Tissue engineering

Tissue engineering = regenerative medicine

Aim :

repair or replace portions or whole tissues

How:

combination of cells/engineering/materials

the tissues involved require certain mechanical and structural properties for proper function

Tissue engineering

End point :

-Restoration of continuity (REPAIR)

- (partly) functional REPLACEMENT of diseased or damaged tissue/organ – no replication of natural structure

- Generation of new tissue structurally & functionally analogous to the tissue/organ (REGENERATION)

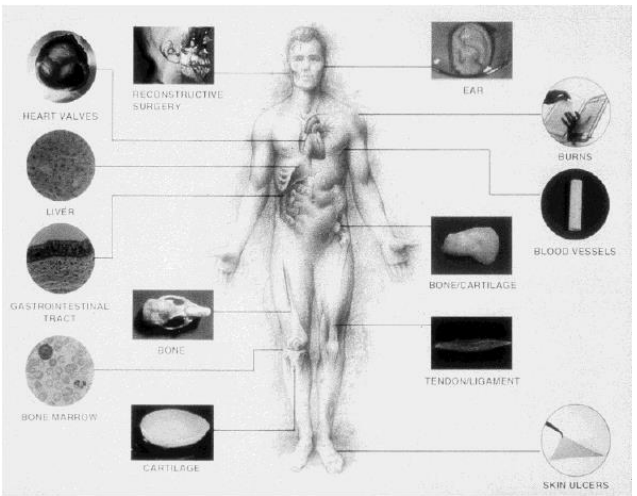
Tissue engineering

Repair : use of natural processes regulated by drugs, devices, biologics

Replacement: devices (prostheses, artificial organs transplants, grafts)

Regeneration: in progress

Tissue engineering



Tissue Engineering

Cellular
- -stem cells
- -therapeutic cloning
- -cell transplantation

Metabolic
- -bioartificial
  - liver
  - pancreas
  - kidney
- -encapsulated cell therapy

Structural
- -skin
- -cardiovascular
- musculoskeletal

Tissue engineering approaches

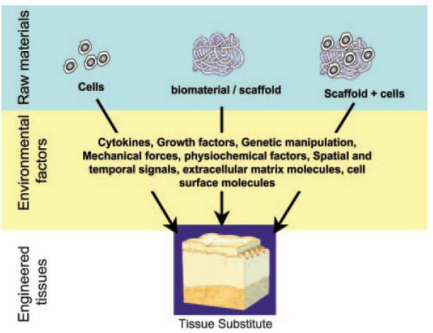


Fig. 1. Tissue engineering approaches. Tissue engineering approaches are classified into three categories: (*i*) cells alone, (*ii*) cells with scaffolds, and (*iii*) scaffolds alone. Each one of these approaches can be enhanced by *in vitro* microenvironmental factors before application as a tissue substitute.

Tissue engineering – cells

Autologous: from the same individual

Allogenic: donor of the same species

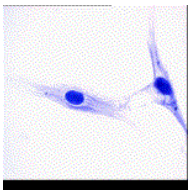
Isogenic/syngenic: from genetically identical organisms

Xenogenic : other species

Tissue engineering – cells

Cell types:

Stem Cells



Differentiated Cells

Tissue engineering – cells

Autologous cells:

pro	contra
-Fewest problems with rejection -Pathogen transmission	-Genetic disease -Proliferation/regeneration issue -surgery -slow - cell culture

Tissue engineering – cells

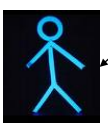
Autologous cells:

- Mesenchymal stem cells
- bone marrow
- Fat
- fibroblasts

Bone
Cartilage
Fat
nerve

Stem cells??

Tissue engineering – cells



Allografts
harvesting tissue from a donor, transplanting in a patient, deceased or living donors – heart, kidneys, lungs, liver, bone marrow, tendons, ligaments, cartilage, cornea, blood vessels

(+) can be a good match
(-) anti-rejection drugs required, shortage of donor tissues/organs

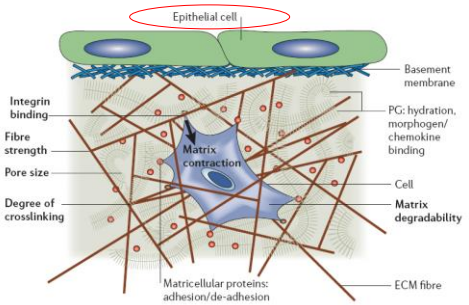
Tissue engineering

Xenografts – removing tissue from animals for transplantation into a human
(+) available supply, standardized products
(-) rejection, disease transmission (virus, proteins)

Man-made Materials & Devices – artificial hearts, heart valves, prosthetic hips…
(+) fill short term needs
(-) material fatigue, fracture, toxicity, degradation, remodeling

Tissue engineering – cells

Importance of 3D structure



Basement membrane
-Mechanical stiffness
-Transport regulation

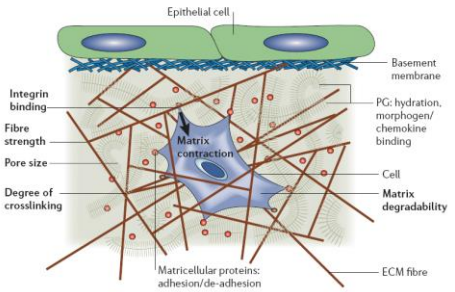
ECM
Cell adhesion and migration
Sensitivity to proteolitic enzymes (remodeling)
Storage of grow factors & effector molecules

Fine tuning of local Concentrations & gradients

Griffith et al; 2006

Tissue engineering – cells.

