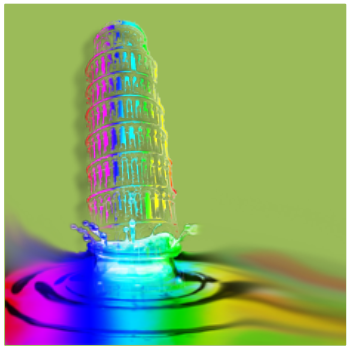

Dynamynic cell culture systems: bioreactors

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New testing systems: why?

Overcoming problems:

- ethical
- scientific

Cell culture

PROBLEM: cells taken out of their biological context

Cell culture conditions

- nutrient supply
- oxygen and CO_2 supply
- temperature

Bioreactors

Static Culture System

Low Shear Perfusion System

Rocking Culture System

Spinner Bioreactor System

Spinner-Air Lift Bioreactor System

Rotary Cell Culture System

Airlift Bioreactor

Hollow-Fiber Bioreactor

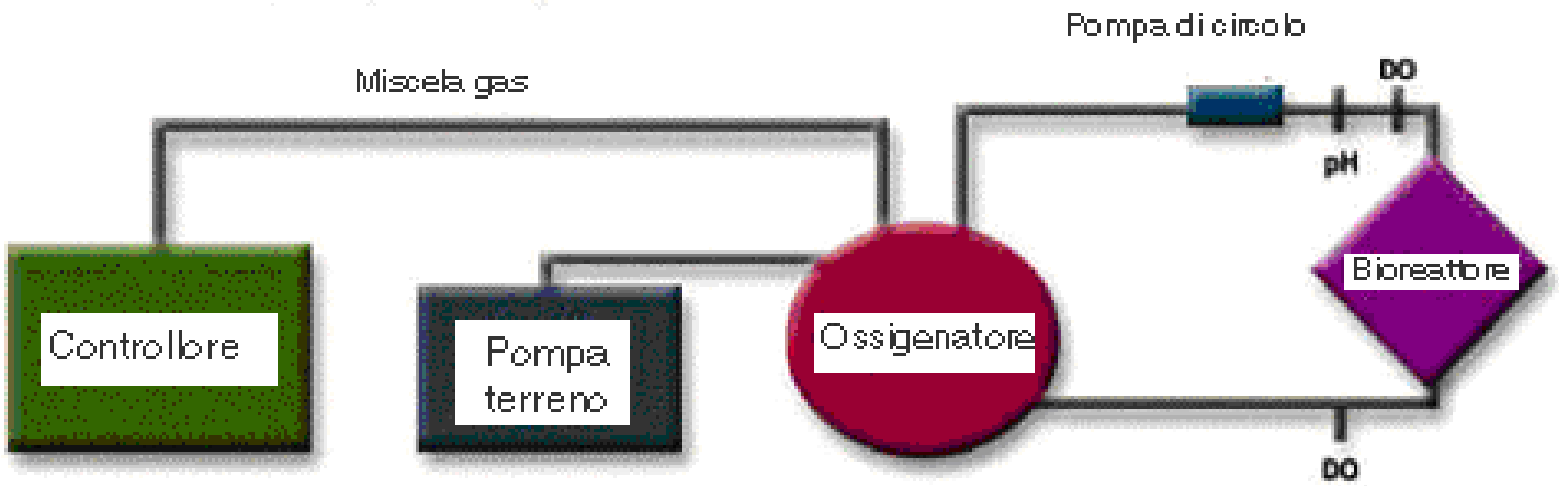
Static Culture System

- Simpler culture cells on the market
- They guarantee a homogeneous growth of the crop, allow visualization under a microscope thanks to their transparency.
- They are made of high quality plastic (generally polystyrene)

Low Shear Perfusion System 1/3

- Flow bioreactors designed for high-density cultures: nutrient flow allows easier perfusion and more effective removal of cellular catabolites Cell mono-layer growth rate increased by 100-200%

- Controller enters the right mixture of air and CO2 in the oxygenator to manage pH: it can manage system temperature and pump speed that circulates the culture medium



