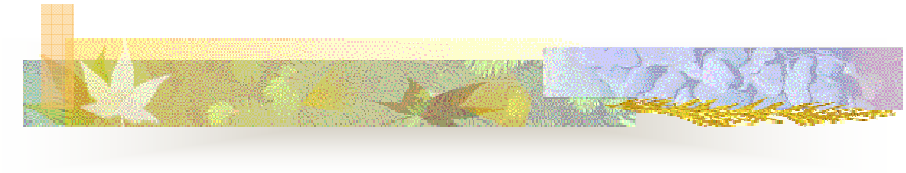
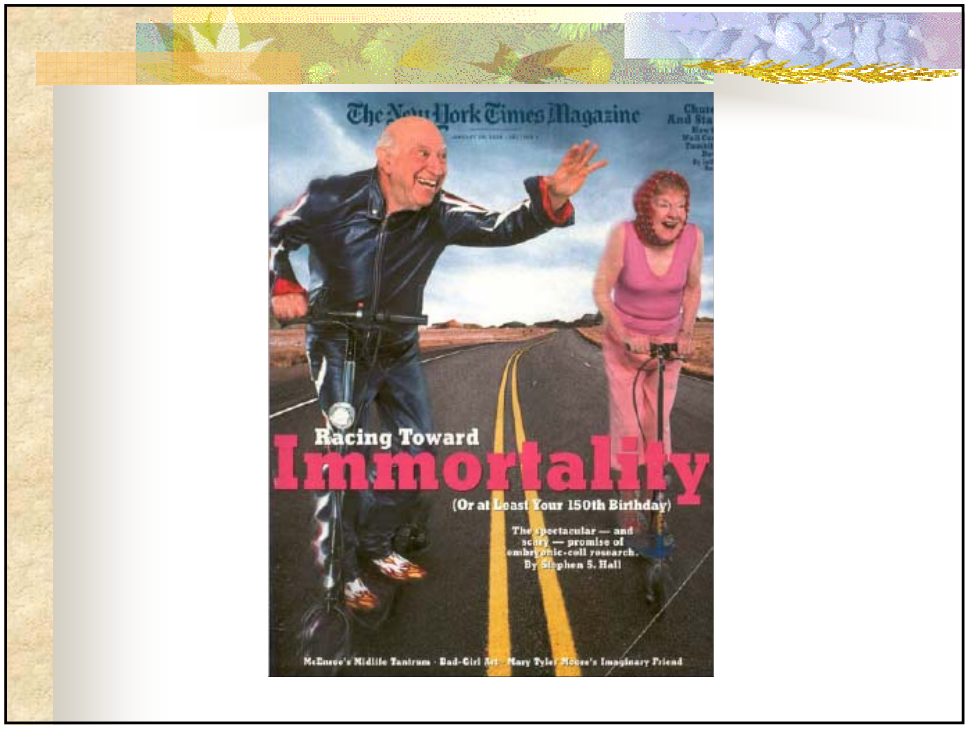
C6/BME2 Tissue Engineering



Prof. Zhanfeng Cui and Dr Xia Xu
4 Lectures and 1 Class

Contents

- Principle of Tissue Engineering
- Biomaterials for Tissue Engineering
- Mass Transfer
- Bioreactors for Tissue Engineering



To live longer, stronger and smarter !

- System biology and synthetic biology
- Preventive medicine
- Diagnostic technology
- Regenerative medicine
- Silver bullets and drug delivery
- Personalised medicine/therapy
- Tele-medicine/healthcare
- Life elongation and aging

Anything to do with Engineering ?

Regenerative Medicine

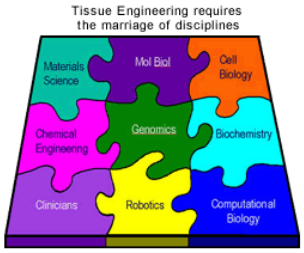
- to promote regeneration of cells, tissues and even organs
- to prevent and cure diseases
- to repair and replace diseased and lost tissues
- Methodologies
  - Gene therapy
  - Cell therapy
  - Functional biomaterials
  - Tissue engineering
  - Stem cell transplantation

Principle of Tissue Engineering

TISSUE ENGINEERING

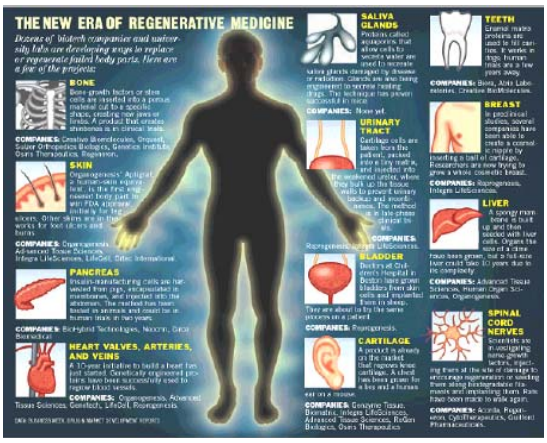
- **Definition**
  - Application of principles and methods of engineering and life sciences
  - Development of biological substitutes
  - Method of restoring, maintaining or improving biological functions

- **Multi-disciplinary field**



- **Applications**
  - Virtually every human tissue!

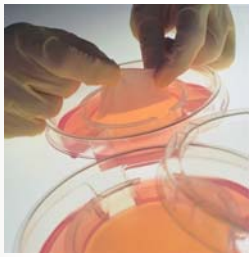
Do we need it?

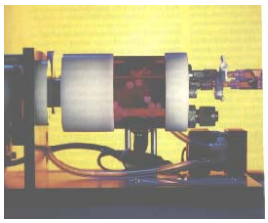


Organ and Tissue Deficiencies

• Tissue	• Procedures/Patient pa
– Skin	– 4,750,000
– Bone	– 1,340,000
– Cartilage	– 1,150,000
– Tendon & Ligament	– 123,000
– Blood Vessels	– 1,360,000
– Pancreas	– 728,000
– Urological	– 82,000
– Dental	– 10,000,000

Can we do that?

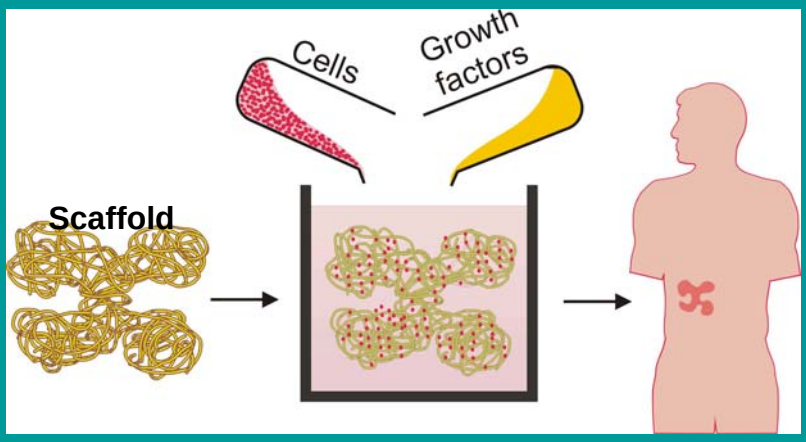




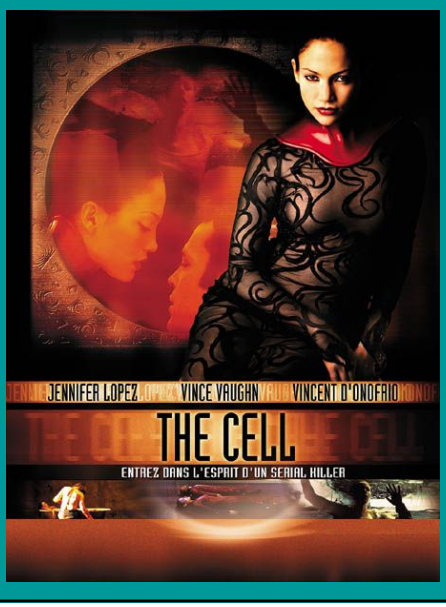
Neomorphogenesis

- To create an environment where the cells would be close enough to form structures, which can function and be implanted.
- Approach: donor cells are placed and cultured on a highly porous, biodegradable, polymer matrix.
- Examples: skin, cartilage, tendons, ligament, liver, ureters, etc.

Tissue Engineering

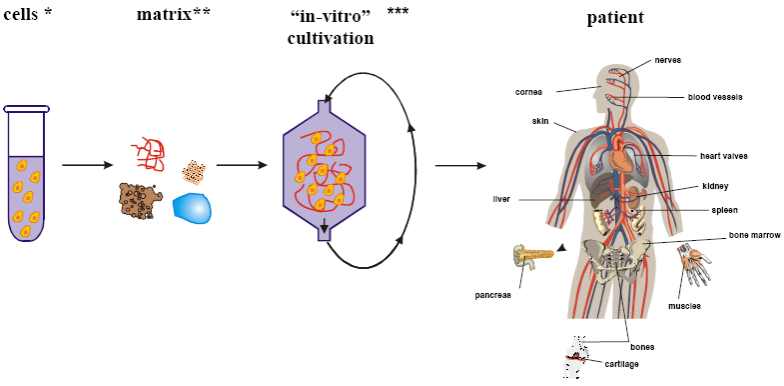


Cell + Matrix



Principle of Tissue Engineering

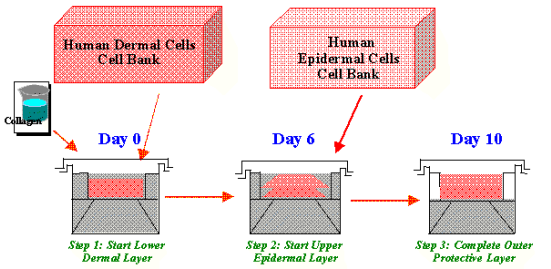
(1) Processes of TE



Engineered Skin

A New Era for Apligraf

How Apligraf is Made



Human dermal cells (fibroblasts) are combined with collagen, the primary protein of skin. The dermal cells move through the collagen, rearranging it, while also producing human collagen.

Human epidermal cells (keratinocytes) are placed on top of the dermal layer. These cells multiply to cover the dermal layer.

The epidermal cells are prompted, by exposure to air, to complete formation of the outer protective layer of skin - the stratum corneum.