Importance of 3D structure



In vivo ECM component	Functions
Collagen: fibrillar (I, II, III, V, XI, XXIV, XXVII)	Structural scaffold Controls stiffness, resists tension Binds adhesion factors (for example, fibronectin) Binds some growth factors (for example, BMP2) Porous: allows amoeboid migration strategies
Collagen: nonfibrillar (I–XXVII, except fibrillar types)	Broadly serve many ECM and cell-adhesion functions, including: binding other ECM proteins and proteoglycans to aid ECM organization and stability; aiding fibrillar collagen formation; forming networks as barriers for solute transport, including basement membrane (VI); modulating cell migration and proliferation
Fibrin	Structural matrix in wound healing Controls stiffness, resists tension Binds adhesion molecules Easily degraded and remodelled
Elastin	Provides elastic recoil
Proteoglycans	Resists compression Hinders water transport Hinders macromolecular transport Binds growth factors and chemokines Electrokinetic effects
Matricellular proteins	Intermediate, weak adhesion (see REF. 127)

Griffith et al; 2006

Importance of 3D structure – local gradients

Living cell : production/consumption

From basic nutrients to effector molecules

↓

Diffusion/convection

↓

Local concentration

1. Effects local cell behavior (middle vs. surface)
2. Initiation of chemotaxis (examples?)

Oxygen gradient

Why so important?

*high o_2 concentrations are toxic for many cells

*Low o_2 concentrations may trigger differentiation of many cells (stem cells)

* Regulation of activity of signaling molecules

Additional things to keep in mind……

*Protein gradient
Protein concentration and modification of cell behavior

*Mechanical loads
Regulation of growth (connective tissue, muscle)
Regulation of cell behavior (non-mechanically active – active)
Stimulation of signaling networks

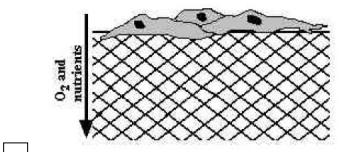
*Coupling by interstitial flows
(between blood and lymph)

Tissue engineering – cells - scaffolds

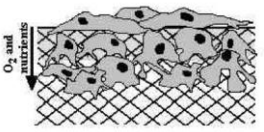
Cells are often implanted or 'seeded' into an artificial structure capable of supporting three-dimensional tissue formation.

Scaffolds recapitulate the *in vivo* milieu and allow cells to influence their own microenvironments.

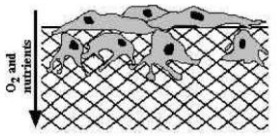
Why not just a simple scaffold ?



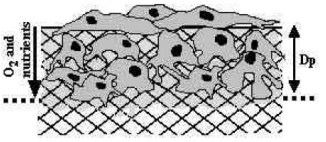
Cell seeding on scaffold.



The cells fully colonise the pores and start to lay down their own extracellular matrix.



Cells start to proliferate and migrate into the pores of the scaffold.



The top layer of cells consumes most the oxygen and nutrients in addition to limiting the diffusion of these components, thus reducing the amount available for pioneering cells migrating deep into the scaffold. Eventually, cellular migration is halted due to the lack of oxygen and nutrients supply. The layer of cells that can survive on the diffusion of oxygen and nutrients from the medium is called the cellular penetration depth (Dp).

Tissue engineering – scaffolds

*Allow cell attachment and migration

*Deliver and retain cells and biochemical factors

*Enable diffusion of vital cell nutrients and expressed products

*Exert certain mechanical and biological influences to modify the behavior of the cell phase

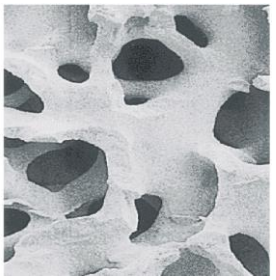
Scaffolds: Mimic Role of the ECM

- Space formation (hydrogel)
  - Direct and guide tissue formation and growth
  -
- Mechanical support
  - Tension (collagen)
  - Compression (PGs)
  - Elasticity (elastin)

- Cell-cell, cell-matrix interactions
  - Attachment, proliferation, migration, differentiation
  - Cell function

Tissue engineering – scaffolds

Natural
Synthetic



Biodegradable
Permanent

Synthetic non biodegradable polymers
Natural biopolymers
Self-assembled biological structures
Tissue derived structures
Bioactive ceramics and glass ceramics
Composites
Multilayered structures

Tissue engineering – scaffolds

	Advantages	Disadvantages
Natural ▪ collagen ▪ fibrin ▪ hyaluronan ▪ agarose	▪ Cell recognition ▪ Natural degradation	▪ Immune response ▪ Batch-batch variability
Synthetic ▪ PLA ▪ PGA ▪ alginate	▪ Batch-batch consistency ▪ Custom-tailored	▪ Biocompatibility? (FDA) ▪ Degradation

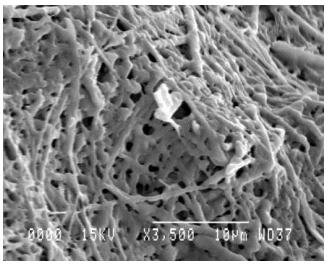
Tissue engineering – scaffolds

*High porosity & pore size (?)