Low Shear Perfusion System 2/3

• Bioreactor cell consisting of two parallel polystyrene plates, provide high surface area for cell adhesion and growth

• Coupling for the cell culture medium inlet

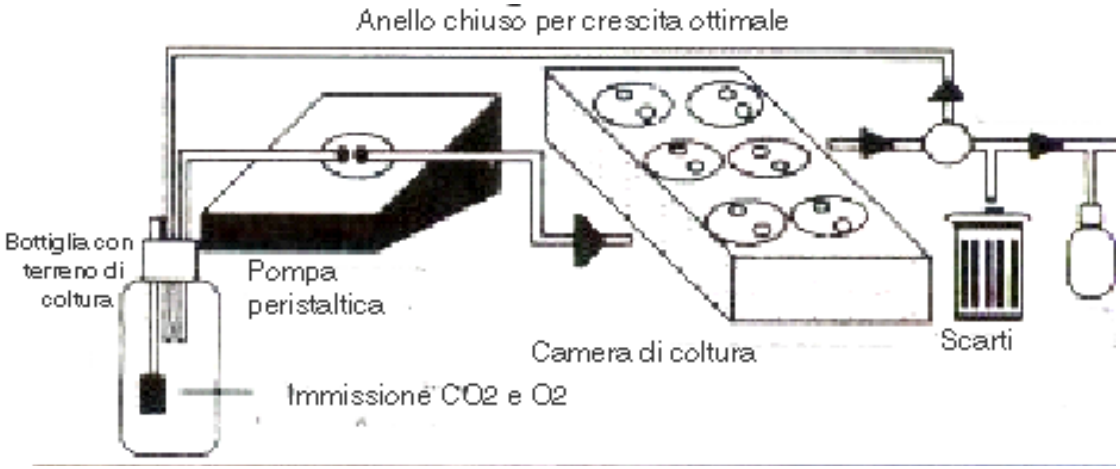
• The shape of the cell chamber does not allow high flows due to the turbulent motions that are created and that make the culture environment not ideal for 3D cell growth: easily sterilizable cell and also "disposable".

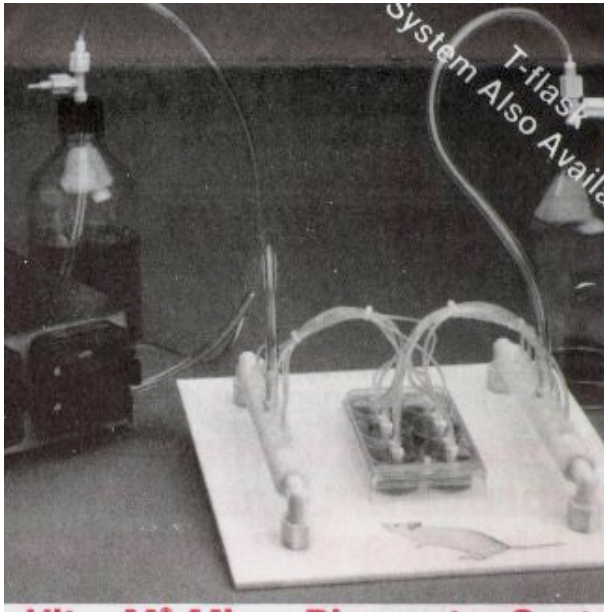
Low Shear Perfusion System 3/3

Model has two interesting features:

- selection of up to 6 reagents to flow in the 6 culture chambers with constant flows over time
- possible collection of catabolites for analysis
- Low flow rates (flow rates from 1ml / s to 10ml / s)

Totally bio-compatible (silicone, polystyrene, polycarbonate)





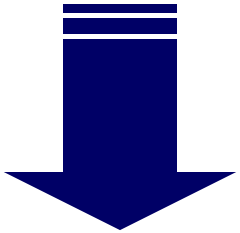
Rocking Culture System 1/2

Cell culture devices, such as T-Flask or similar devices, have volumes lower than one liter due to limited O2 transfer

For larger volumes Stirred-Tank bioreactors modified to limit shear are needed; they are steel and expensive.