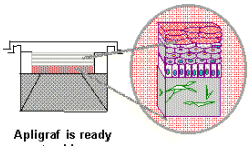
Apligraf Facts

Product size and shape are determined by configuration of cell culture dish.

Apligraf is made in 3-inch diameter size.

With the cells from one postage stamp-size piece of skin, up to 200,000 pieces can be made.

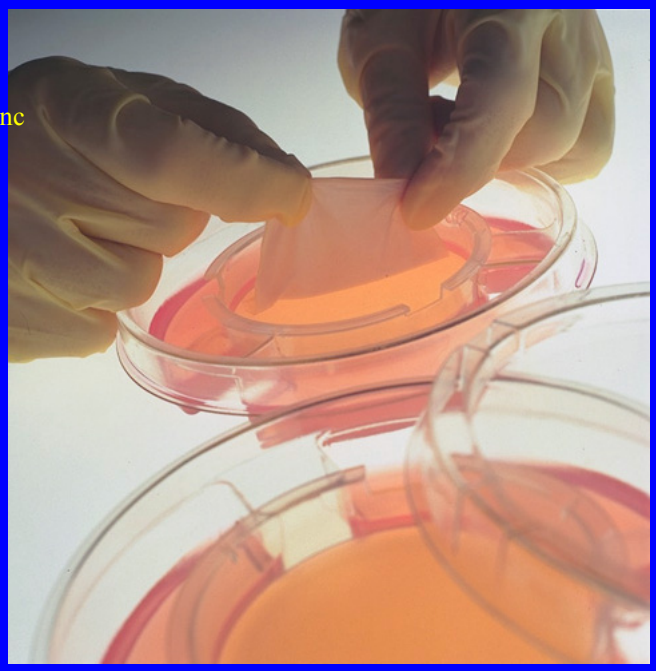
Day 20



Apligraf is ready to ship.

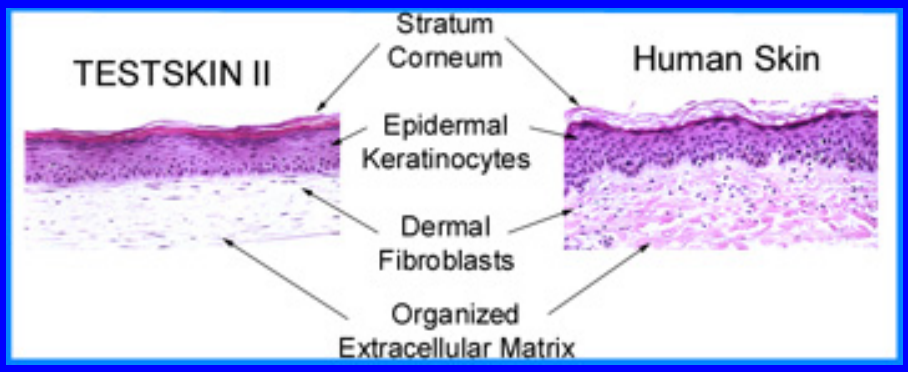
Apligraf

by Organogenesis Inc



TESTSKIN™ II

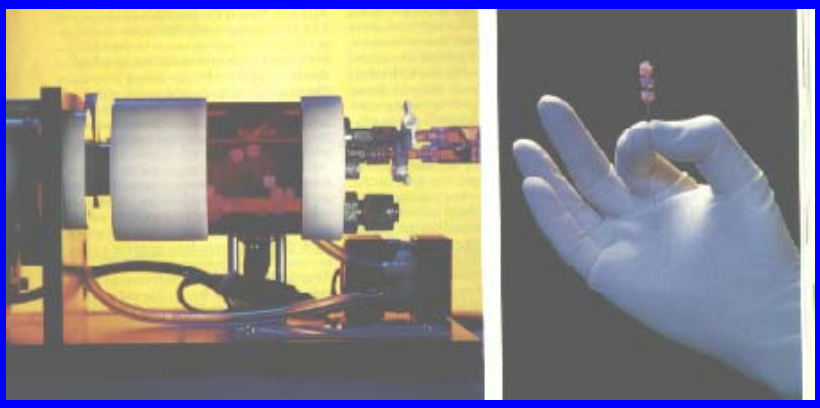
Living Skin Equivalent for In Vitro Testing



Organogenesis Inc

Dermagraft - by Advanced Tissue Science

Engineered Cartilage



Engineere

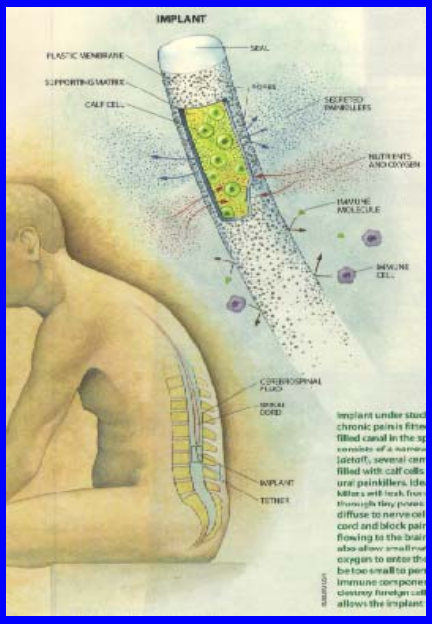
Engineered Soft Tissue

Immunoisolation Membranes

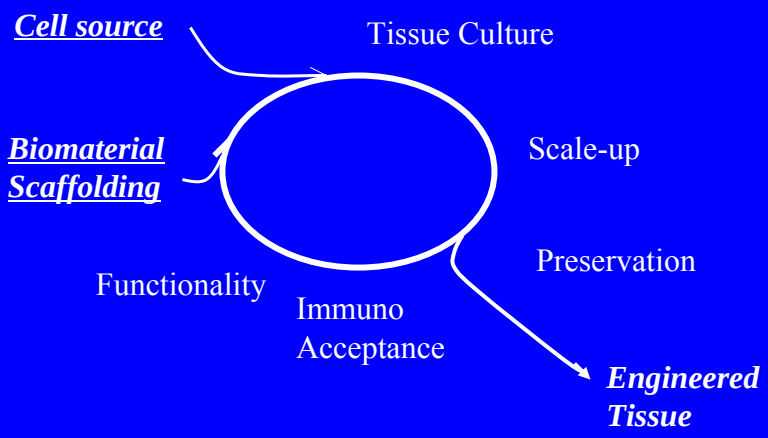
- Living cells are encapsulated with a polymeric membrane
- The membrane allows the molecules of interest to diffuse through, but rejects large antibodies and immune cells to protect the cells
- Examples: livers and pancreas

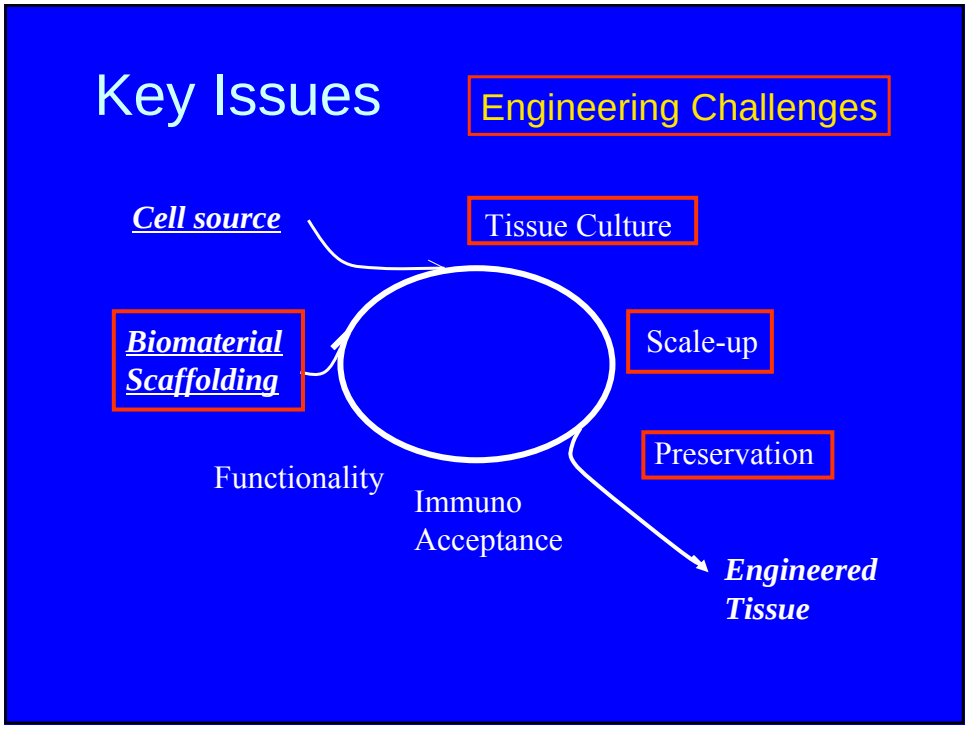
Cell Encapsulation

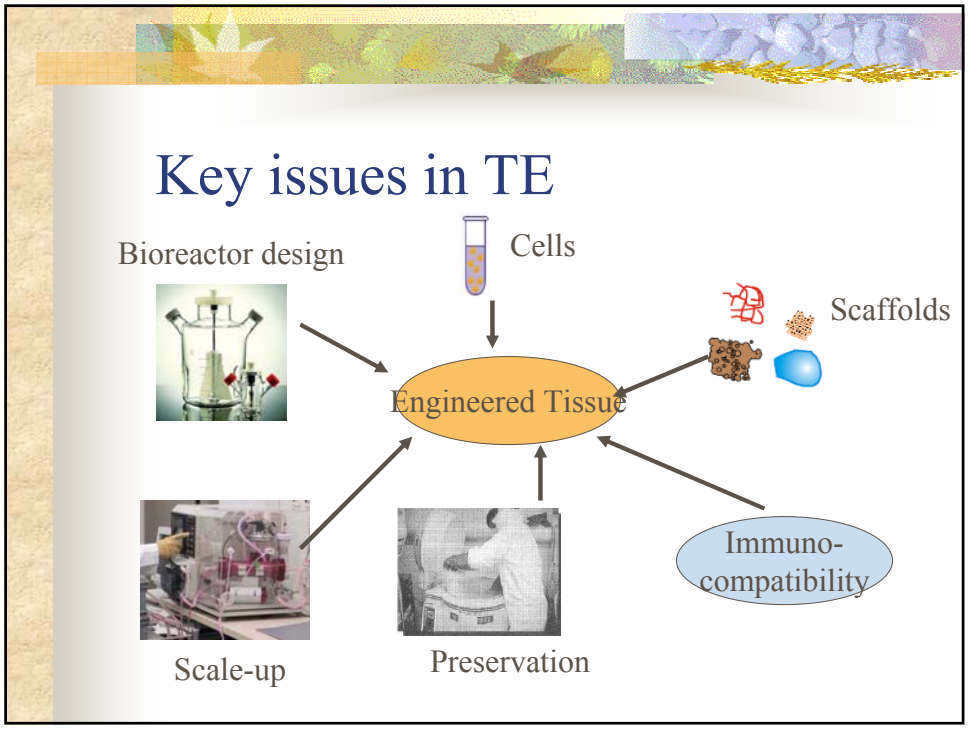
Cells secreting pain killer are encapsulated in a hollow fibre membrane and implanted into the spinal cord for the treatment of chronic back pain.