*Biodegrability – no surgical removal

rate of degradation ~ rate of tissue formation

*Injectability



Tissue engineering – scaffolds

Bulk material composition & microstructure

-Surface chemical composition

-Corrosion parameters

- surface topography and porosity

-Degradation product toxicity

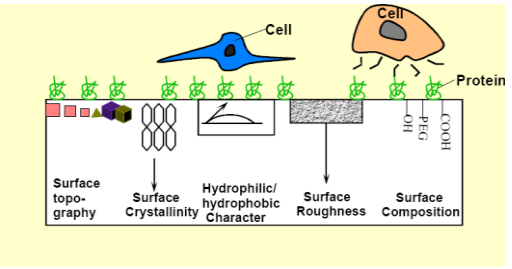
-Ion release profile

-Metal release profile

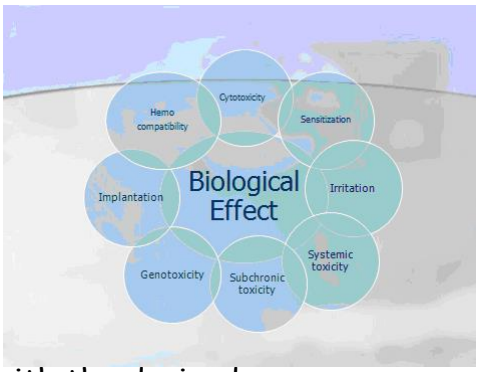
-sterility

-Water content

-Hydrophobic-hydrophilic balance



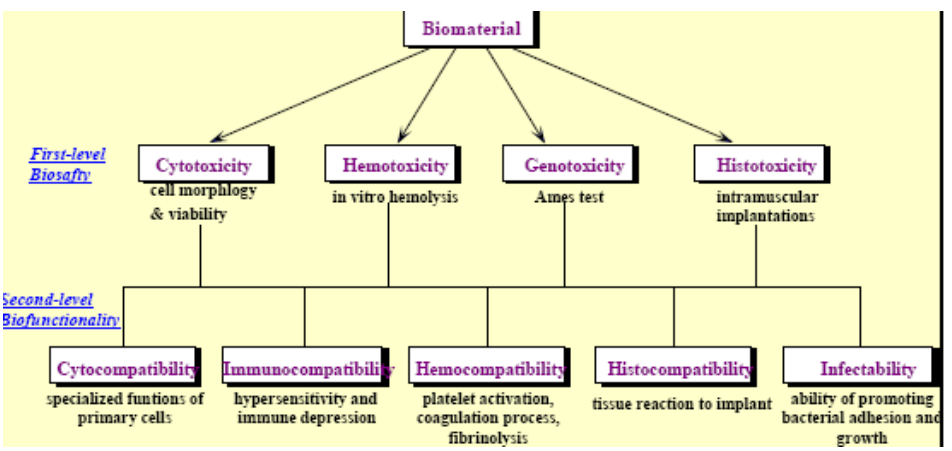
Biocompatibility



• Biocompatibility is species specific.

The biocompatibility of a long term implantable medical device refers to the ability of the device to perform its intended function, with the desired degree of incorporation in the host, without eliciting any undesirable local or systemic effects in that host.

Biocompatibility Tests



New generation of scaffolds

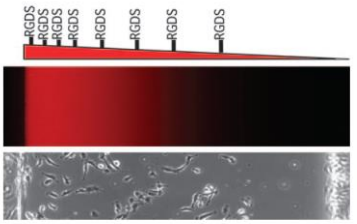


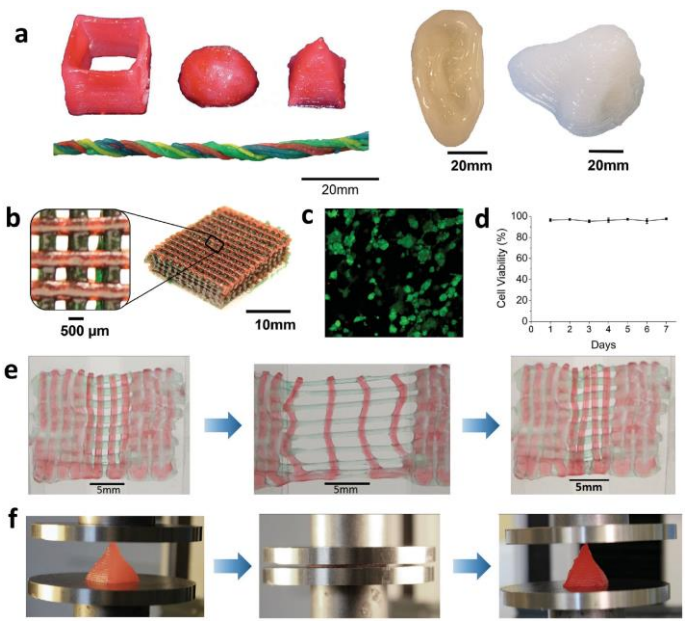
Fig. 3. Gradient hydrogels for tissue engineering. (*Top*) Hydrogels can be fabricated with control over the spatial properties of the materials by embedding a gradient of materials, such as RGD peptide, directly into the material. (*Middle* and *Bottom*) The shape of the gradient can be visualized by using fluorescent molecules (*Middle*), and its function can observed by imaging endothelial cell adhesion after a few hours on the gels (*Bottom*) (39).

Prefabricated hydrogel with controlled localization of growth factors

Specific regions within an agarose gel were tethered with RGD (Arg–Gly–Asp) peptide

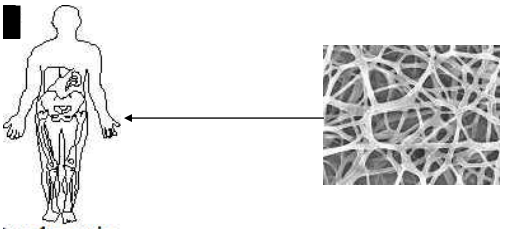
Khademhosseini et al; 2006

New generation of scaffolds



Host response

Complement
neutrophil
macrophage activation



Platelet adhesion, activation, degradation

Activation of clotting cascade

Lymphocyte behavior

Antibody production

Host response - the reality

*The host response, involving both humoral and cellular components is extremely complex

