Aalto University
School of Chemical Technology

CHEM-E3225, Cell- and Tissue Engineering, 5 cr

TOPIC 6:
Bioreactors for Tissue Engineering
Birla Chapter 6

- **Today we will**

1) Remind ourselves of some culture systems
2) Review some terminology
3) Examine Bioreactors

Why do we need to do this ?
Because scaling-up from laboratory culture is (usually) needed for products to become commercially feasible

Because in the group work on 23.4. and 24.4. you will need to understand the biological and technological needs that your product (the case that you are working on in the groups work)should take into account

In vitro cellular environment – what should we take into account when growing cells (in bioreactors)?

- Cell-cell interaction
- Cell-matrix interaction
- Soluble factors
- Cell shape and polarity
  - Cell shape changes under suboptimal conditions
  - Cell becomes polarized under optimal conditions.
- Dynamic stress
  - Skeletal myocytes require tensile stress
  - Cardiac myocytes require pulsatile tensile stress
  - Chondrocytes and osteocytes require compressive stress
- Oxygen tension
  - The higher oxygen tension is achievable at the air-liquid interface

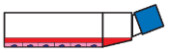
Four ways to view growing of cells

1) Static culture (conventional, 2D)
2) 3D Culture
3) Dynamic culture
4) Bioreactors

To remind you…

1. Static culture (conventional system) in the laboratory

- The 1st *in vitro* cell culture was established in 1907.

Features:

1. The upper side of cells gets nutrients but not the bottom side.
2. Mass transfer occurs by molecular diffusion.
3. Cell-substrate interaction is non-specific, mediated by the adsorbed proteins.

More advanced static culture systems:

1. Transwell insert allows the cells to have access to the nutrients from below.
2. The substrate is coated with ECM or biomaterials before seeding cells to induce specific interaction.

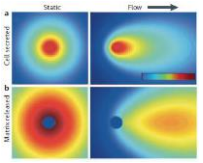
To remind you…

2. 3D culture

- The significant difference between 2D and *in vivo* was first realized by cancer biologists, followed by tissue engineers and stem cell biologists.
- 3D culture system is ”something between a Petri dish and a mouse”---meaning it is more predictive of *in vivo system than 2D.*
- In 3D culture, cells are cultured in a biomaterial scaffold. For example: cells cultured within Matrigel, within a polymeric hydrogel, or within a porous scaffold.

3. Dynamic culture

- The culture medium is not on a stationary condition.
- It couples both biophysical and biochemical cues.
- Perfusion of the tissue culture will promote nutrient transport and can also produce mechanical stresses on the cells, as well as alter local biochemical gradients.



2006 Nature Rev Molecul Cell Biol 3 :211

4. Bioreactors

- Bioreactors are devices that provide a tightly controlled environment for biological processes, e.g. cell expansion, differentiation, and tissue formation.

- The aim of bioreactors is to
  - Support the 3D tisse development
  - Maintain cell viability within the tissue
  - Maintain cell function within the tissue
  - Provide appropriate physical and molecular cues

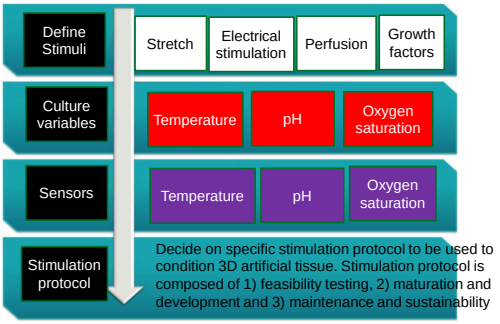
Bioreactors for tissue engineering

- Key functions:
  - Increase cell-seeding efficiency into a 3D scaffold
  - Provide efficient mass transfer of gases, nutrients, and regulatory factors to the engineered tissue constructs
  - Expose the developing tissue constructs to physiologically relevant mechanical and electrical stimuli.