Key Issues




Key Issues
Engineering Challenges
Cell source
Tissue Culture
Biomaterial Scaffolding
Scale-up
Preservation
Functionality
Immuno Acceptance
Engineered Tissue


Key issues in TE
Bioreactor design
Cells
Scaffolds
Engineered Tissue
Immuno-compatibility
Scale-up
Preservation

Cell Source

- Autogetic: *Patient's own cells; immune acceptable, does not lend itself to off-the-shelf availability*
- Allogenic: *Cells from other human source; lends itself to off-the-shelf availability, but may need to engineer immune acceptance*
- Xenogentic: *From different species; not only need to engineer immune acceptance, but must be concerned with animal virus transmission*
- Stem cells: *Great potential but little is known*

Cells

Cell sources:

- From the patient (autologous cells)
- From a donor not identical with the patien (allogeneic cells)
- From a different species (xenogeneic cell

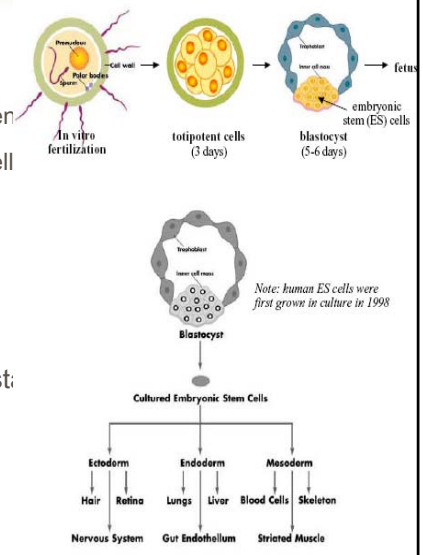
And Stem cells can be from

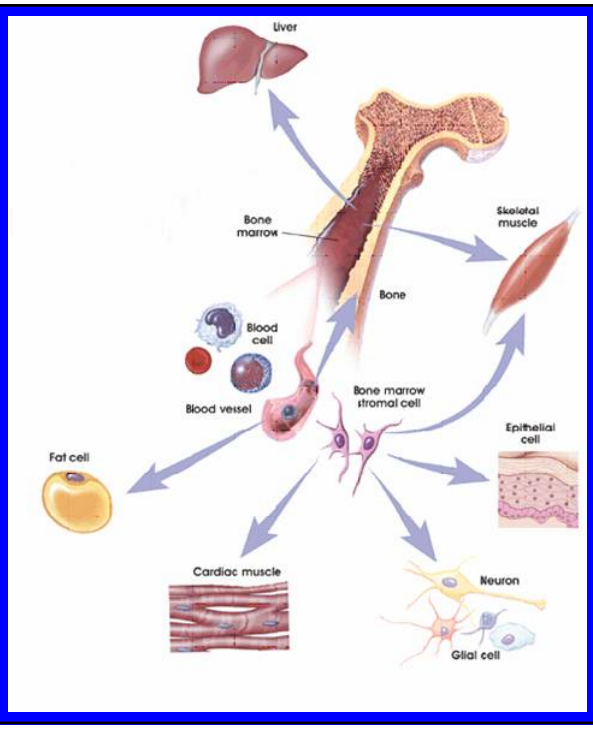
- The patient or another donor

(autologous or allogeneic adult stem cells)
Include:
Embryonic stem cells and adult stem cells

Characteristics:

- renew themselves for long periods
- Differentiation under certain circumst

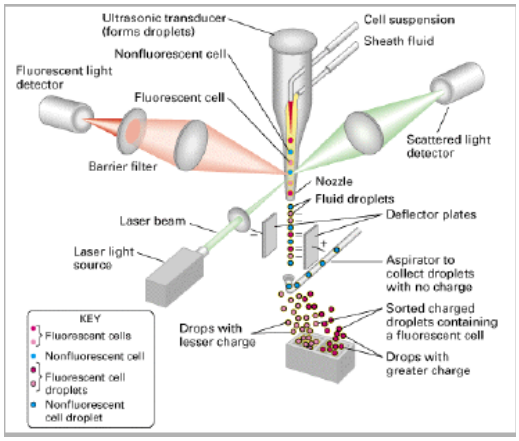




Cells

- Cell isolation:
  - From tissue:
    - Mechanical desegregation
    - Enzyme digestion: collagenase, trypsin, elastase, Hyaluronidase, papain, *Chymotrypsin*
  - From suspension:
    - Centrifuge, Flow cytometry

Fluorescence-activated cell sorting (FACS)

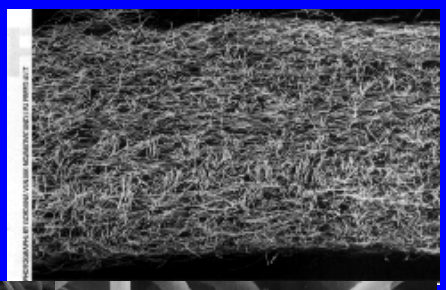


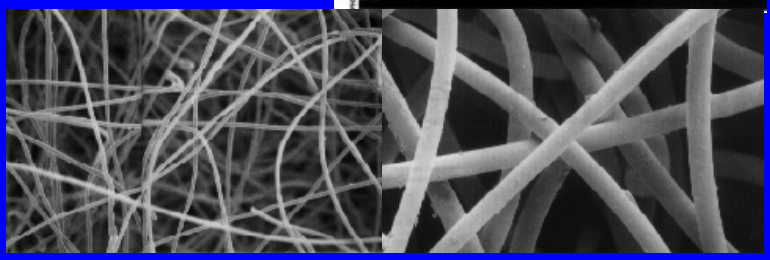
Detection and isolation of cells expressing specific surface proteins which can be detected by fluorescence monoclonal antibodies

Scaffolding Materials

- Natural Biopolymers
  - e.g collagen, alginate
- Synthetic Polymers
  - biocompatibility
  - biodegradability
  - mechanical properties
  - surface properties

Biopolymer Scaffolding

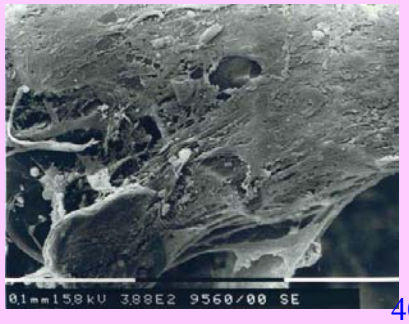




Growth of bone stem cells on 3 different materials after 21 days

20 X

20 X



400 X

Scaffolds

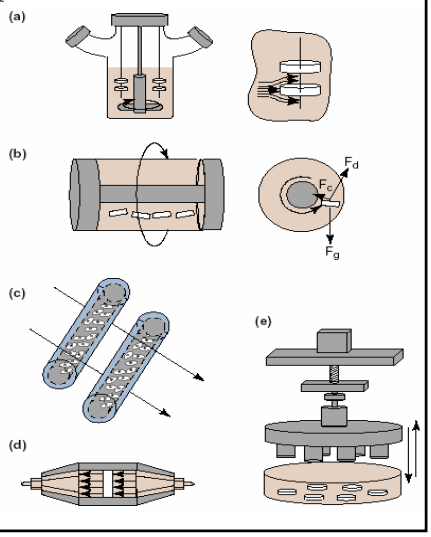
- Requirements:
  - Good adhesion, differentiation and proliferation
  - Good biocompatibility
  - Biodegradability
  - Non-toxicity
  - Larger surface for cell-polymer interaction
  - Interconnected pores
  - Easily fabricated
  - Mechanical properties

Scaffolds

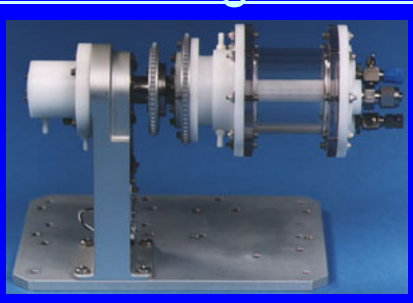
- Synthetic scaffolds
  - PLA, PGA
- Biological scaffolds
  - Collagen
- Hydrogel scaffolds
  - Agarose, alginate

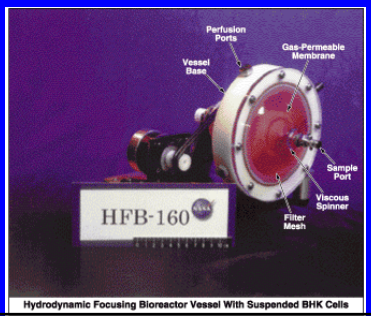
Bioreactor design

- Provide:
  - Nutrients and oxygen
  - Mechanical stress
  - Efficient mass transfer