These systems have been used for proliferation of neuronal stem cells, for the study of cell proliferation within porous polymeric structures

The design of the oxygenation and agitation system of the cell medium is therefore important, as the formation of vortices can cause damage to the cells and alter their functionality.

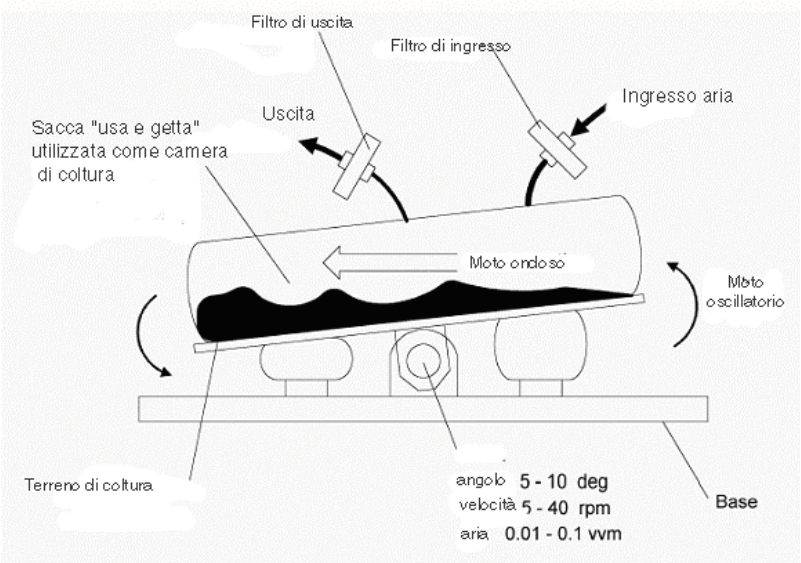


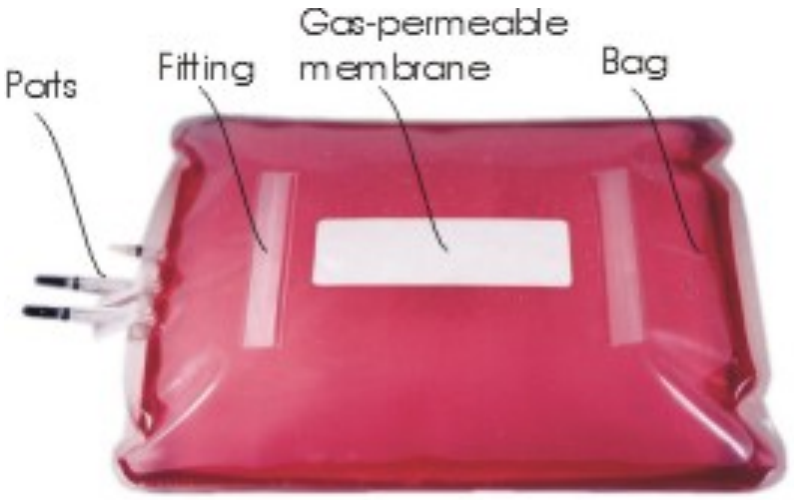
The Rocking Culture System is a low-cost bioreactor for making large cell and virus cultures.

Rocking Culture System 2/2

Rocking-bioreactor consists of two components:

- fixed base with an oscillating shelf;
- "disposable" culture bag (polyethylene), flexible, impermeable to gases.
- Gas flows through connectors on the top of the bag
- Mass transfer and soil mixing achieved through oscillation of the support
- Oscillatory motion generates waves that increase gas transfer, promoting cell mixing and suspension and minimizing thermal gradients.



