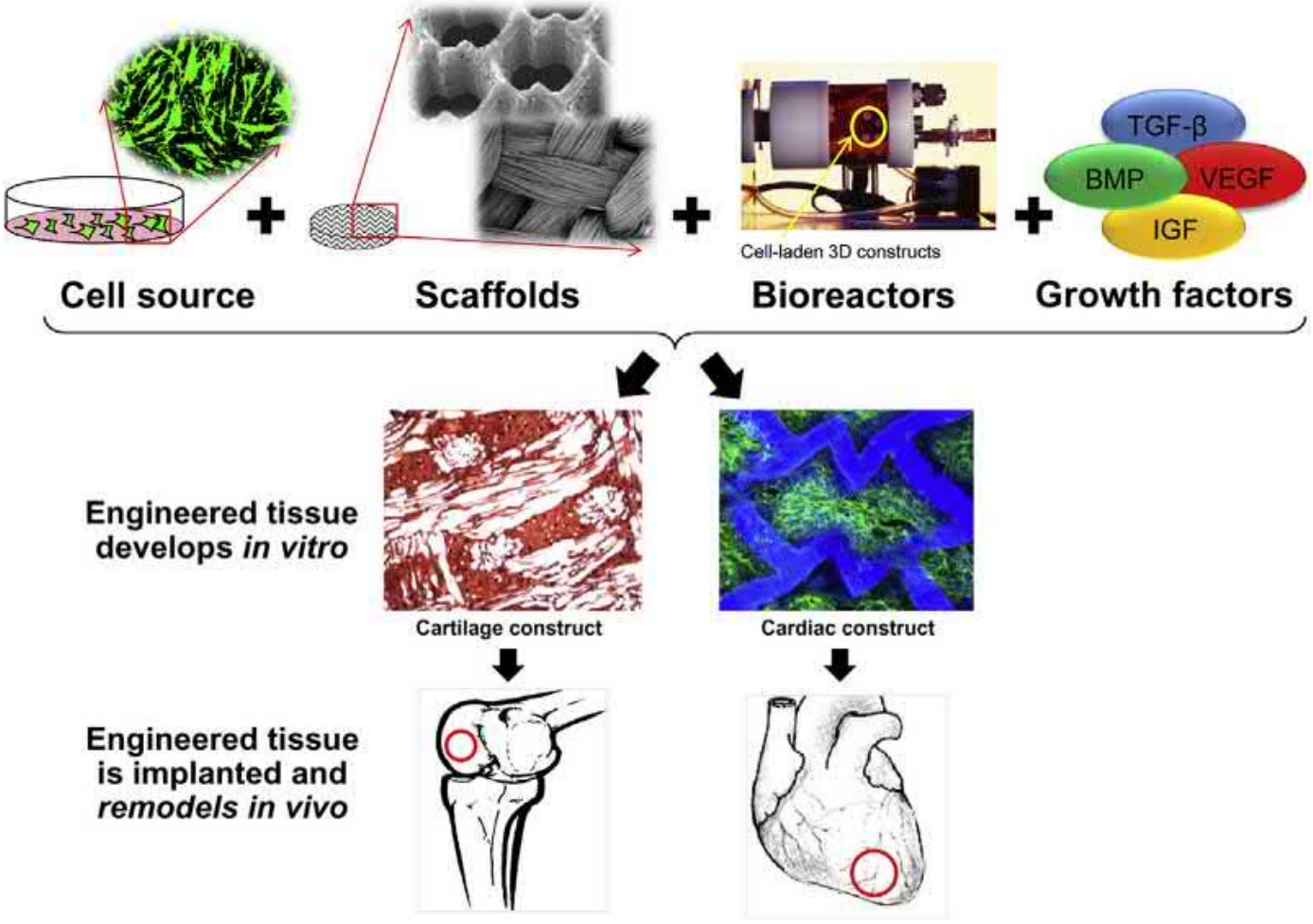