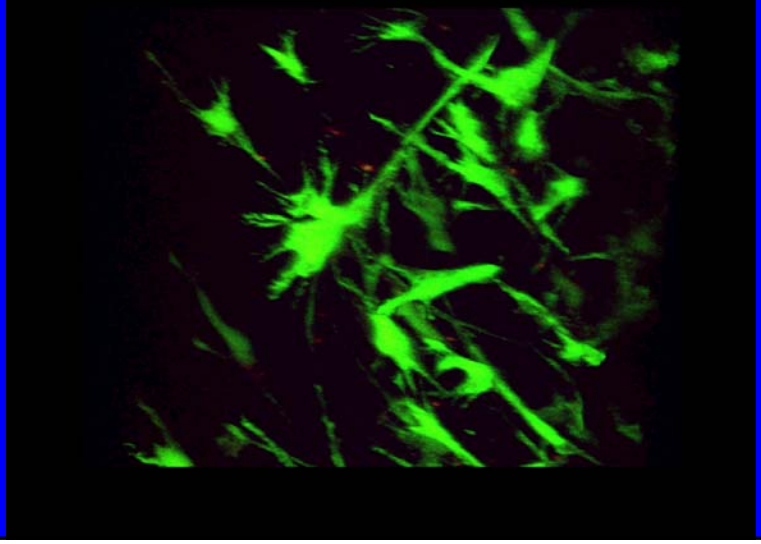
Bioreactors for Tissue Engineering





Hydrodynamic Focusing Bioreactor Vessel With Suspended BHK Cells

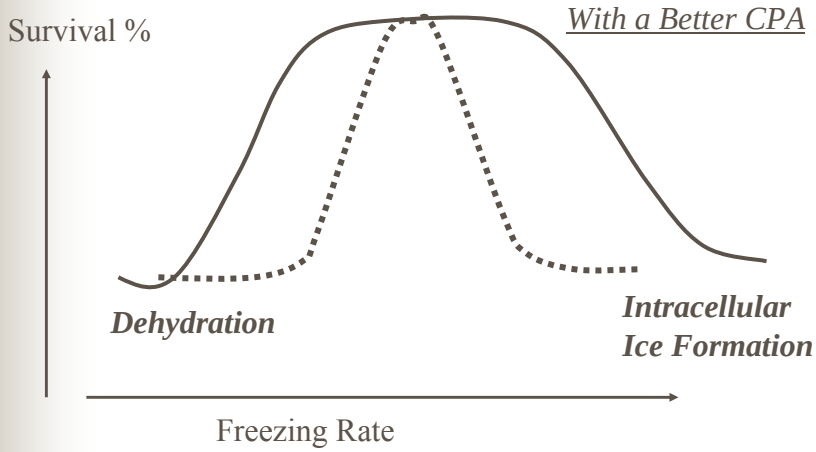
Multiphoton 3D Resolved Rotational Views of Bone Marrow Stem Cells Expressing EGFP Gene Grown in Matrigel



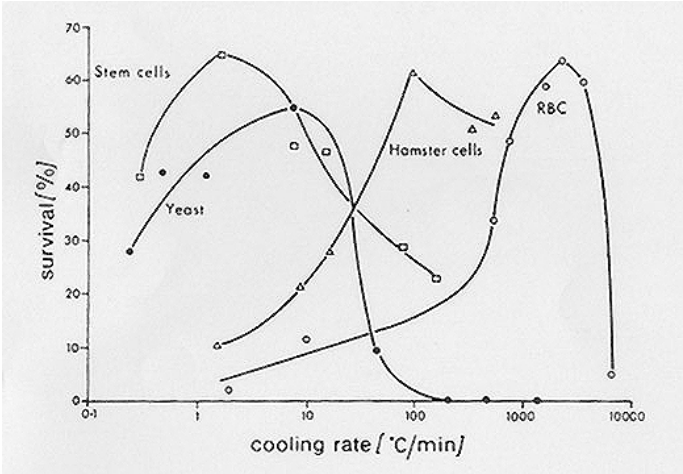
Preservation

- Needs for cell preservation – e.g. stem cell banking
- Needs for engineered tissue preservation
  - Off-the-shelf availability
  - Long production cycle
- Needs for organ preservation – matching availability of organ and recipient
- Quality control
- Product distribution

Freezing Damage to Cells

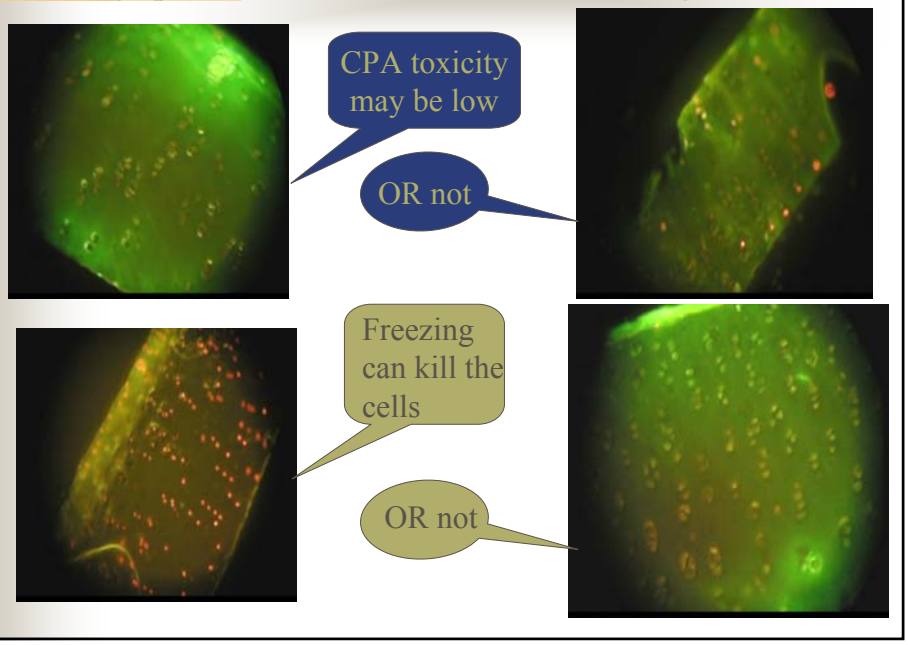


Effects of cooling rate on cell survival



Cooled to -196 C and thawed rapidly (from Mazur 1970)

Cryopreservation of Articular Cartilage

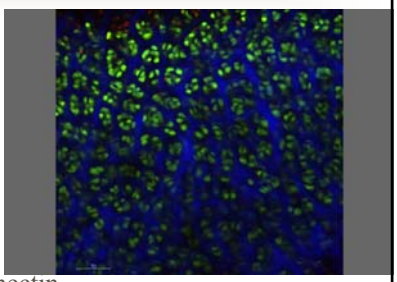


Biomaterials for Tissue Engineering

Scaffolding Materials

- Natural Biopolymers
  - e.g collagen, alginate
- Synthetic Polymers
  - biocompatibility
  - biodegradability
  - mechanical properties
  - surface properties
- Inorganic/ceramic materials
  - Bioglass, HA
- Biocomposite
  - PLA-HA
  - Collagen-HA

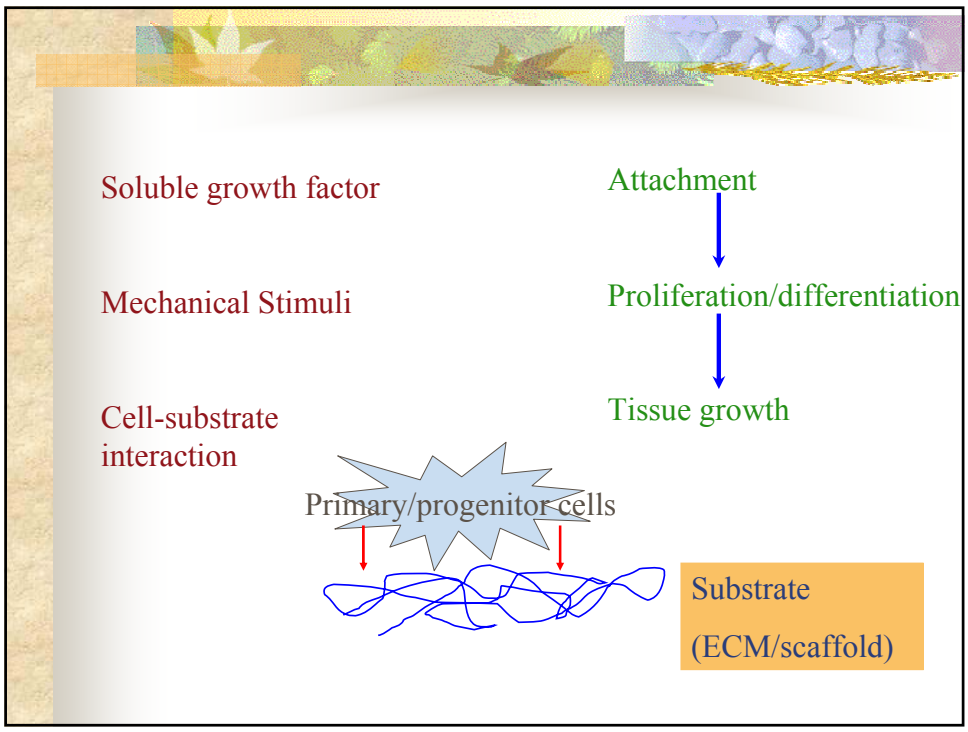
Extracellular Matrix



- Compositions of the ECM:
  - The polysaccharides:
    - proteoglycans
  - The proteins:
    - collagen, Elastin, Laminin, Fibronectin
- The role of ECM:
  - Provide 3D environment for cells to organize in tissues
  - Importance in cell-cell signaling and cell-ECM interaction to regulate cell adhesion and tissue function.