*There is often a two-way relationship between the material variable and the host response

e.g. a degradation process is pro-inflammatory and the products of inflammation enhance the degradation process

Host response - the reality

*Mechanical stability influences the host response, and in many situations the host response determines the stability

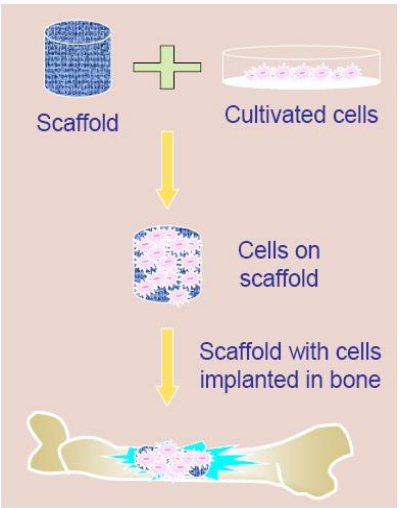
*The host response is time dependent

*The host response is patient specific, depending on age, sex, health status, pharmacological status etc.

Tissue engineering of :

1. Bone
2. Skin

Bone-grafting the gold standard



- **Patient-derived bone cells onto a macroporous man-made scaffolds to create completely natural new tissues**
- **Scaffolds gradually degraded and eventually eliminated**

Methods of reconstructing bone defects

1. Autograft /Allograft

2. BONE GRAFT SUBSTITUTES
- biomaterials
- synthetic materials
- licon-based compounds
- biologic/synthetic composite grafts

* Have a disadvantage compared to autografts

Bone regeneration/graft interaction

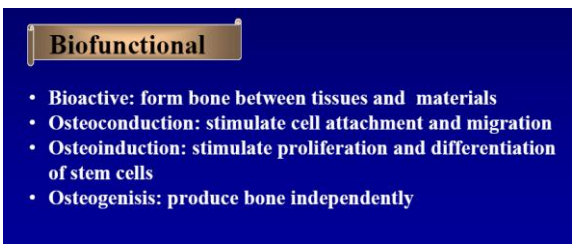
Osteogenesis – osteoprogenitor cells proliferate to osteoblasts and osteocytes

Osteoinduction –stimulation and activation of host cells mesenchymal cells to differentiate into bone-forming osteoblasts (TGF-beta super family of proteins)

Osteoconduction- facilitation and orientation of new Haversian systems into the bone

Properties of bone-graft substitutes

- osteoinduction/osteoconduction
- biocompatible
- Bio-resorbable
- structurally similar to bone
- Easy to use
- Cost-effective

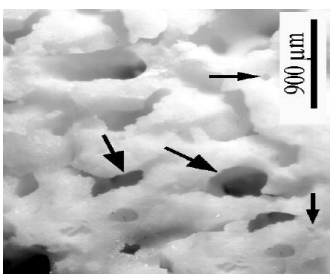


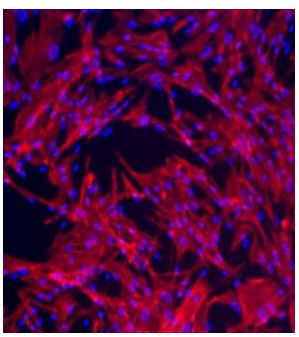
Ideal bone-graft substitutes

Chemotaxis	Mesenchymal stem cells and other bone forming cells migrate to the site of implantation.
Proliferation	Mesenchymal and other bone forming cells divide and increase in number.
Morphogenesis	Cells begin to take on the form and structure of bone.
Neo-angiogenesis	New blood vessels are formed in the immature callus.
Calcification	Osteoblasts produce new mineralized tissue under biologic influences like mechanical loading and growth factors.
Maturation	Some osteoblasts transform into the osteocytes, the body continues to remodel under local environmental and mechanical forces, leading to formation of a normal trabecular bone pattern.

Under the microscope..

an artificial bone scaffold and the scaffold filled with bone forming osteoblast cells





Methods of reconstructing bone defects

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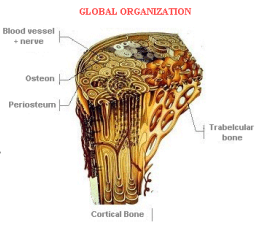
Bone graft substitutes - biomaterials



DBM (demineralised bone matri

-decalcificated cortical bone
- processed to reduce infection and immunogenicity

Mineral phase removed to improve availability of bone growth factors

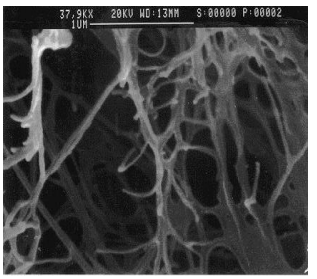
"bone graft extender"

InterGro DBM

Bone graft substitutes - biomaterials

Collagen
-EMC component
-Contributes to mineral deposition, vascular ingrowth
growth factor binding

Alone poor graft material (why?)
Coupled with BMPs
(bone morphgenic proteins)
or other materials enhances
graft incorporation



"bone graft extender"

Bone graft substitutes - synthetic materials

Disadvantages:

- unpredictable resorption
- difficulty in handling
- Poor clinical results (foreign body reaction)

Bone graft substitutes - synthetic materials

Ceramics

Tricalcium phosphate

Stoichiometry similar to amorphous bone precursors

Hydroxyapatite

Stoichiometry similar to bone mineral, slowly resorbable

Injectable calcium phosphate

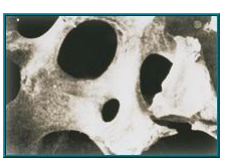
No osteogenic or osteoinductive properties, biocompatible