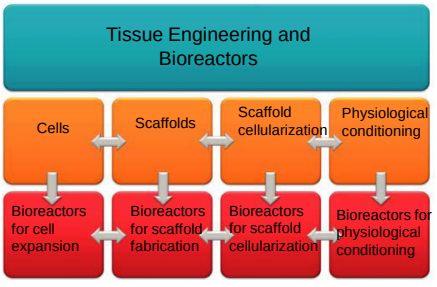
Principles of bioreactor design

- Mass transport of nurtients, biochemical factors and oxygen to the cells, and rapid removal of metabolic products
  1) External mass transfer:
     - Transport from the medium to the tissue/scaffold surface
     - Depends on hydrodynamic condition of the bioreactor
  2) Internal mass transfer:
     - Transport through the tissue/scaffold to the cells
     - Depends on both diffusion and convection. This significantly depends on tissue/scaffold structure.
- Biophysical signaling considerations
  - Stress, strain, compression, tension, fluid flow, electrical signals

Design considerations for Bioreactors, Birla figure 6.3.



Integration of Bioreactor Technology with Tissue Engineering (Birla, Figure 6.5)



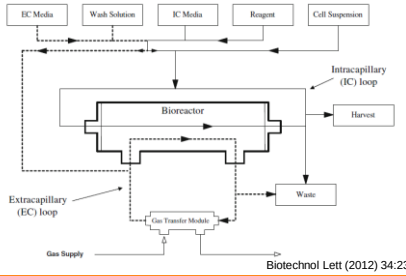
Two types of bioreactors

1. Cell- and tissue-specific bioreactors for tissue engineering

2. General bioreactors for screening, research, and small trials before engineering large tissues:

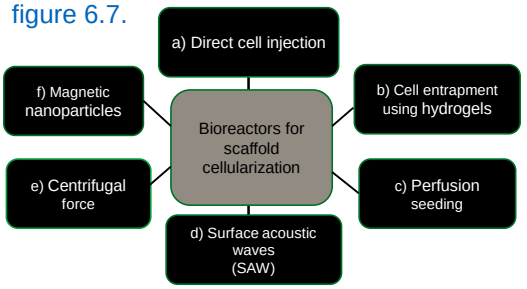
   - these are modular, miniscaled, and multiparametric, not cell- and tissuespecific. For example, micro-bioreactors are not used for engineering clinical-size grafts.

Schematic of bioreactor for (stem) cell culture



Biotechnol Lett (2012) 34:2307–2315

Bioreactors for Scaffold cellularization; Birla figure 6.7.



Different types of bioreactors

(A) tissue culture flasks
(B) spinner flask
(C) rotary wall bioreactor (is a type of perfusion bioreactor)
(D) hollow fiber bioreactor
(E) fibrous bed bioreactor
adapted from the spinner flask
(F) wave bioreactor (GE Healthcare)

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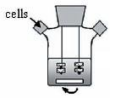
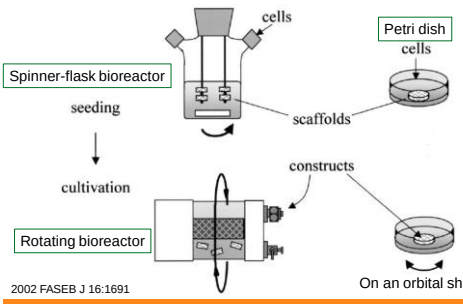
1. Spinner-flask bioreactor

- uses a magnetic stir bar to mix cell suspension around a stationary scaffold, aiding in cell distribution through the scaffold.
- Stirring the medium produces mass transfer through turbulent convection, generates shear stress that enhance cell and tissue growth.
- It provides gas exchange via side arms with loose screw caps.
- But the turbulent flow produces high shear stress that may result in cell damage or ECM loss. It can also induce the formation of fibrous tissue on the surface of the scaffold.



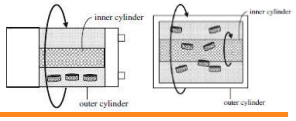
Bioreactor vs. Petri dish



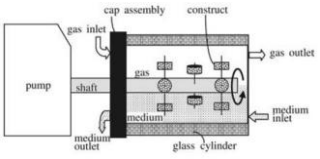
2002 FASEB J 16:1691

2. Rotating bioreactors

- The bioreactor is completely filled with medium.
- The outer cylinder rotates around its central axis and the inner cylinder remains still (A) or rotating (B).
- Cell-scaffold constructs are freely suspended in the rotating culture medium.
- The inner cylinder is made of a silicone membrane that allows gas exchange.