ECM functions

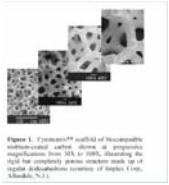
- Support cells
- Regulate:
  - Polarity
  - Cell division
  - Adhension
  - Molity
- Development
  - Migration
  - Differentiation
- Growth factors

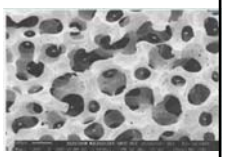


Biomaterials for Tissue Engineering

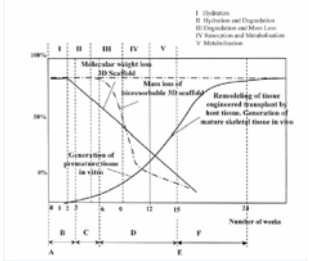
- In order to grow engineered tissues with proper functions, it is necessary to mimic native ECM
- Biomaterials for tissue Engineering should provide a 3D structure for cells to form new tissues, and allow for the delivery of cells and appropriate bioactive factors to the desired site

Properties of biomaterials for TE





- Material properties:
  - Mechanical support
  - High porosity, high surface area to volume ratio
  - Uniformly distributed and interconnected pores
  - Easy to make desired shape
  - Biodegradability
  - Sterilizable

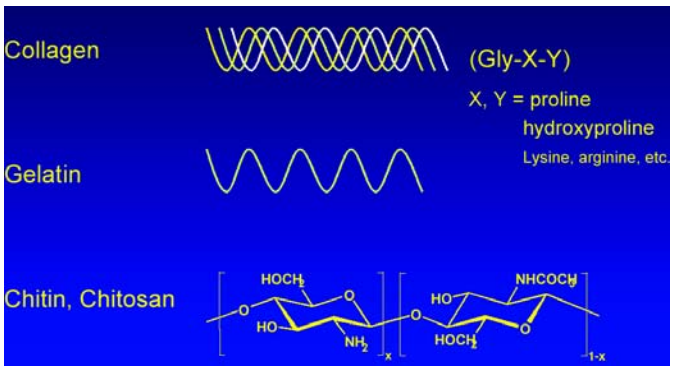


Properties of biomaterials for TE

- Biocompability
- Cell adhesion
- Cell growth
- Retention of cell functions

Types of Biomaterials for TE

- Natural Materials: low toxicity, low chronic inflammatory response



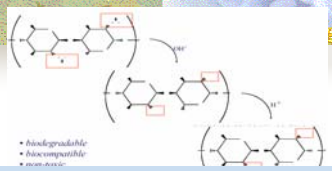
Collagen

- Structure protein with triple-strand helical structure
- Extracted by enzyme treatment and salt/acid extraction
- Can be resorbed into the body
- Degradation rate cab be altered by crosslinked with chemicals
- Excellent for attachment and biological interaction with cells
- Disadvantages:
  - Poor mechanical properties
  - Undergo contraction

Alginate

- A polysaccharide isolated from seaweed
- Gelling in the presence of calcium ion
- Used as injectable cell delivery vehicle and a cell immobilization matrix
- Biocompatible
- FDA approved for human use
- Disadvantages:
  - Poor mechanical strength
  - Poor cell adhension

Chitosan



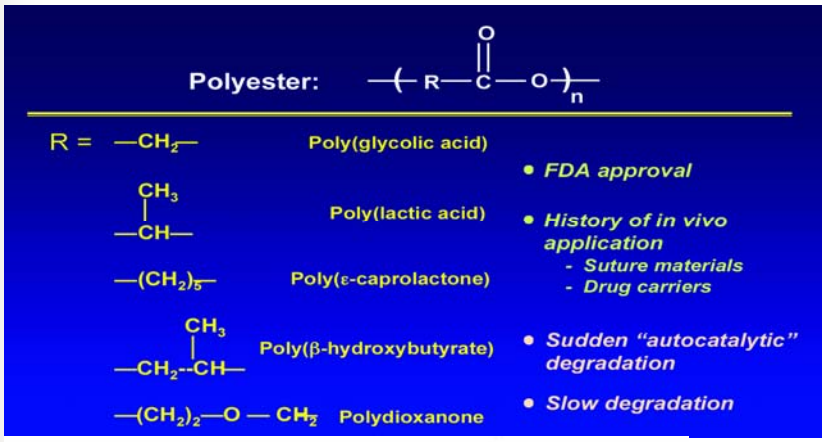
•Derived from chitin, the most abundant polysaccharide (marine organism) after cellulose in nature
•Linear polyamine (poly-D-Glucosamine)
•Reactive amino and hydroxyl groups available
•Advantages:
  •Biocompatible and biodegradable
  •Mild process conditions
  •Controllable mechanical/biodegradation properties
  •Availability of chemical side groups for attachment to other moleules
  •Accelerates the formation of osteoblasts responsible for bone formation
•Disadvantage:
  •Cell attachment

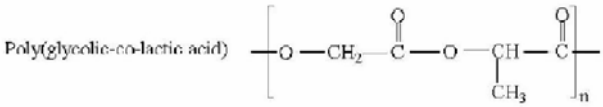
Synthetic Materials: Bioceramics and bioactive glasses

- Inorganic/non-metallic
- Bioactive glasses based on silica network structure with Ca, P, Na, form bond with living bone
- Bioceramics:

Bioceramic	Bone tissue attachment
Single crystal Al_2O_3 Polycrystalline Al_2O_3	Dense non-porous nearly inert ceramics. Bone growth into surface irregularities by cementing the device into the tissues or by press fitting into a defect (morphological fixation).
Polycrystalline Al_2O_3 Hydroxyapatite (HA)-coated porous metals Bioactive glasses Bioactive glass ceramics HA Calcium sulphate Tricalcium phosphate (TCP) Calcium phosphate salts	Porous inert implants – bone ingrowth occurs that mechanically attaches the bone to the material (biological fixation). Dense porous/non-porous surface-reactive ceramics, glasses and glass ceramics attach directly by chemical bonding with bone (bioactive fixation). Dense porous/non-porous resorbable ceramics slowly replaced by bone.

Synthetic Materials: Polymers

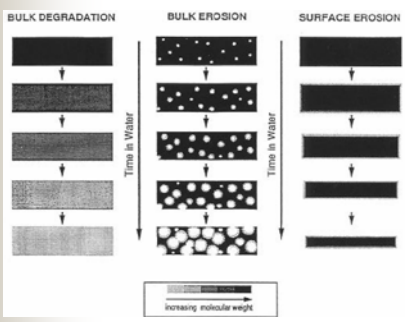


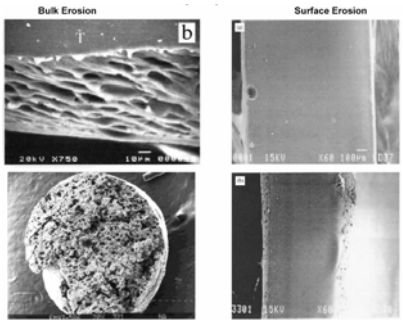


Synthetic Materials: Polymers

- PGA, PLA and PLGA are FDA approved
- Degradation by nonenzymatic hydrolysis
- The degradation products are nontoxic natural metabolites and eliminated from the body in the form of carbon dioxide and water

Mechanism of dissolution

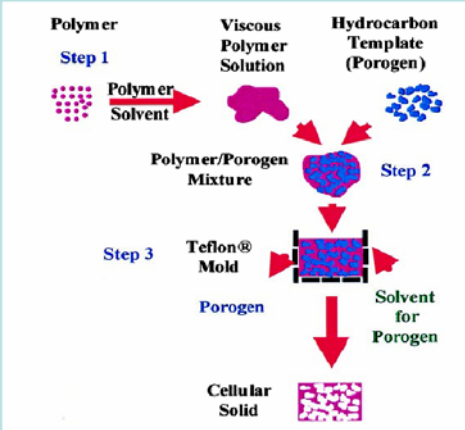




Scaffold Fabrication Techniques

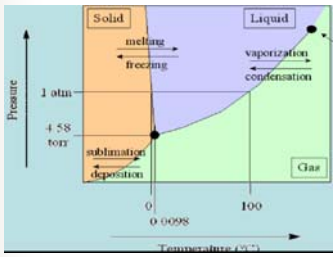
- Convention techniques:
  - Gas foaming, freeze drying, solvent casting and particulate leaching, fibre bonding, electrospinning melt molding, phase separation
- Rapid prototyping techniques:
  - Fused deposition modelling, 3D printing, selective laser sintering

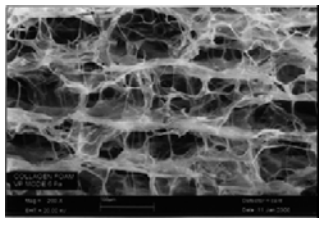
Solvent casting and particulate leaching



- Not easy to achieve uniformly distributed and interconnected pores
- Not solvent free

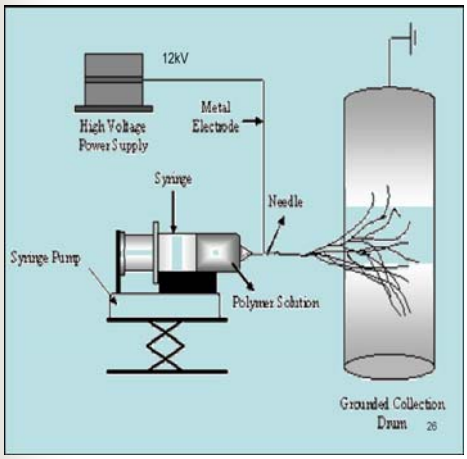
Freeze drying

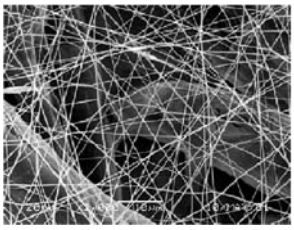


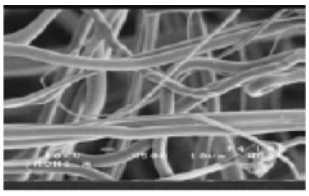


- The size of pores is determined by the cooling rate and volume fraction of materials, for example collagen
- The process is generally good for heat sensitive materials

Electronspinning

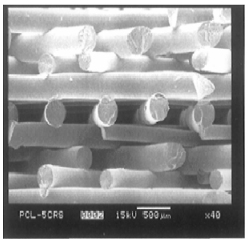


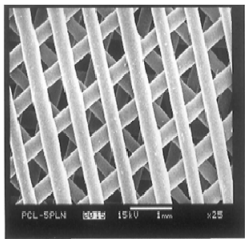




Fibres in non-woven matrix

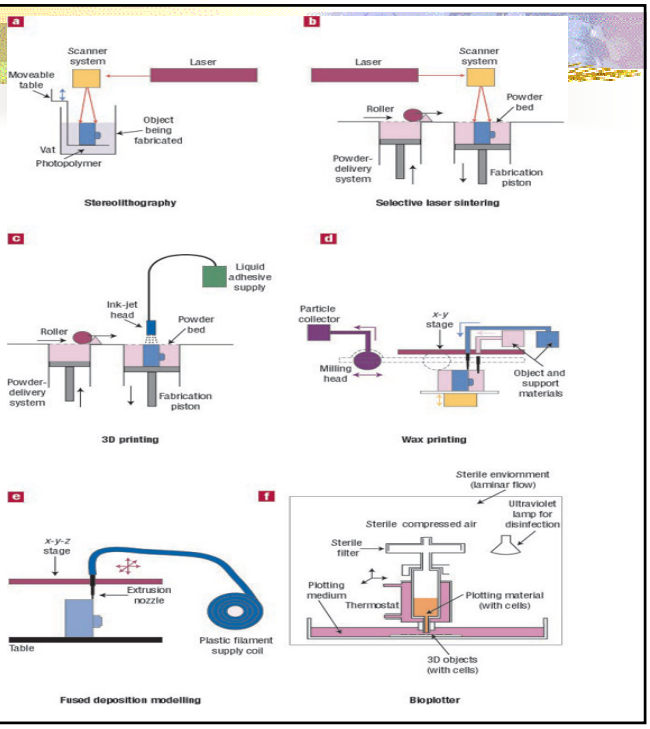
Rapid prototyping





- 3D scaffold is produced from a 3D representation devised in CAD
- Layer-layer slicing of the shape into consecutive 2D layers, which can be fed to the control equipment to fabricate the part