Bone graft substitutes - synthetic materials

Coralline hydroxyapatite (calcium phosphate)

Derived from sea coral
Osteoconductive properties
High compressive but low tensile strength
Natural or manufactured (coralline-HA)

Scanning electron microscope image (SEM)



human cancellous (spongy) bone

coralline hydroxyapatite

Bone graft substitutes – licon based products

Bioactive glasses

Solid, non –porous, hard

Consist of : Ca, P and silicate (silicon dioxide)

Good mechanical strength but difficult to fix to the skeleton (vs. ceramics)

Osteointegrative

osteoconductive

Bone graft substitutes – licon based products

Glass ionomers

Used in dentistry

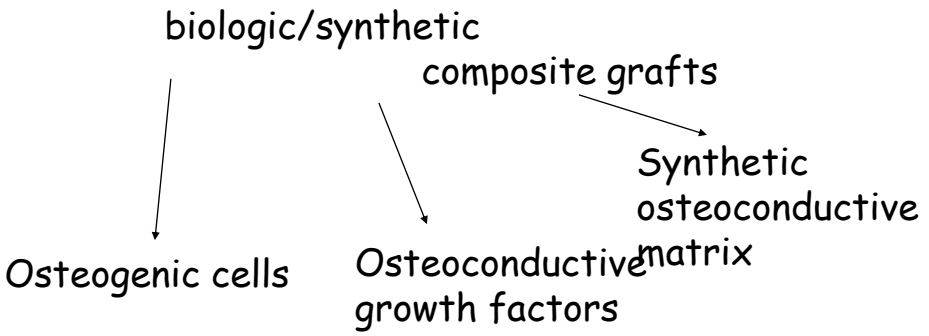
calcium/aluminium/fluorsilicate glass powder mixed with polycar-boxylic acid

Porous cement paste that hardens after 5 min - osteoinduction and bone ingrowth

Non-resorbable

Can be impregnated with antibiotics or high molecular weight molecules for slow release

Bone graft substitutes



Bone graft substitutes – summary

Type	Graft	Osteoconduction	Osteoinduction	Osteogenesis	Advantages
Bone	Autograft	3	2	2	"Gold standard"
	Allograft	3	1	0	Availability in many forms
Biomaterials	DBM	1	2	0	Supplies osteoinductive BMPs, bone graft extender
	Collagen	2	0	0	Good as delivery vehicle system
Ceramics	TCP, hydroxyapatite	1	0	0	Biocompatible
	Calcium phosphate cement (CPC)	1	0	0	Some initial structural support
Composite grafts	β-TCP/BMA composite	3	2	2	Ample supply
	BMP/synthetic composite	–	3	–	Potentially limitless supply

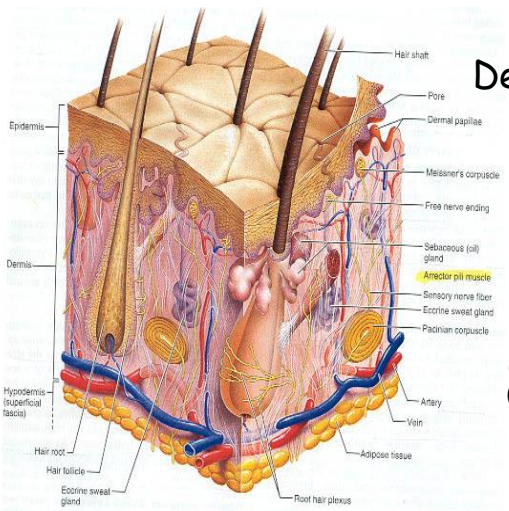
Score: 0 (none) to 3 (excellent). DBM: demineralised bone matrix, TCP: tricalcium phosphate, BMA: bone marrow aspirate, BMP: bone morphogenetic protein.

Giannoudis et al., 2005

Tissue engineering of :

1. Bone
2. Vessels
3. Skin

SKIN



Epidermis

Keratinocytes provide protective properties.

- Melanocytes provide pigmentation.
- Langerhans' cells help immune system.
- Merkel cells provide sensory receptors.

Dermis

Collagen, glycoaminoglycans, elastine, ect.
Fibroblasts are principal cellular constituent.
Vascular structures, nerves, skin appendages.

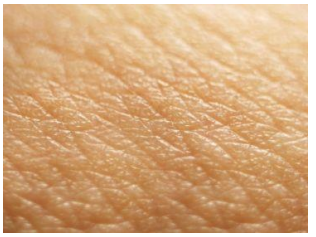
Hypodermis

(fatty layer)

- Adipose tissue plus connective tissue.
- Anchors skin to underlying tissues.
- Shook absorber and insulator

Function of the skin

*Protection of underlying tissues from injury: mechanical, heat, cold, biological.



*Prevention of excess water loss.

*Act as a temperature regulator.

*Serve as a reservoir for food and water: adipose tissue

*Assist in the process of excretion: Salt, Urea etc

*sense organ for cutaneous senses: pain, heat, cold etc

* entrance of foreign bodies: microorganisms.

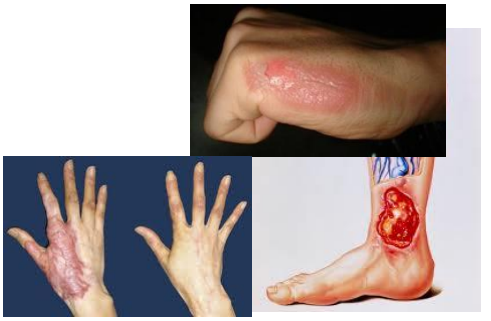
*Serve as a seat of origin for Vitamin D.

Skin replacement – the cause …

*Burn injuries – massive tissue damage

*non-healing ulcer – chronic wounds

* Reconstructive surgery



*Burns are one of the most expensive catastrophic injuries to treat.