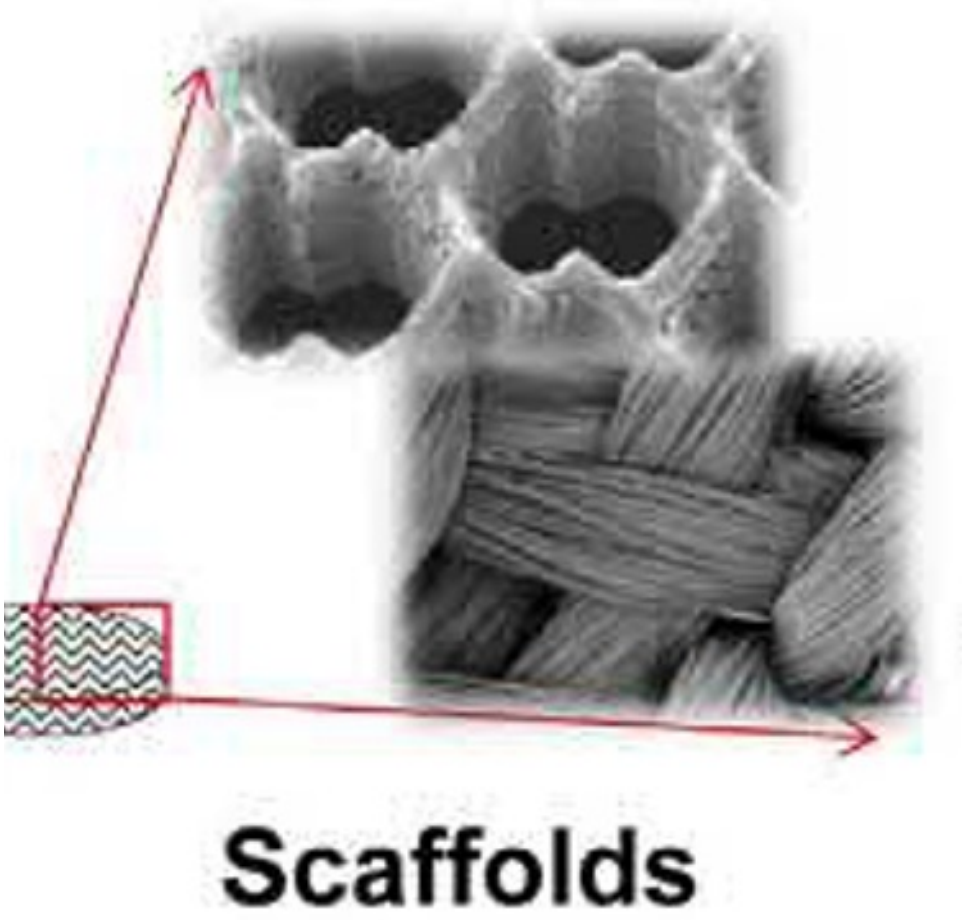