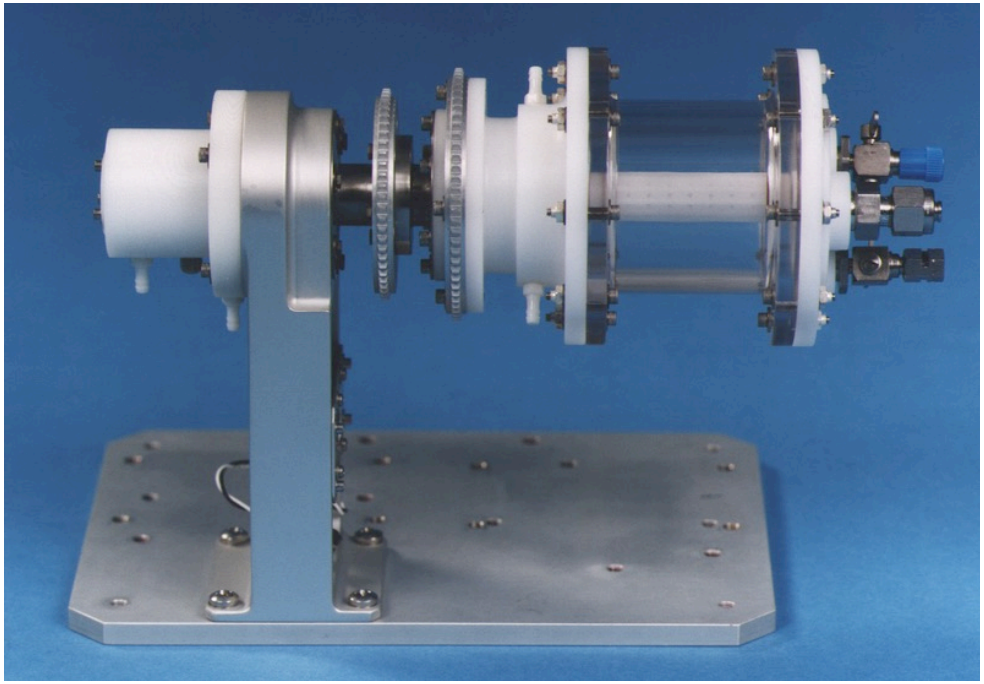


Rotary Cell Culture System 1/2

The rotating wall bioreactor was developed for:

- improve the physical parameters of the culture environment
- allow good interactions between cells and cells, cells and polymeric support matrix and develop 3D cultures without sacrificing catabolite removal and nutrient and oxygen supply.

Consisting of a rotating cylindrical chamber containing a co-rotating cylindrical membrane that performs the functions of oxygenation: a system without air bubbles and placed horizontally with respect to the ground.



Rotary Cell Culture System 2/2

✓Culture medium, cells and cellular aggregates rotate inside the chamber without colliding with the walls of the same or with other objects that could damage them.

✓ Minimized shear forces due to the absence of blades, bubbles or stirrers.

✓ Cells (in suspension or anchored on microcarrier) establish uniform orbit around the central axis of the cell, pushed by the low forces determined by the motion of the fluid; when the cell masses grow, increased rotation speed to compensate for cell sedimentation.

✓ **Advantage:** system allows to cultivate tissues with very complex 3D structures, favoring the reorganization of the same cell structure present in the tissue in vivo.

✓ Bioreactor also used for the study of the effect of microgravity on cellular organization, on cell-cell and cell-substrate interactions and on the use of important elements useful for the growth of the tissue under examination, in particular of the bone tissue.

Airlift Bioreactor

✓ Elongated chamber arranged in a vertical position, introduction of gas from the base

use:

✓ production of a wide variety of monoclonal antibodies,

✓ analysis of substances of bacterial origin

✓ study of cells of plant origin

✓ differentiation and proliferation of hematopoietic cells

✓gas introduced from below causes a reduction in the density of the soil contained in the pipe, favoring its circulation

Advantages:

✓ absence of moving mechanical parts that can cause damage to the cells in culture.

✓ oxygen supply is sufficient and non-controlled shear stresses tend to be low.

Hollow-Fiber Bioreactor

Working principle: network of semipermeable artificial capillaries which, wetted by the culture medium, due to diffusion phenomena, supply O2 cells and nutrients and remove the catabolites.

