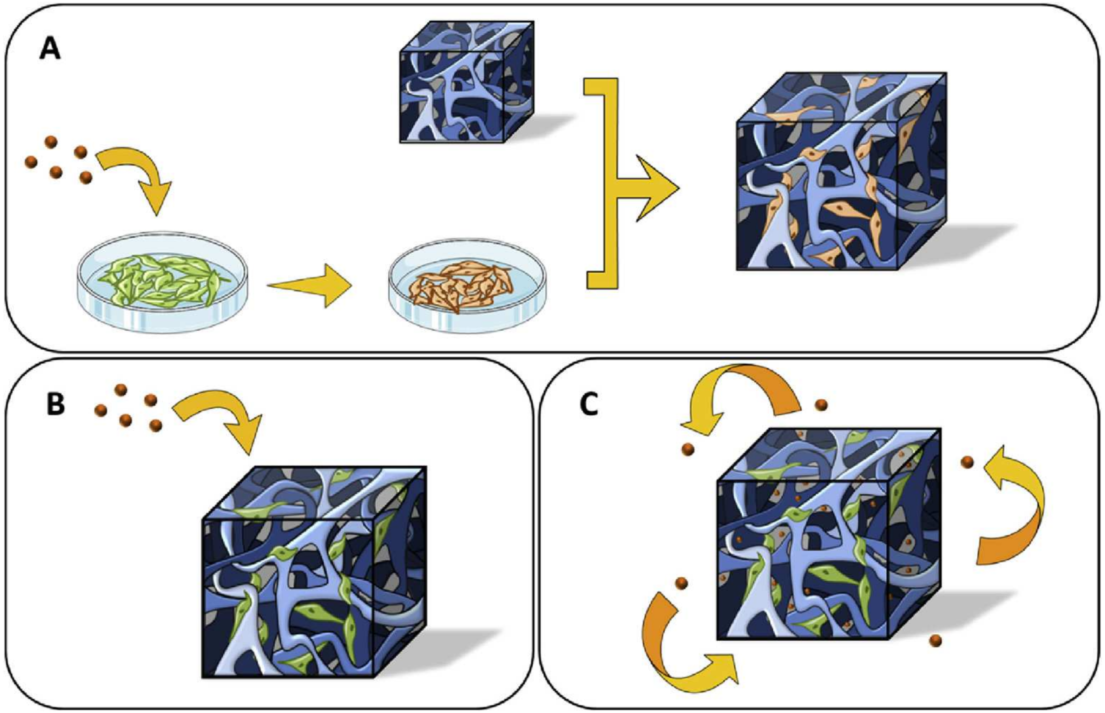
IPS technology





“Gene-Activated Matrix (GAM)” approach

- A direct, local and sustained delivery of nucleic acids from a scaffold to ensure efficient and durable cell transfection



Raisin et al., Biomaterials 104 (2016) 223e237

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What is the future?

Little attention has been paid to:

- Patient-specific implant architecture
- Mechanical function
- Site-specific function

Combination of two or more of novel approaches may hold promise for production of more clinically relevant, vascularised and functional BTE constructs with high shape complexities, and possibly patient- and/or defect specific implants.

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Tissue Engineering & Regenerative Medicine Research Group

- Bioengineering
- Biomedical Technologies
- Materials Science and Engineering
- Neuroscience

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aylin.sendemir@ege.edu.tr

- Same-day isolation of bone marrow-derived cells has been employed in a number of clinical applications [39,40]. Regenexx™ (Regenerative Sciences, Inc., Broomfield, CO, Canada) provides bone marrow-derived stem cells for nonunion fractures. Autologous bone marrow-derived osteoprogenitor cells along with allograft material (demineralized bone matrix and cancellous bone) have been used clinically as Osteocel plus® (NuVasive, Inc., CA, USA) [41,42]. Some other examples of commercially available, cell-based allograft products include ‘Ovation OS’ (Osiris Therapeutics, Inc., MD, USA), which contains allogeneic osteoblasts and MSCs, growth factors and extracellular matrix within cancellous bone matrix. Another such product is ‘Trinity evolution’ which comprises allogeneic cancellous bone along with osteoprogenitor cells and osteogenic cells inside a matrix with demineralized cortical bone [43].

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