TE *in vitro* studies

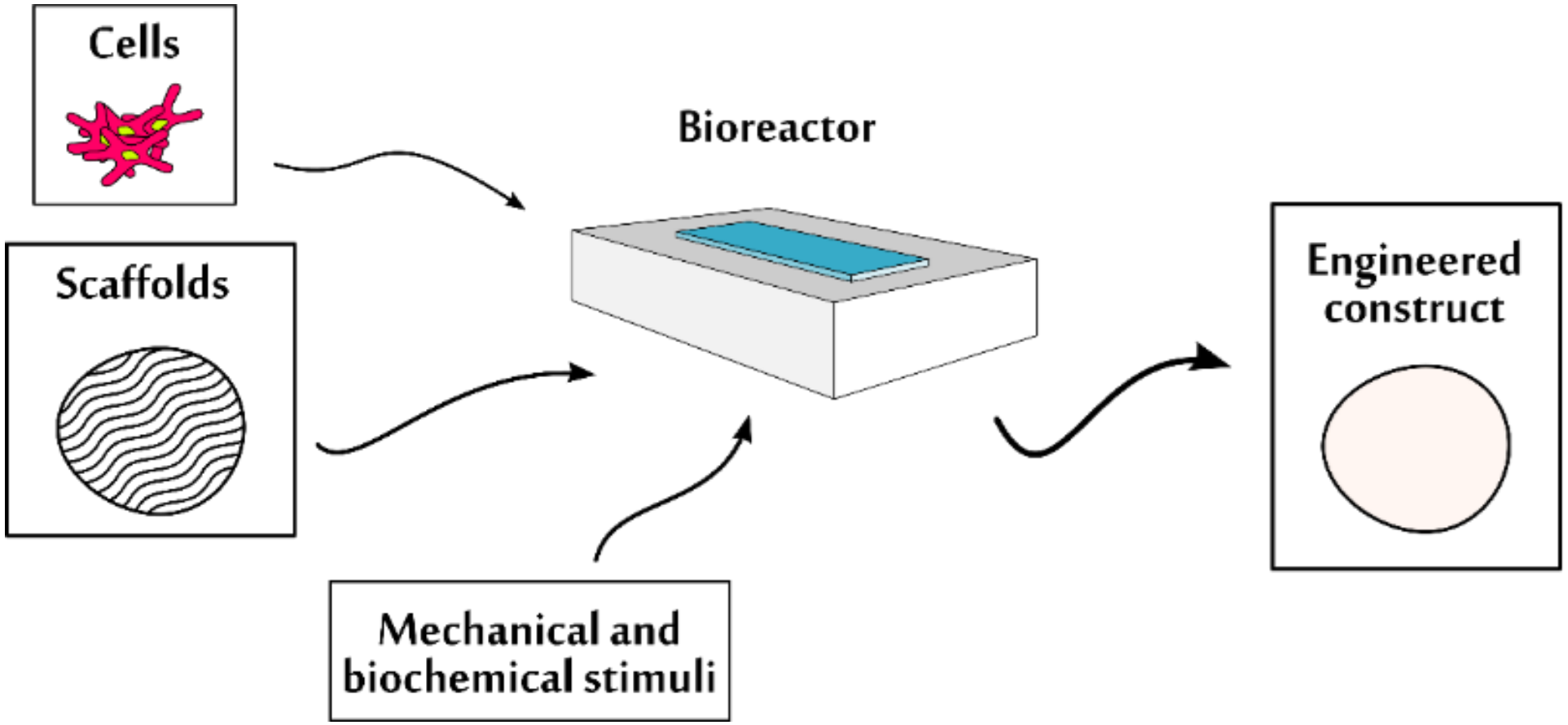

TGF-β
BMP
VEGF
IGF
Cell-laden 3D constructs
Cell source
Scaffolds
Bioreactors
Growth factors
Engineered tissue develops *in vitro*
Cartilage construct
Cardiac construct
Engineered tissue is implanted and *remodels in vivo*



Scaffolds

- promote cell-biomaterial inter- actions, cell adhesion, and extracellular matrix (ECM) deposition;
- permit sufficient transport of gases, nutrients, and regulatory factors to allow cell survival, proliferation, and differentiation;
- biodegrade at a controllable rate that approximates the rate of tissue regeneration under the culture conditions of interest; and
- provoke a minimal degree of inflammation or toxicity in vivo

to produce
functional biological tissue



Bioreactors

Bioreactors are essential in tissue engineering because:
-they provide an in vitro environment mimicking in vivo conditions for the growth of tissue substitutes
-they enable systematic in-vitro studies of the responses of living tissues to various mechanical and biochemical cues

Essential parts of bioreactors:

- pH sensor
- pO_2 sensor
- Temperature sensor

sensors

- Heating system
- Peristaltic pump
- Actuators

Active components

Closely -controlled culture conditions (biological, physical, and mechanical)