- Better simulation of real conditions in the cellular environment in vivo
- Additional features of the system are:
- cultivation of numerous cell types;
- large-scale production of proteins;
- large-scale production of monoclonal antibodies;
- study of endothelial cells under flow;
- co-crops;
- production of retroviral vectors.

- Very high flows in the culture medium without the use of peristaltic pumps

The design and construction of the gas-permeable hollow fibers is importnat , and the study of the cell medium flow and oxygenation system so that the cells are always well oxygenated and supplied with appropriate nutrients.

Equations used for the bioreactor design

Temperature:

$$\nabla^2 T(r,t) = \frac{1}{\alpha}\frac{\partial T(r,t)}{\partial t}$$

Fourier equation

Pressure:

$$p \cdot V = n \cdot R \cdot T$$

State equation of gases

$$v = 4\sqrt{P_d}$$

velocity of fluid as function of differential pressure

$$dn = \frac{dP_c}{RT}V$$

N. of moles of gas introduced into the cell chamber

Equations used for the bioreactor design

Nutrients supply

$$\frac{\partial L}{\partial t} = D\frac{\partial^2 L}{\partial x^2} - \frac{\rho k L}{K_M + L}$$

Thiele Modulus

$$\Phi = \left[\frac{\rho k X^2}{D}\right]^{\frac{1}{2}}$$

D = Diffusion Coefficient
K = Cinetic constant
L(x) = Concentration of nutrients
ρ = cell density

where X = maximum distance from the source of nutrients
If Φ <1 then the intake of nutrients is insufficient, therefore the distance between the source and the most distant cell is excessive since the diffusion is slowed by a low D and a high ρ

Equations used for the bioreactor design

Transport of molecules in the extracellular matrix

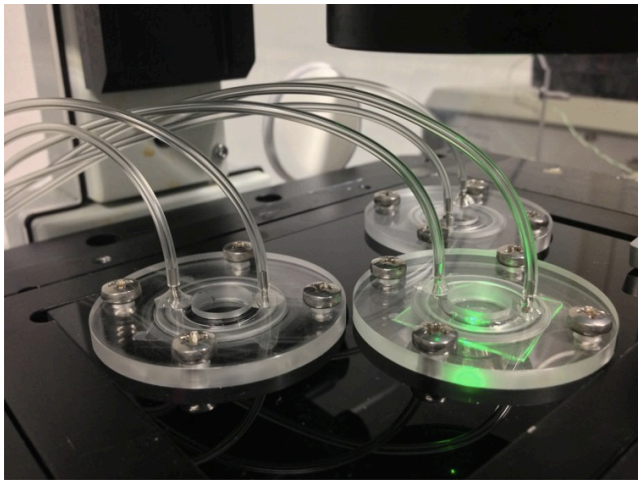
$$N_i = \Phi\left[-D_i \frac{\partial c_i}{\partial x} + \frac{z_i}{|z_i|} u_i c_i E \right] + W_i c_i U$$