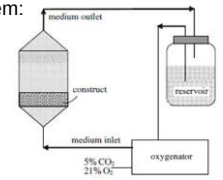
Rotating-shaft bioreactor



2006 Biotechnol Lett 28:1415

Perfusion bioreactor

- It perfuses culture medium through tissue constructs
- It produces higher density and more uniform distribution of cells than with spinner-flask bioreactor.
- Components of the whole system:
  - Medium reservoir
  - Pump
  - Oxygenator
  - Tubing
  - Bubble trap
  - Perfusion chamber



Engineering Skin: Dermagraft®

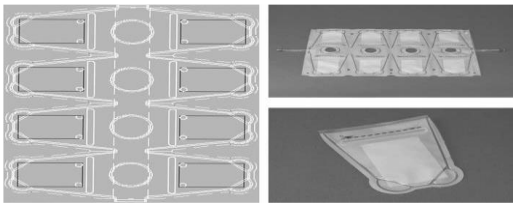


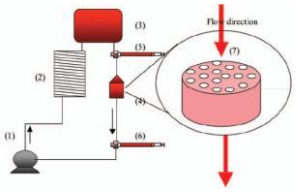
Fig. 1 Design of the Dermagaft bioreactor. Left-hand panel: black rectangles knitted lactate/glycolate scaffold. White lines are welds and the white broken lines cuts. Left-hand upper panel: completed bioreactor with inlets and outlet. Right-hand lower panel: following tissue growth, pockets containing individual pieces of Dermagraft are sealed and then cut out of the bioreactor
(Mansbridge, 2006. Journal of Anatomy)

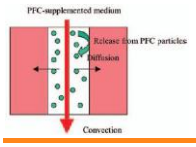
Example: Bioreactor design for cardiac muscle tissue engineering

- The native cardiac muscle (cardiac myocytes):
  - Densely packed cells
  - High metabolic activity
  - High oxygen consumption supplied by a dense capillary network
  - Synchronous contraction
- Considerations in bioreactor design:
  - Perfusion through the scaffold: direct perfusion bioreactor
  - Oxygen supply by synthetic oxygen carriers
  - Cyclic mechanical stretch
  - Cardia-like electrical stimuli

A prefusion bioreactor for cardiac tissue engineering

(1) Multichannel peristaltic pump
(2) Gas-permeable silicone tubing
(3) Reservior bag
(4) Perfusion cartridge
(5) Bubble trap
(6) Bubble trap
(7) Channeled poly(glycerolsebacate) scaffolds seeded with cardiac fibroblasts and cardiac myocytes





Oxygen was released from perfluorocarbon (PFC) particles into the medium within the tissue construct

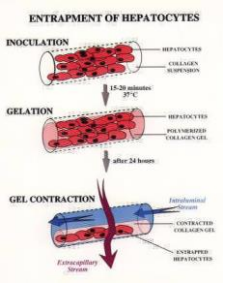
Perfusion, channeled scaffold, and oxygen supply

2006 Tissue Eng 12:2077

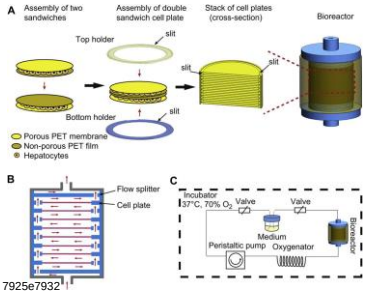
Bioreactors used in patients

- Extra-corporeal system, not permanent replacement
- Two types of systems
  - Hollow fiber system
  - Flat membrane sheet system
- Examples
  - Bioartificial liver (BAL) devices
  - Bioartificial kidney (BAK) devices

Hollow fiber system



Flat membrane system



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Summary

Bioreactors are used to

a) Grow cells
b) Grow cells on materials (scaffolds)
c) Grow cells to form tissues
d) Do research on cell behaviour under different conditions (biochemical, biophysical, mechanical)