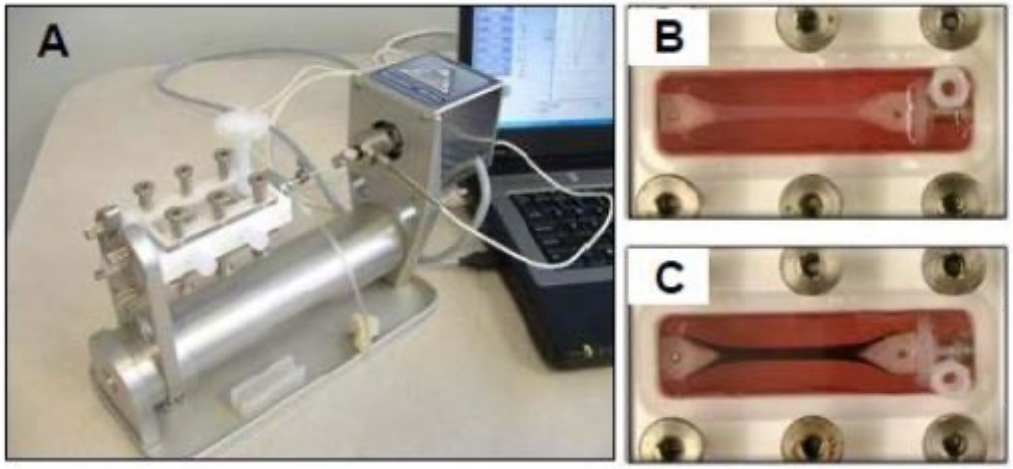
Closer in vitro replication of native tissues through bioreactor technologies

Bioreactors

For example, ligaments and tendons are dense connective tissues dominated by fibroblasts. It is known that rapid turnover of collagens in the matrix of ligaments and tendons is essential.

ECM in tendons and ligaments reacts to mechanical stress (axial stretch/compression) by a molecular adaptation. In healing ligaments and tendons, mechanical loading has been shown to affect the organization of collagen fibers and alignment of fibroblasts.

A) Image of stimulator and bioreactor chamber assembly.



B) and C) show details of bioreactor chamber with constructs.

Drive tissue development by mimicking the native physical environment

Classification of bioreactors

Bioreactors can also be classified in:

- Shaken bioreactors (rotating, lift, spinner flask, orbital shaker, etc)
- Bioreactor for applying physical stimuli (shear, pressure, stretch, compression, etc)

Physical stimuli that the bioreactor is able to perform depend on the functional requirements of the tissue to be engineered

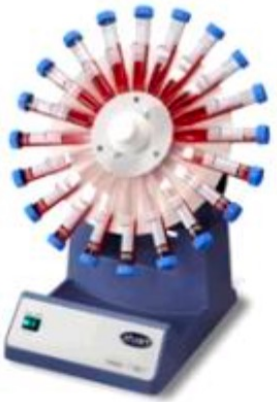
Specific mechanical forces, which are known to be important modulators of cell physiology, might increase the biosynthetic activity of cells in bioartificial matrices and, thus, possibly improve or accelerate tissue regeneration in vitro

Shaken bioreactors

•La cultura è agitata meccanicamente.

•L’agitazione ha come scopo quello di permettere alle cellule l’adesione a strutture come scaffold, e migliorare il trasporto dei soluti.

•L’agitazione può essere studiata o dimensionata per imporre stimoli di intensità nota