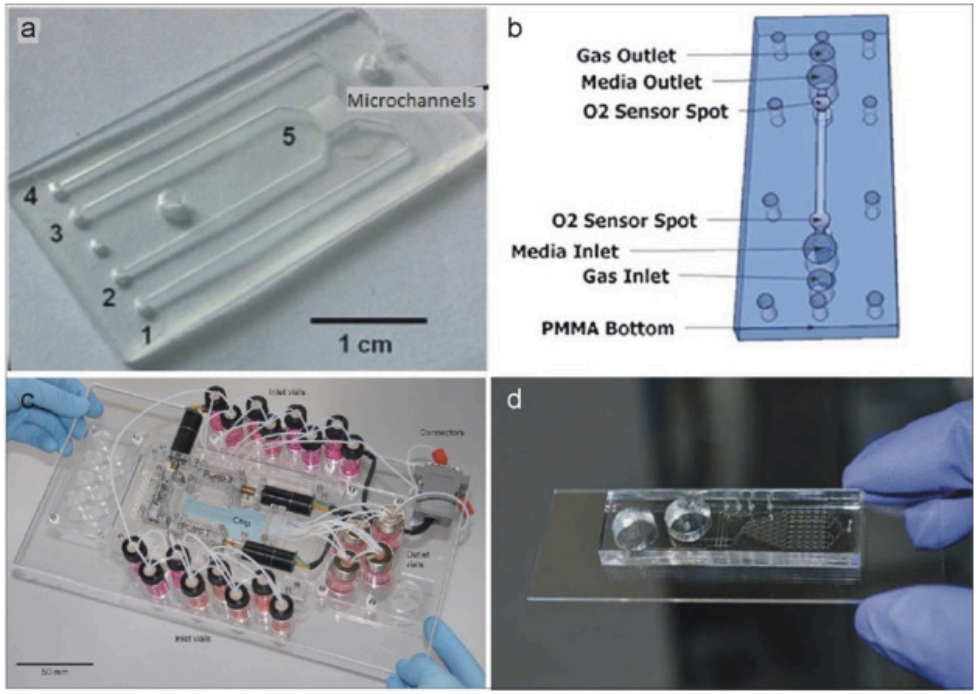
ci = concentration of i-th coumpound
zi = valence of compound
U = fluid velocity respect the tissue or scaffold
E = Electric field Induced in the tissue
μ = electric mobility
Wi = interaction factor between the molecules of the i-th compound and the surrounding environment
(<1 for big molecule).

$$Pe = X\left[\frac{W_i U + \Phi \mu_i E}{\Phi D_i} \right]$$ Peclet Number

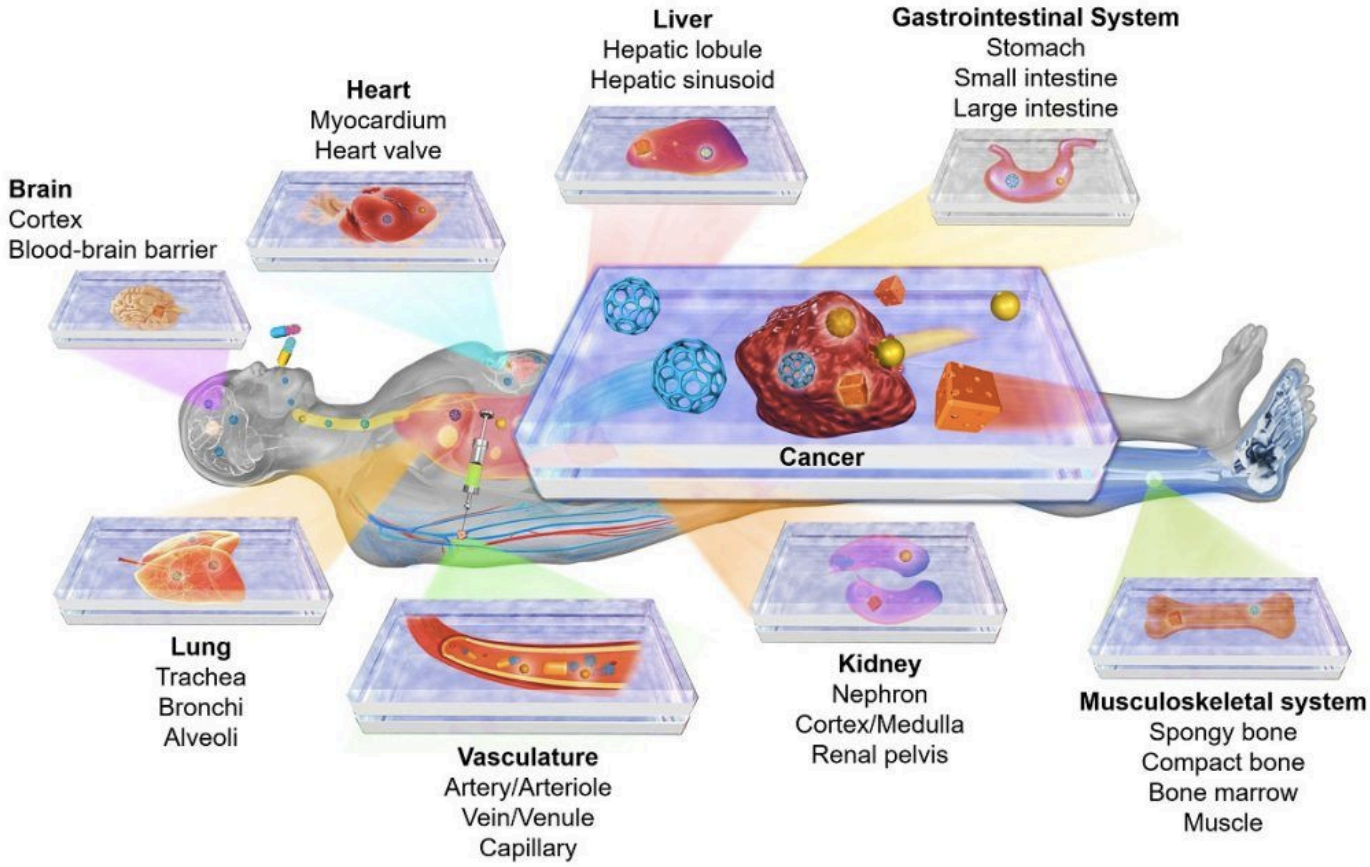
ratio of migratory and convective flows to diffusive ones