A burn of 30% of total body area can cost as much as $200,000 in initial hospitalization costs and physicians fees.

Skin replacement

- Physical Characteristics
  - It should be easy to manipulate the product, i.e. easy to place and dress the skin substitute effectively (Beele)
  - It should improve the cosmetic appearance of the scar (Beele)
- Availability
  - It should be readily available off the shelf and custom made.
- Cost
  - Cost should not preclude the use of the device.

SKIN SUBSTITUTES

temporary - material designed to be placed on a fresh wound (partial thickness) and left until healed

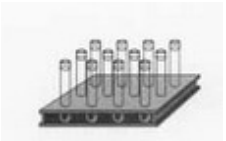
•Bilayer with purpose being to protect wound and optimize healing

semi-permanent-material remaining attached to the excised wound, and eventually replaced by autogeneous skin grafts

permanent incorporation of an epidermal analog, dermal analog, or both as a permanent replacement

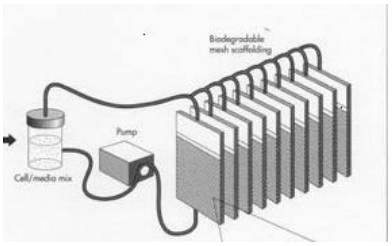
•replacement for one or both layers

Preparation of artificial skin

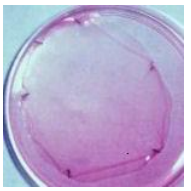


Vials of frozen fibroblasts

3-4 week rotation culture



3-4 weeks, flow back and forth, attachment to scaffold and growth, formation of a few skin layers and disintegration of the scaffold



storage

Synthetic skin substitutes

Acellular	Cellular-allogenic	cellular autologous
Biobrane Intergra Alloderm	TransCyte Dermagraft Apligraft	Epicel Epidex
Silicone membrane Collagen matrix	Fibroblasts + nylon mesh	Keratynocytes fibroblasts

Available Bioactive Temporary Skin Substitutes

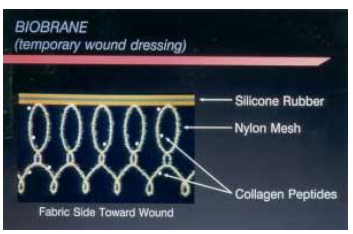
Product	company	origin	layers	uses
Human Allograft	skin banks	cadaver	epidermis dermis	temporary coverage of large excised burns

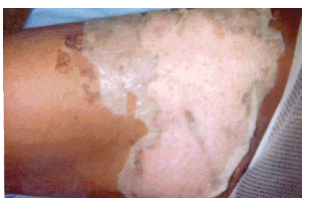
Major issue is safety-

- To reduce risk to patients need to use skin from accredited skin banks
- Even with extensive screening there will always be some level of risk of disease (viral) transmission
- Also availability of skin is a major problem in practice

Available Bioactive Temporary Skin Substitutes

Product	company	origin	layers	uses
Biobrane®	Dow Hickam/Bertek Pharmaceuticals	Synthetic with added denatured bovine collagen	synthetic dermis & epidermis	Superficial partial thickness excised burns

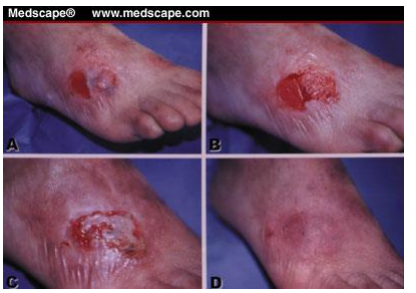




Bilayer product

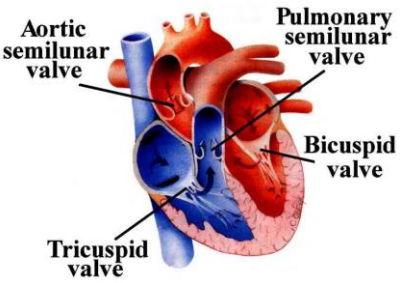
Available Permanent Skin Substitutes

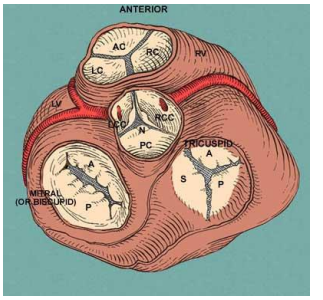
Product	company	origin	layers	uses
Apligraf	Organogenesis Inc Novartis Pharmaceuticals Corp	Allogenic Composite	epidermis dermis	Chronic wounds



Collagen matrix seeded with human neonatal keratinocytes and fibrobla

Tissue engineering of heart valves





The heart has four chambers. The upper two are the right and left atria. The lower two are the right and left ventricles. Blood is pumped through the chambers, aided by four heart valves.