Rapid prototyping



Acellular tissue matrices

- Collagen-rich matrices prepared by removing cellular components from tissues
- The matrices slowly degrade
- Replaced and remodelled by ECM protein synthesized and secreted by ingrowing cells

Biomimetic scaffolds-modification

- Surface modification
  - Incorporate bioactive ligands via chemical or physical modification
  - Ligands minic protein found in ECM that are recognized and bind to specific cell surface receptors, such as integrins
  - Ligands can activate the cellular responses
- Bulk modification
  - Recognition sites are present not only on the surface but also in the bulk of the materials.
  - Mimic complicated events associated with in vivo environments.
  - Bulk modification with enzymatically degradable sequences.

Modification

By chemical attachment or physical adsorption

- Short chain peptide sequences
  - Surface density and orientation controlled more easily
  - Nearly all available for cell binding
  - Easily synthesized, purified, and inexpensive
- Long chain ECM proteins
  - Include fibronectin (FN), laminin (LN), and vitronectin (VN)
  - Proven to promote cell adhesion and proliferation

Mass transfer in Tissue Engineering

Transport in Biological system

- Molecular level
  - Characteristic length scale: 1nm
  - Ion channel in lipid layer
  - Transport at the level of a single ion channel
- Cell Level
  - RNA transport out of the cell's nucleus, L~1μm
  - Transpot of G-actin to psuedopod of leukocytes to form F-actin and allow for extension, L~10μm
- Microvascular level
  - Oxygen transport through the vasculature, L~10-100μm
- Organ level
  - L~1cm-10cm

Ficks' Law and Transport Properties

The law governing molecular diffusion of species was first formulated by Adolph Fick in 1855, using the analogy with heat transfer.

$$N_{Ay} = -D_A \frac{\partial CA}{\partial y}$$

where N_A is the main flux of A in y- direction, D_A is the diffusivity of A (m^2/s), $\frac{\partial C_A}{\partial y}$ is the concentration gradient.

Fick's Law applies to the diffusion of a single, dilute, species through a quiescent fluid, or through a liquid.

The diffusivity of gases at room temperature and pressure is around 10^{-5} m^2/s and is inversely proportional to pressure (why?). The much greater rate of intermolecular illusions in liquids means that liquid phase diffusivities are much lower – around 10^{-9} m^2/s.

Newton's Equation of Viscosity

$$\tau_{yx} = -\mu \frac{\partial V_x}{\partial y}$$

$\mu - N.s/m^2$ *or* $Pa.s$

$$\tau_{yx} = -\nu \frac{\partial(\rho V_x)}{\partial y}$$

ν - m^2/s

ρV_x - *momentum*/m^3

Fourier's Law of Conduction

$$q_y = -k \frac{\partial T}{\partial y}$$

$k - W/(m.K)$

$$q_y = -\alpha \frac{\partial(\rho CT)}{\partial y}$$

α - m^2/s

ρCT – *Thermal energy*/m^3

Fick's Law of diffusion

$$N_{Ay} = -D_A \frac{\partial C_A}{\partial y}$$

$D_A - m^2/s$

C_A – *mass*/m^3

General rate equation

Mass, heat, Momentum } Flux = mass, heat, momentum } diffusivity x

mass, heat, momentum } Concentration gradient

ν (μ), α (k) and D are called the transport properties, and they depends on temperature, pressure and concentration (for D)

For gases. $T \uparrow \rightarrow k \uparrow, \mu \uparrow, D \uparrow$

$P \uparrow \rightarrow D \downarrow$

For liquid $T \uparrow \rightarrow k \uparrow, \mu \uparrow, D \uparrow\uparrow$

D heavily depends on composition as well.

Convective Mass Transfer

Film theory

For the convective mass transfer between a surface with a concentration of C_{As}, and a fluid with a bulk concentration C_{Ab}, the rate of the mass transfer can be evaluated by assuming the mass transfer resistance is only confined within a thin laminar layer with a thickness of δ close to the surface. Then the mass flux

$$N_A = \frac{D}{\delta}(C_{As} - C_{Ab}) = k_c (C_{As}\text{-}C_{Ab})$$

where $k_c = D/\delta$ (m/s) is named as the mass transfer coefficient.

As one can expect, the more turbulent, the thinner of this laminar layer, and hence the higher the k_c.

If we look at the mass transfer in a circular pipe

$k_c = f(D, \mu, \rho, V, d)$

By dimensional analysis, we have

$$\frac{k_c.d}{D} = f\left(\frac{\rho Vd}{\mu}, \frac{\mu}{\rho D}\right)$$

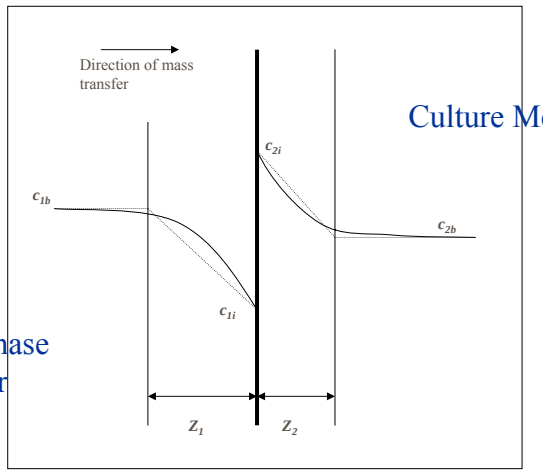
$$\frac{k_c d}{D} = \frac{k_c}{D/d} = Sh \quad Sherwood\ Number \rightarrow \frac{convective\ transfer\ rate}{diffusive\ transfer\ rate}$$

$$\frac{\rho Vd}{\mu} = \text{Re} \qquad \text{Re}\ynolds\ number$$

$$\frac{\mu}{\rho D} = \frac{\gamma}{D} = Sc \quad Schmidt\ number \rightarrow \frac{momentum\ transfer\ BL\ thickness}{mass\ transfer\ BL\ thickness}$$

$$[compare\ to\ Nu = f(Re, Pr),\ and\ Pr = \frac{\mu C_P}{\kappa} = \frac{\mu/\rho}{k/\rho C_p} = \nu/\alpha\ ]$$

Interface Mass Transfer



NA = kc1 (C1b – C1i) = kc2 (C2i – C2b)

At the interface the two phases must be in equilibrium with each other. For example, in a gas/liquid system, Henry's Law will apply

$$P_{Ai} = H\, c_{Ai}$$

where **H** is the Henry constant. In the gas phase (assumed to be phase 1, and ideal) the molar concentration of A is P_A/RT. We thus have two equations for the flux through the gas and liquid films

$$N_A = k_G\,(P_{Ab} - P_{Ai})/RT$$

$$N_A = k_L\,(c_{Ai} - c_{Ab}).$$

We can also define **overall mass transfer coefficients** based on the overall driving forces. There are two of them

$$N_A = K_G\,(P_{Ab} - P_A^*)/RT = K_G\,(P_{Ab} - H\,c_{Ab})/RT$$

$$N_A = K_L\,(c_A^* - c_{Ab}) = K_L\,(P_{Ab}/H - c_{Ab}).$$

The quantity Hc_{Ab} is the equilibrium partial pressure P_A^* that would occur over a solution of concentration c_{Ab}, and P_{Ab}/H is the equilibrium concentration c_A^* for a partial pressure P_{Ab}. Note that neither P_A^* nor c_A^* actually occur in the system - they are hypothetical values. We can use the condition for equilibrium at the interface, $P_{Ai} = H\,c_{Ai}$, to eliminate the interface concentration and partial pressure and hence obtain the law of addition of resistances

$$\frac{1}{K_G} = \frac{1}{k_G} + \frac{H}{RTk_L}$$

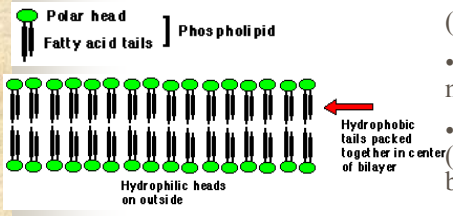
$$\frac{1}{K_L} = \frac{RT}{Hk_G} + \frac{1}{k_L}$$

The distribution of resistances between the gas and liquid phases therefore depends on the solubility. For a sparingly soluble gas (large H) the mass transfer resistance is in the liquid phase; for a highly soluble gas (small H) a greater proportion of the resistance is in the gas phase.

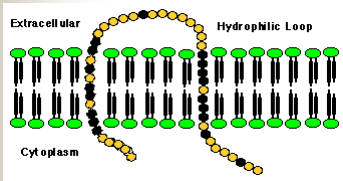
Mass transport through cell membranes

- **Cell membranes**
  - is a selectively permeable lipid bilayer coated by proteins which comprises the outer layer of a cell.
  - In essence membranes are essential for the integrity and function of the cell.
  - control the input and output of the cell

Cell Membranes



- Cell membranes are phospholipid bilayers (2 layers)
- Bilayer forms a barrier to passage of molecules in an out of cell
- The hydrophobic tails of the phospholipids (fatty acids) are together in the center of the bilayer. This keeps them out of the water



- Proteins that penetrate the membrane have hydrophobic sections ~25 amino acids long
- Hydrophobic = doesn't like water = likes lipids

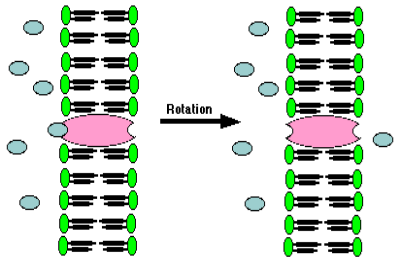
Functions of cell membranes

- Attaches parts of the cytoskeleton to the cell membrane in order to provide shape.
- Attaches cells to an extra-cellular matrix in grouping cells together to form tissues.
- Transports molecules into and out of cells
- Acts as receptor for the various chemical messages that pass between cells
- Part of the body's defense mechanism.