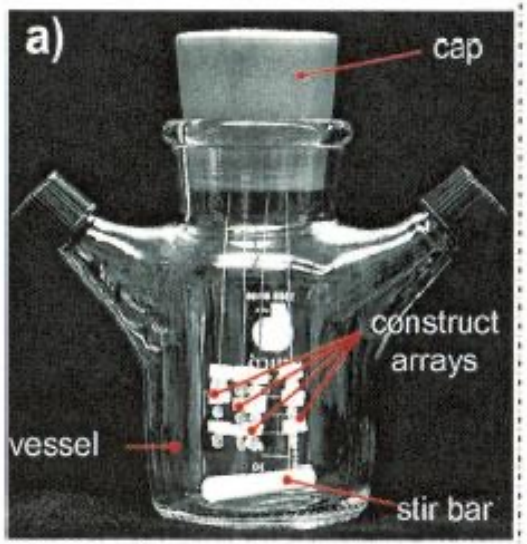


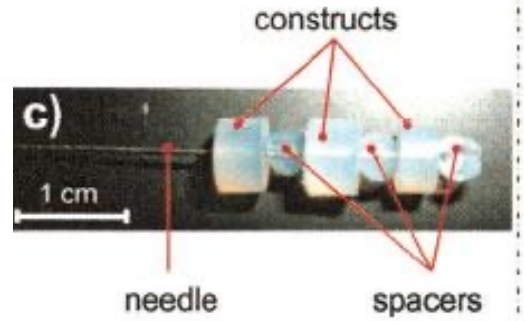
Shaken bioreactors: spinner flask

- Usato per mantenere in sospensione le cellule evitando che si depositino sul fondo del contenitore colonizzando gli scaffold che vengono tenuti sospesi grazie ad appositi supporti

- Molto usate per la colonizzazione di scaffold porosi

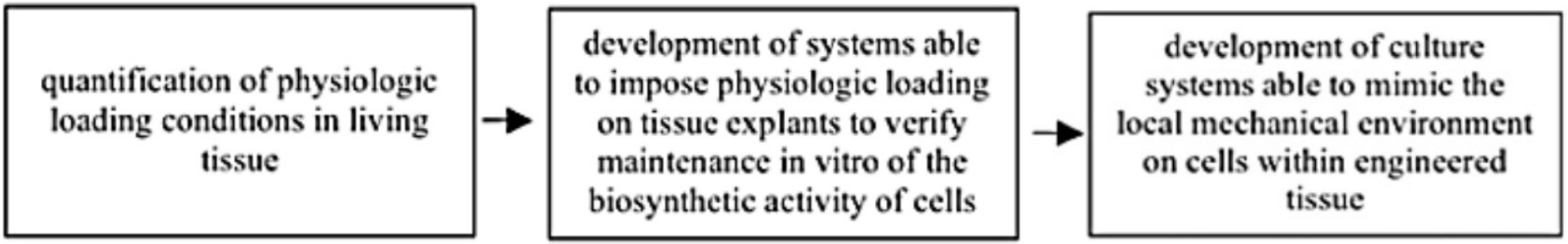
- Vengono usate anche dopo la colonizzazione per stimolare le culture (Shear Stress)





Volume di terreno utilizzato: 20-500 mL

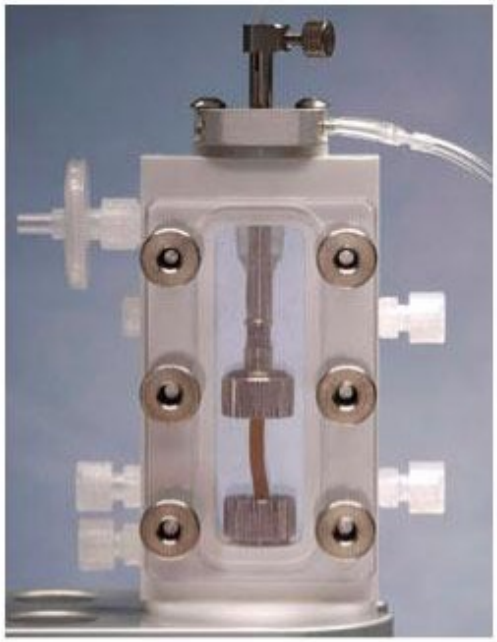
Bioreactors for physical stimuli

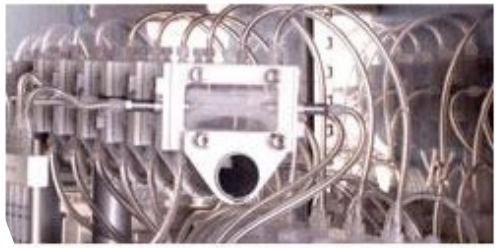


Bioreactors for physical stimuli

- E’ possibile applicare alle culture stimoli specifici
- La stimolazione è nota, modellata e finemente controllabile
- Si possono applicare diverse tipologie di stimolazione:
- Shear stress
- Pressione idrostatica
- Compressione
- Trazione
- Torsione
- Pressione differenziale ...