The valves open and close to let the blood flow in only one direction

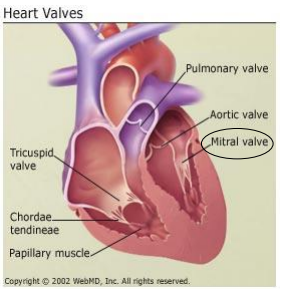
Heart Valve Diseases

Blood can leak back through the valve in the wrong direction, which is called **regurgitation**

the mitral valve, sometimes has "floppy" flaps and doesn't close tightly. This is called mitral valve prolapse

when mitral valvedoesn't open enough, which blocks blood flow, it is called stenosis

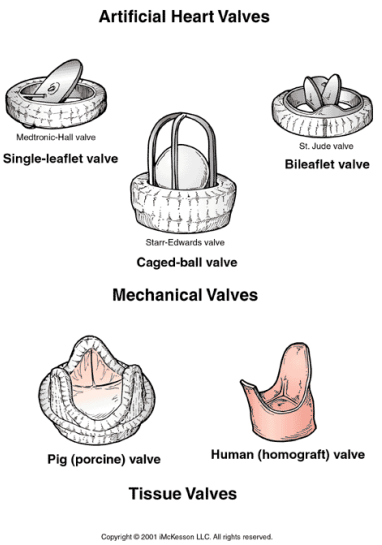
*infections
*heart attacks & heart diseases
*genetic defects



Available prostheses & substitutes

first operation 1965

-Enhanced quality of life
-Imperfect functional
-restitution



Disadvantages specially in pediatric applications:
very small valve size
subsequent and repetitive operations to accommodate growth of the patient

Heart valve surgery complications:

thromboembolic complications
- ischemic limb
- splenic infarct
- renal infarct
- ischemic bowel

infections

limited durability

lack of growth potential

In the year 2000, 87,000 valve replacement procedures were performed in the US

The aim …

generating completely biological, autologous "living" valves with functional features of their native counterparts that may overcome the present limitations

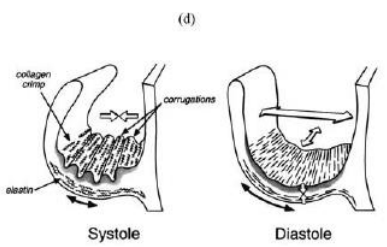
Two main concepts

(i) engineering of completely artificial valves from biocompatible and degradable synthetic polymers or biomaterials

(ii) decellularization of native heart valves from xenogeneic/allogeneic donors an extension of clinically employed bio-logical valves. (repopulation and adaptive remodeling)

The challenges:

valves are vital and dynamic tissues composed of specialized cells and extracellular matrix (ECM) that respond and remodel in response to changes in local mechanical forces

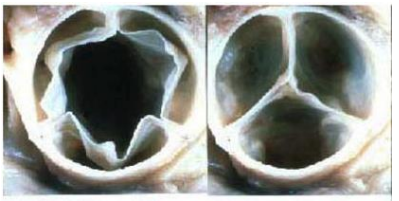


Mendelson et al; 2006

The challenges:

Approximately 40 million times a year,

opening and closing of the leaflets induces repetitive changes in the shape, dimensions, and stress of the leaflets and supporting valvular structures

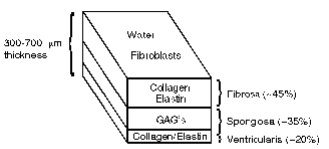


sheer stress due to blood flow (open valve), flexure (opening and closing), and tension (closed valve). Mendelson et al; 2006

Structure of heart valves

(1) the *ventricularis* closest to the inflow surface, rich in elastin
(2) the *fibrosa* closest to the outflow surface, primarily composed of densely packed collagen
(3) the centrally located *spongiosa*, largely composed of glycosoaminoglycans (GAGs).

Together, collagen, elastin, and GAGs comprise the valvular ECM



Aortic Cusp Cross-Section

Structure of heart valves

TABLE 2. Key structural elements of heart valves.

Element	Sub-structure	Function
Extracellular matrix	Collagen	Provides strength and stiffness to maintain coaptation during diastole, when cusp has maximal area
	Elastin	Extends in diastole; contracts in systole to minimize cusp area
	Glycosaminoglycans	Accomodates shear of cuspal layers, cushions shock during valve cycle
Cells	Interstitial	Synthesize ECM; express MMPs and TIMPs that mediate matrix remodeling
	Endothelial	Maintain nonthrombogenic blood-tissue interface; regulate immune and inflammatory reactions
Blood vessels		Few and focal; valve cusps and leaflets sufficiently thin to be nourished by diffusion from the heart's blood
Nerves		Present, with uncertain function
Other principles	Corrugations	Accordian-like folds in cusps; allows cuspal shape and dimensions to vary with cardiac cycle
	Crimp	Microscopic collagen folding, allows lengthening at minimal stress
	Anisotropy	Permits differences in radial and circumferential extensibility
	Cords	Macroscopic collagen alignment; transfers forces from cusps to aortic wall

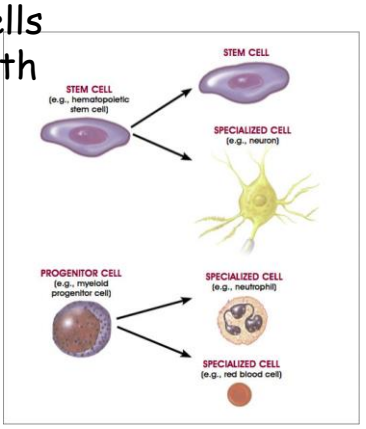
endothelial cells covering the surface and interstitial cells with variable properties of fibroblasts, smooth muscle cells, and myofibroblasts in the interior

Mendelson et al; 2006

Cells in engineering of heart valves

differentiated tissue-specific cells (such as endothelial and/or smooth muscle cells

stem cell



Endothelial progenitor cells (EPCs)

are bone marrow-derived hematopoietic stem cells capable of differentiating into the endothelial cells that line the blood vessels and cardiac valves

Cells in engineering of heart valves

Mesenchymal stem cells (MSCs), have the potential for differentiating into osteogenic, chondrogenic, adipogenic and myogenic lineages.