Transport through cell membranes

- Passive transport
  - Diffusion
    - Simple diffusion
    - Facilitated diffusion
  - Osmosis
- Active transport

Facilitated diffusion



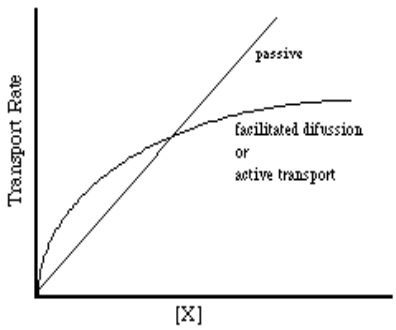
$$\frac{dn}{dt} = \frac{V_{Max}}{1 + K / \left(dC/dx\right)}$$

K and Vmax depend on properties of the diffusing molecule,

maximum rate of diffusion (Vmax): when all the carrier proteins are saturated

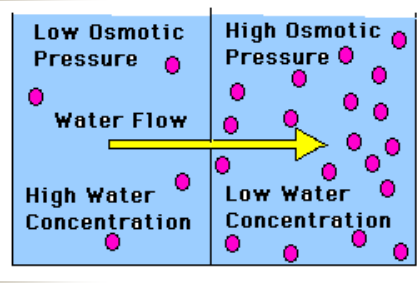
How quickly the carrier proteins become saturated can be described by the variable K, the concentration gradient at which the rate of diffusion is 1/2 Vmax.

Facilitated diffusion-conclusion



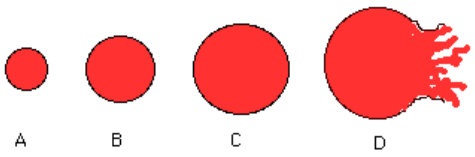
- Simple diffusion of solute into a cell is linearly related to the concentration of solute outside the cell.
- Facilitated diffusion, however, approaches a maximum rate as the carrier proteins become saturated with solute.

Osmosis



- Movement of water from dilute solution to high osmotic pressure concentrated solution
- Osmosis is passive: doesn't require ATP energy
- Osmotic flow through most biological membranes is by bulk flow and is similar to the flow caused by a pressure gradient

Osmosis



- If the external solution balances the osmotic pressure of the cytoplasm it is said to be isotonic.
- If the external solution is more dilute than the cytoplasm it is hypotonic and if the external solution is more concentrated it is hypertonic.
- Osmosis often produces significant volume changes, causing swelling or shrinking when cells are in Hypotonic Solutions or Hypertonic solutions

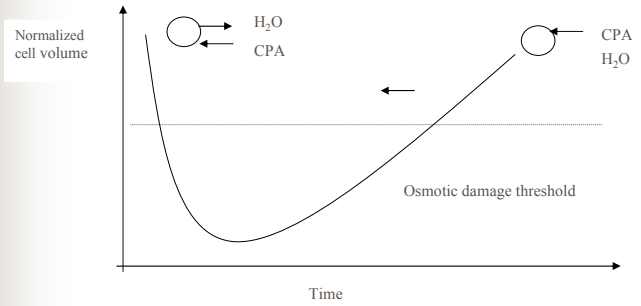
Activated transport

- Primary active transport:
  - use energy (usually through ATP hydrolysis) at the membrane protein itself to cause a conformational change that results in the transport of the molecule through the protein.
    - Na+-K+ pump.
- Secondary active transport:
  - use energy to establish a gradient across the cell membrane, and then utilizing that gradient to transport a molecule of interest up its concentration gradient.

Comparison of Simple Diffusion, Facilitated Transport & Active Transport

Property	Simple Diffusion	Facilitated Transport	Active Transport
Requires special membrane proteins	No	Yes	Yes
Highly selective	No	Yes	Yes
Transport saturates	No	Yes	Yes
Can be inhibited	No	Yes	Yes
Hormonal regulation	No	Yes	Yes
Uphill transport	No	No	Yes
Requires ATP energy	No	No	Yes

Mass transfer in cryopreservation



Once cell volume change is beyond the osmotic threshold, it will result in irreversible damage.

CPA transport through cell membranes

- Kedem-Katchalsky formalism (K-K model)

$$J_v = \frac{1}{A}\frac{dV_{w+c}}{dt} = -Lp\{(C_s^e - C_s^i) + \sigma(C_c^e - C_c^i)\}RT$$

$$J_{CPA} = \frac{1}{A}\frac{dN_c}{dt} = \overline{C}_c(1-\sigma)J_v + \omega(C_c^e - C_c^i)$$

$$C_s^i(t) = C_s^{e,0}\left(\frac{V_{cell}^0 - V_b - v_{CPA}N_c^{i,0}}{V_{cell}(t) - V_b - v_{CPA}N_c^i(t)}\right)$$

$$C_c^i(t) = \left(\frac{N_c^i(t)}{V_{cell}(t) - V_b - v_{CPA}N_c^i(t)}\right)$$

2-P model

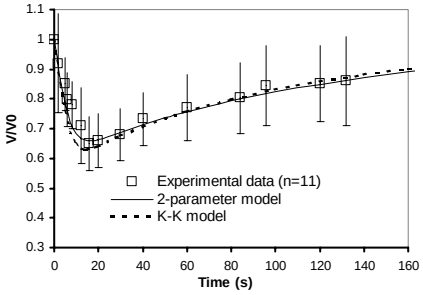
$$J_w = \frac{1}{A}\frac{dV_w}{dt} = -Lp(C^e - C^i)RT$$

$$J_{CPA} = \frac{1}{A}\frac{dN_c}{dt} = \omega(C_c^e - C_c^i)$$

$$V = V_w + V_c + V_b$$

$$dV_c / dt = \nu_{CPA} dN_c / dt$$

K-K vs 2-P



Normalized volume change of chondrocytes during 1,2-propanediol addition (1.4 M, 21oC)

Modelling Tissue Growth

Features of a Growing Engineered Tissue

- Cells consume nutrient and produce waste
- Mass transport is by diffusion, and/or perfusion, and/or convection
- Cells can proliferate
- Cells make extracellular matrix
- Cells can degrade scaffold

Mass Transfer Modelling

$$\frac{\partial C_i}{\partial t} = D_{e,i}\nabla^2 C_i + \Phi_i + C_i \nabla \bullet V$$

i = 1,2, … N with N the number of concerned substances (nutrients, metabolites, growth factors ect)
C the concentration
V the perfusion velocity vector
Φ is the consumption rate or production rate of i per unit volume.

PLUS BCs and ICs

Challenges

The consumption/productions rates

Φi = ϕ[C1, C2, ……, CN, ρ (x,y,z), P(t), St]
ρ (x,y,z) the cell density distribution
P(t) the hydrostatic pressure time profile
St other factors, e.g. mechanical/electrical stimuli

The effective diffusivities

depend on cell density, ECM production
scaffold degradation and non-uniformity;
are difficult to evaluate

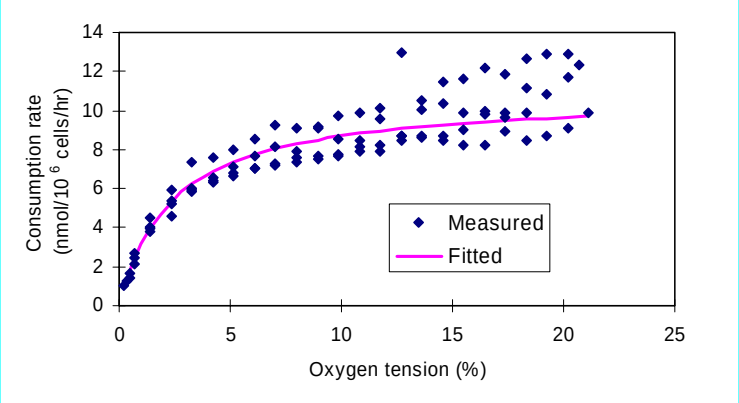
Perfusion

also depends on applied driving force

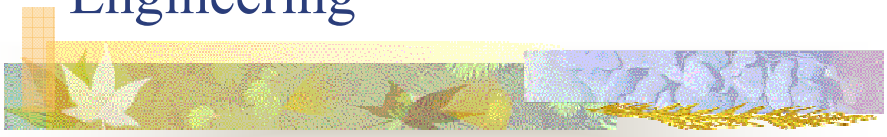
Good News – dilute, the interactions among solutes can be ignored !

Oxygen Consumption of Chondrocytes

$$\Phi = \frac{K \cdot C}{M + C}$$



Bioreactors in Tissue Engineering



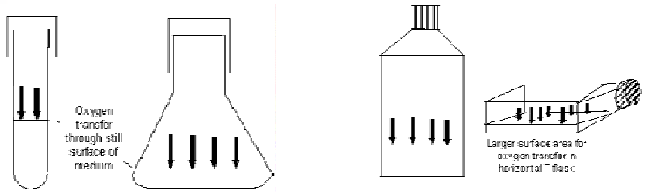
1. Description of Bioreactors

- Objectives of bioreactors in Tissue Engineering
  - establish spatially uniform cell distributions on three dimensional scaffolds, to maintain desired concentrations of gases and nutrients in the culture medium, and to expose developing tissue to appropriate physical stimuli.
- Bioreactors are able to mimic physiological conditions in order to maintain and encourage tissue regeneration.