LigaGen L30-4C Chamber

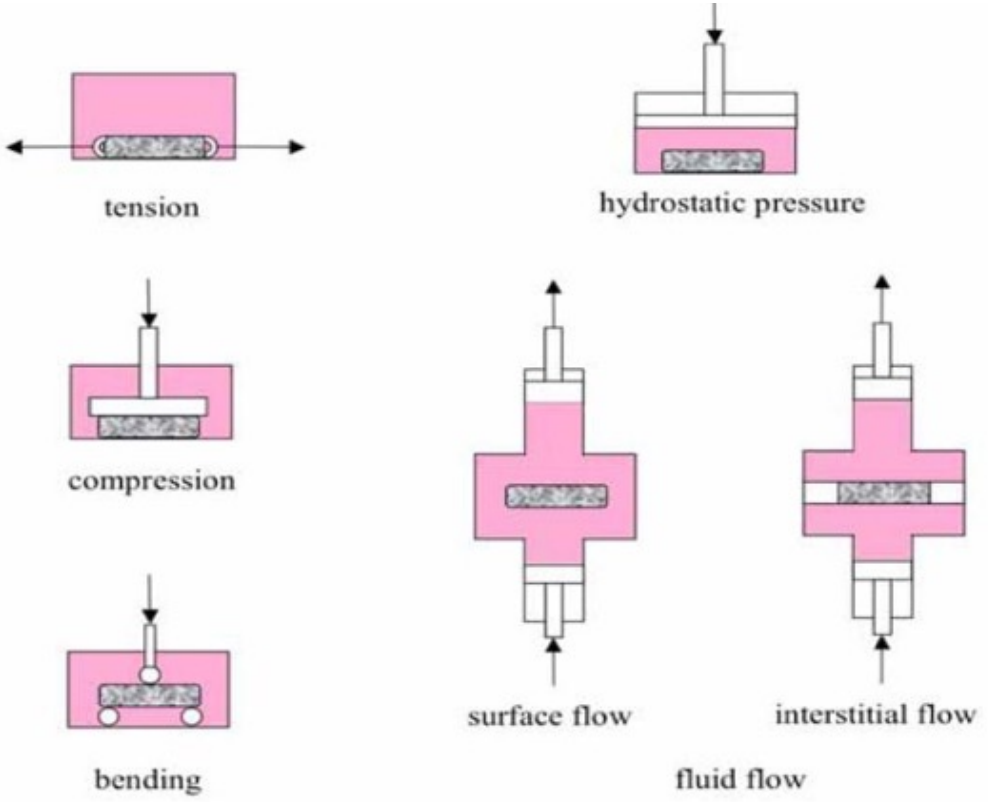




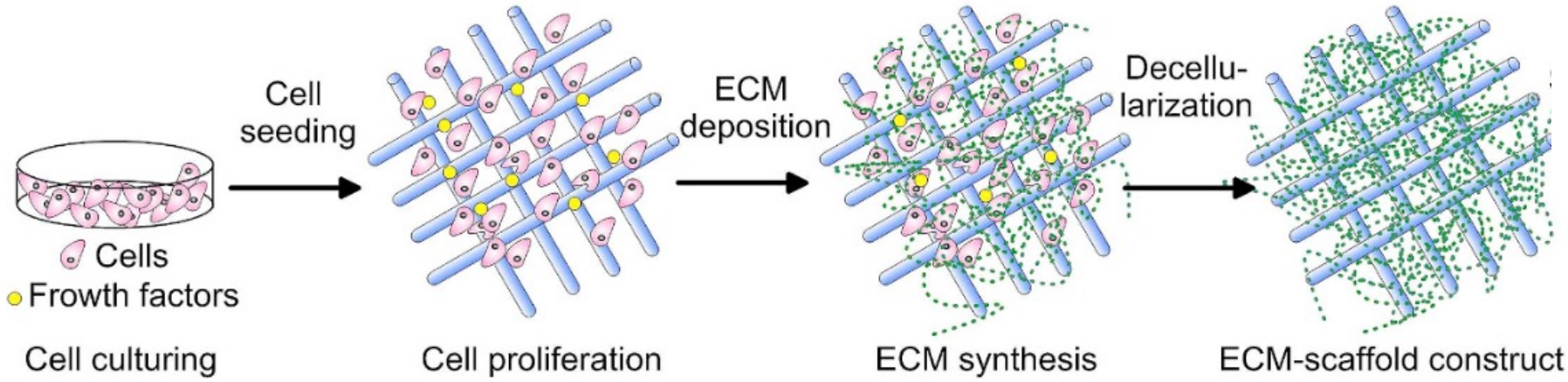
LumeGen V60 Chamber: 6VC System

LumeGen V60 Chamber

Different kind of stimulation



TE In vitro preparation



Scaffolds sterilization

Table 1. Microorganism inactivation ability of different sterilization techniques.

Category	Technique	Inactivation level	Mycobacteria	Vegetative bacteria	Bacteria spores	Nonenveloped virus	Enveloped virus	Prions	Fungal
Heat	Heat treatment	High	✓	✓	✓	✓	✓	✓	✓
Irradiation	Gamma	High	✓	✓	✓	✓	✓		✓
	E-beam	High	✓	✓	✓	✓	✓		✓
	UV	Medium		✓			✓		
Plasma	Plasma	High	✓	✓	✓	✓	✓	✓	✓
Chemical sterilization	EtO	High	✓	✓	✓	✓	✓	✓	✓
	Peracetic acid	High		✓	✓	✓	✓	✓	✓
	Ethanol	Medium	✓	✓			✓		✓
	Iodine	Medium		✓	✓	✓	✓	✓	✓
Novel techniques	sCO_2			✓	✓	✓	✓		
	Antibiotics	Low		✓					
	Freeze-drying								

UV: ultraviolet; EtO: ethylene oxide; sCO_2: supercritical carbon dioxide.

Method	Method	Advantages	Disadvantages
Heat	Heat treatment	Simple, fast, effective, high penetration ability, no toxic residues	High temperature, affect the structural properties of biodegradable polymers
Irradiation	Gamma	High penetration ability, low temperature, effective, easy to control, no residue	Induce structural properties changes, dose rate is lower than electron beams, long time
	E-beam	Low temperature, easy to control, no residue, fast	Induce structural properties changes, electron accelerator needed, low penetration ability
	UV	Fast, low temperature, low cost, no toxic residues	Not effective, induce structural and biochemical properties changes of biodegradable polymers under long exposure duration
Plasma	Plasma	Low temperature, improved cell interaction, increasing wettability on surface of biodegradable polymers, fast	May cause changes in chemical and mechanical properties of polymers, leave reactive species
Chemical treatment	EtO	Effective, low temperature	Induce structural property change, leave toxic residue, flammable, explosive, carcinogenic
	Peracetic acid	Low temperature, effective	Structural and biochemical properties change, residual acidic environment
	Ethanol	Low temperature, low cost, no complex equipment, no toxic residue, fast	Not effective, structural and biochemical property change of scaffolds
	Iodine	Low temperature, no structural property change, fast	Affect biochemical property
Novel techniques	sCO_2	No toxic residue, no biochemical property change	May affect porosity and morphology of scaffolds
	Antibiotics	Convenient, simple	Harmful residue, not effective
	Freeze-drying	Low temperature, no structure property change, no toxic residue	Not effective, may affect the biochemical properties of scaffold