Microbioreactors

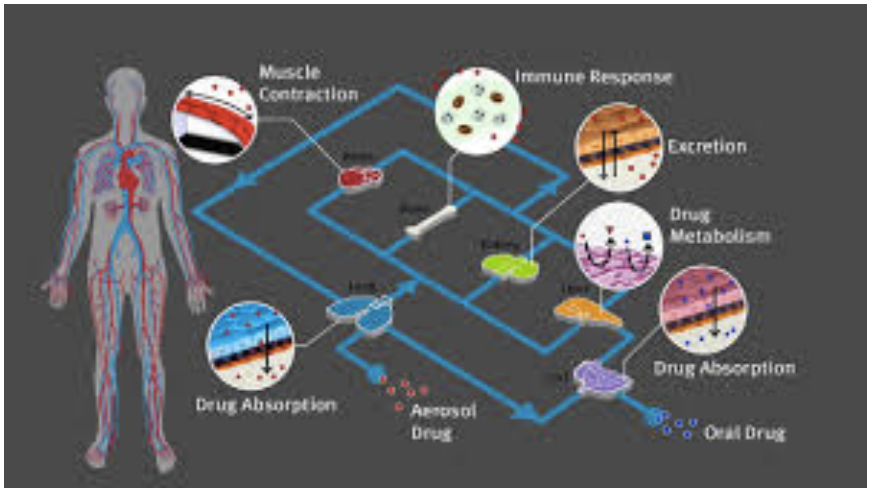


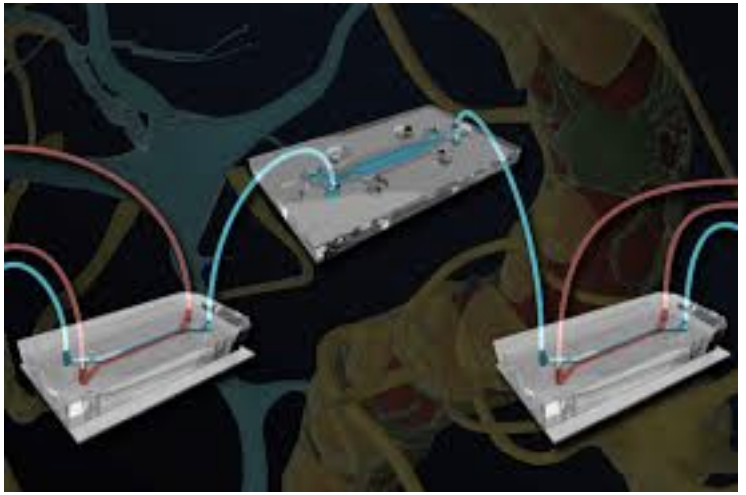


Organ on chip



Organ on chips





Brain on chip