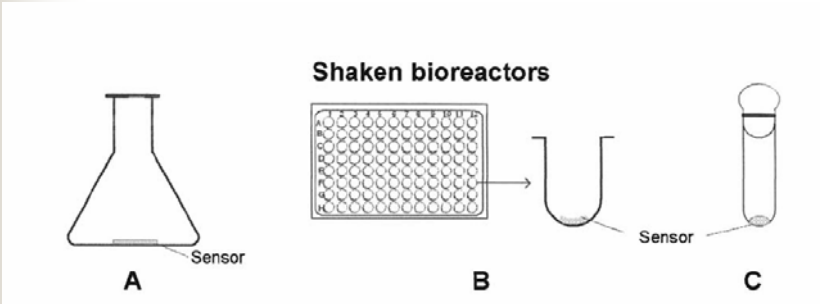
2. Classification and configuration of bioreactors

- Standing cultures
- Shaken bioreactors
- Stirred system
- Hollow fibre bioreactors
- Rotating-wall vessels
- Perfused bioreactors
- Packed bed bioreactors
- Bioreactors with controlled mechanical force

Standing cultures



Shaken bioreactors



Lmitation: O2 limitation and pH excursion may occur without being observed.

Control of experimental parameters such as pH, dissolved O2 and OTR is difficult, cross contamination

O2 transfer is usually low

Stirred System

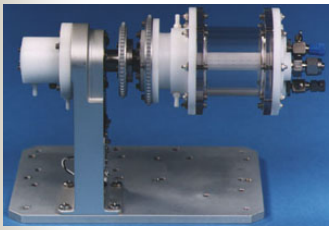
Limitations: high cost compared to other small-scale devices and in the difficulties of their application for high-throughput screening.

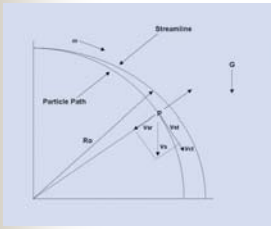
Hollow fiber bioreactors (HFB)

Consisting of bundles of hollow fibers in a plastic cartridge. Cells and tissues are usually seeded outside the fibers in the extra-capillary space.

- Medium is circulated from a reservoir through the fiber intra-capillary space back to the reservoir.
- The walls of the hollow fibers serve as semi-permeable ultra-filtration membranes with a molecular mass cut-off of 10–100 kDa.
- Retain cells and secreted macromolecular products in the relatively small volume of the extra capillary space
- Permit gas, nutrients, and metabolic waste products to diffuse freely across the membrane.

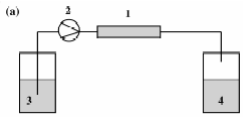
Rotating-wall vessels

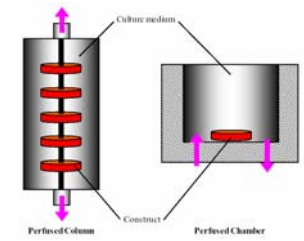


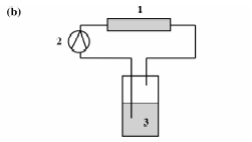


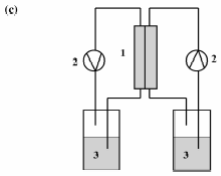
- Solid body rotation:the damaging effects of turbulence and shear stress are minimized.
- The vessel is completely filled with culture media, avoiding the turbulence created by a headspace.
- Delivery of oxygen is accomplished via a coaxial silicone membrane avoiding bubbles, which can create cell-damaging turbulence.
- Gentle mixing of media induced by particle sedimentation.

Perfused bioreactors

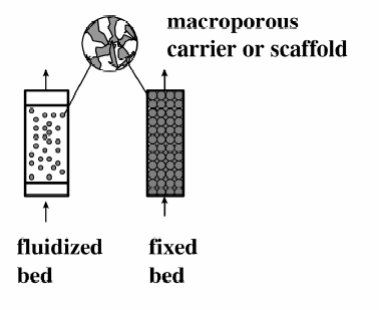




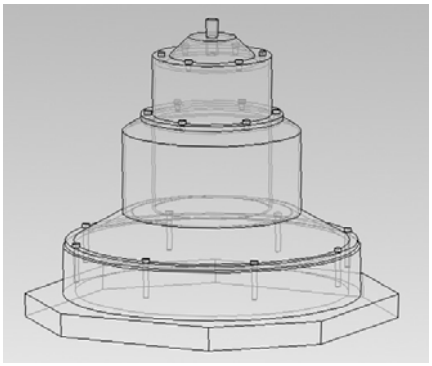




Packed bed Bioreactors

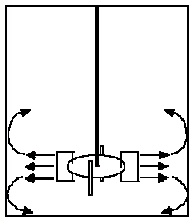


Bioreactors with controlled mechanical forces

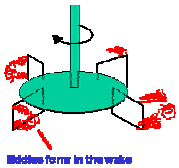


Bioreactor design

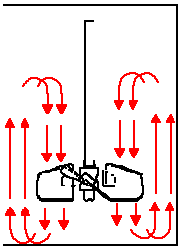
- a. Mixing design for the stirred system
  - Agitator design



With radial flow impellers, the liquid is pushed towards the wall of the tank; that is, along the radius of the reactor

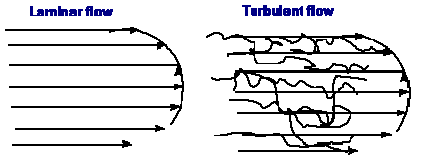


Eddies form in the wake of the impeller blades and generate a high shear environment



With axial impellers, the liquid is pushed in a downward direction; that is, along the axis of the reactor.

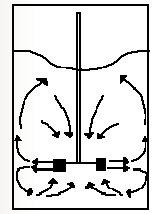
Fluid mixing



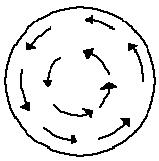
Fluid stays in ordered flow lines. There is no significant mixing between the flow lines

As the fluid moves turbulent eddies are formed. These eddies break down forming smaller eddies. On a microscale, liquid moves in random directions, leading to mixing.

Laminar flow in stirred tank bioreactors



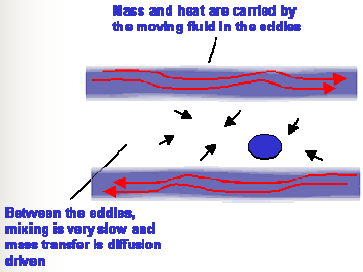
The impeller draws liquid and air towards it, creating a vortex



Even during vortexing, the liquid circulates around the tank in flow lines, leading to poor mixing

Vortex should be avoided as the collisions between the cells, air-bubbles and the impeller can lead to cell damage. Vortexing can be prevented with: Off-centre impellers; Baffles.

Turbulence and turbulent eddies

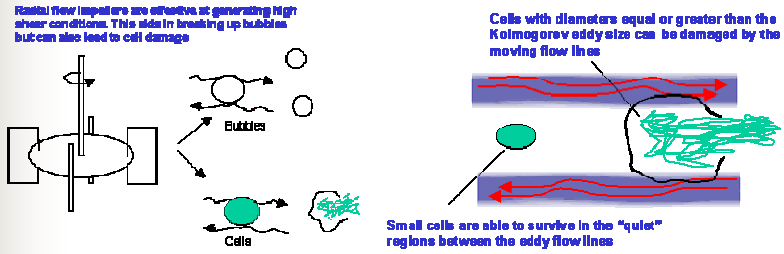


Kolmogorov Eddy Size:

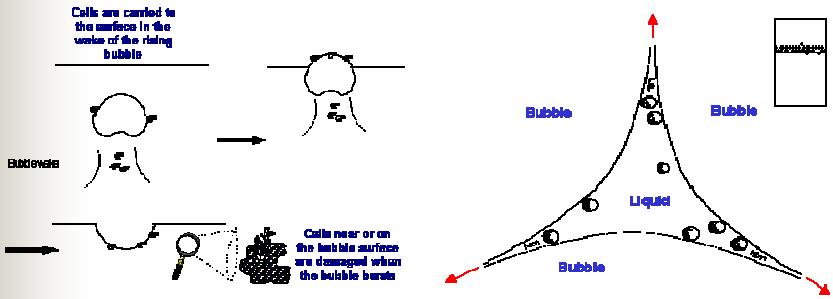
The smallest eddy size that can be formed is called the Kolmogorov Eddy Size

b. Cell damage

- Shear damage
  - Localised shear
  - Shear in the bulk liquid (Kolmogorov Eddy Size)

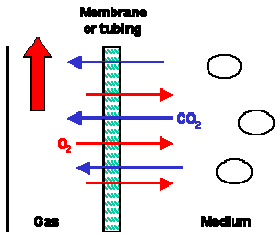


Bubble damage and foam damage



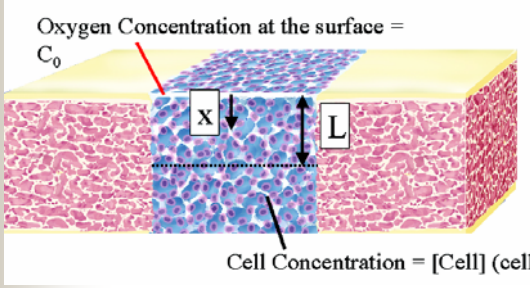
c. Methods of minimizing cell damage

- Impeller design
- Bubble free oxygenation
  - headspace oxygenation
  - external oxygenation
  - direct oxygenation using gas permeable silicone tubing or hydrophobic membranes.



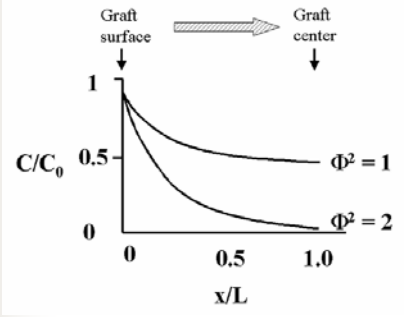
d. Oxygen transfer

The diffusion distance is critical to maintaining the balance between oxygen delivery to a site and consumption of oxygen by cells, both in native tissues and in tissue engineering strategies involving cell transplantation.



✓Few cells tolerate diffusion distances of >0.2 mm.
✓Cartilage is exceptional, maintaining viability in avascular regions >1 mm thick.

$$\frac{d(C/C_0)}{d(x/L)} = \phi^2 = \frac{[Cell]\,Q_{oxygen}L^2}{C_0 D_{oxygen}} \sim \frac{\text{Reaction rate}}{\text{Diffusion rate}}$$



In the model, diffusion of oxygen in the x direction is balanced by cellular consumption.

Tissue Engineering Solutions for Human Problems !