Marrow progenitor cells or multipotent adult progenitor cells (MAPCs)

are reported to expand indefinitely

Scaffolds in heart valve tissue engineering

synthetic

Polyglycolic acid (PGA) - crystalline, linear, aliphatic polyester, with a high melting point and low solubility in organic solvents.

polylactic acid (PLA)

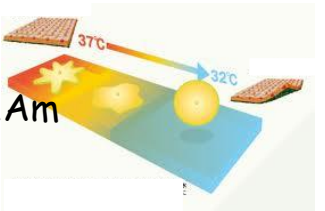
Biodegradable polymers, poor substrates for cell growth *in-vitro*.

Smart bio-material scaffolds

carry spatial and chemical information that affects cellular function and/or responds to changes in the environment.

biodegradable elastic shape-memory polymers predictably alter their shape with changes in temperature and polymers that transition between hydrophobic and hy-
drophilic states in response to electric potential

Temperature sensitive polymer PIPAAm (poly *N*-isopropylacrylamide)



natural scaffolds

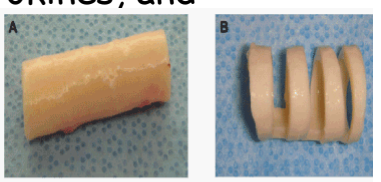
comprise pure ECM components (such as collagen or fibrin) or decellularized but otherwise intact allograft or xenograft tissue (such as heart valve or small intestinal sub-mucosa).

Decellularized porcine small intestinal submucosa (SIS)

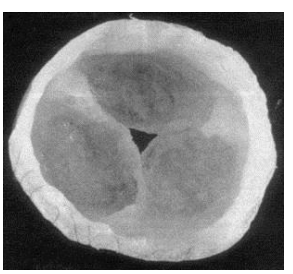
- does not require cell seeding.
-In both animal and human clinical studies, SIS was rapidly remodeled by the host tissue.

has exhibited good vascularization and tissue growth without excessive inflammation and foreign body reaction.

The success of SIS has been attributed to its intrinsic ECM proteins, GAGs, cytokines, and growth factors (VEGF and TGF-¯).



Till 2000



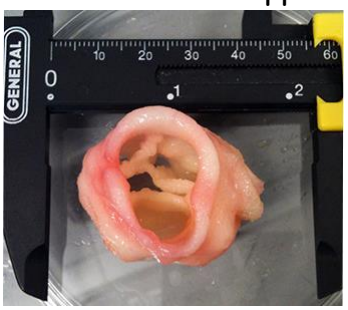
40 tissue engineered heart valve leaflets

(20 xenograft and 20 allograft leaflets) were produced

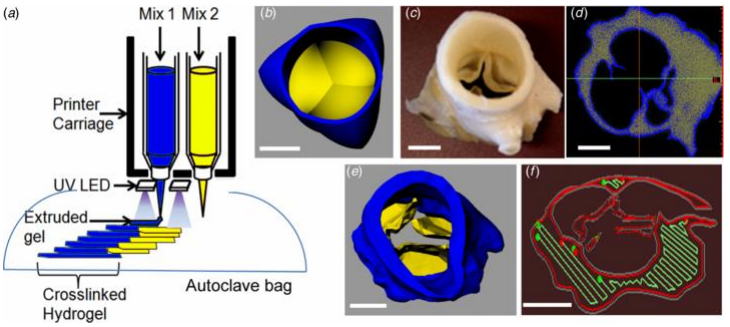
experiments on animal models & small scale on humans to prove their functionality

A huge challenge due to significant mechanical stress the valves need to stand - high pressure aortic flow conditions

Novel approaches in heart valve engineering



Alginate-based hydrogels with encapsulated aortic root sinus smooth muscle cells (SMC) and aortic valve leaflet interstitial cells (VIC) - cells were viable over 7 days in culture.



J Biomed Mater Res A. 2013 May ; 101(5): 1255–1264

The newest approach to 3D printing

RESEARCH ARTICLE

BIOMEDICAL ENGINEERING

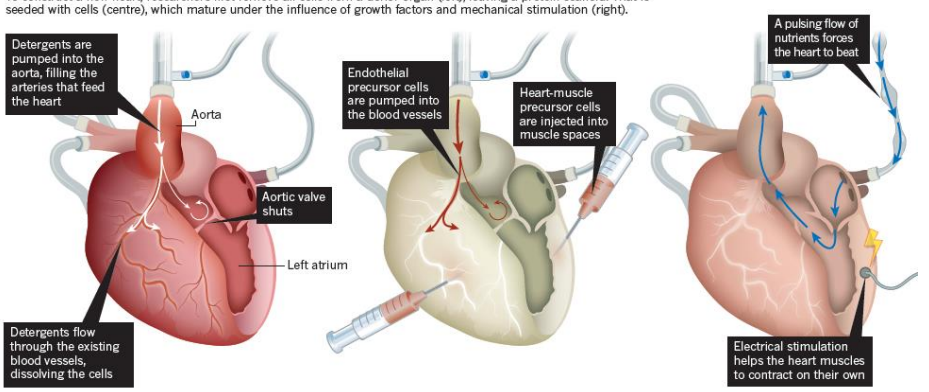
Three-dimensional printing of complex biological structures by freeform reversible embedding of suspended hydrogels

Thomas J. Hinton,[1] Quentin Jallerat,[1] Rachelle N. Palchesko,[1] Joon Hyung Park,[1] Martin S. Grodzicki,[1] Hao-Jan Shue,[1] Mohamed H. Ramadan,[2] Andrew R. Hudson,[1] Adam W. Feinberg[1,3]*

New trends in heart engineering

CUSTOMIZED ORGANS

To construct a new heart, researchers first remove all cells from a donor organ (left), leaving a protein scaffold. That is seeded with cells (centre), which mature under the influence of growth factors and mechanical stimulation (right).



A decellularized human heart awaits rebuilding with an injection of precursor cells.

Different approaches to heart engineering

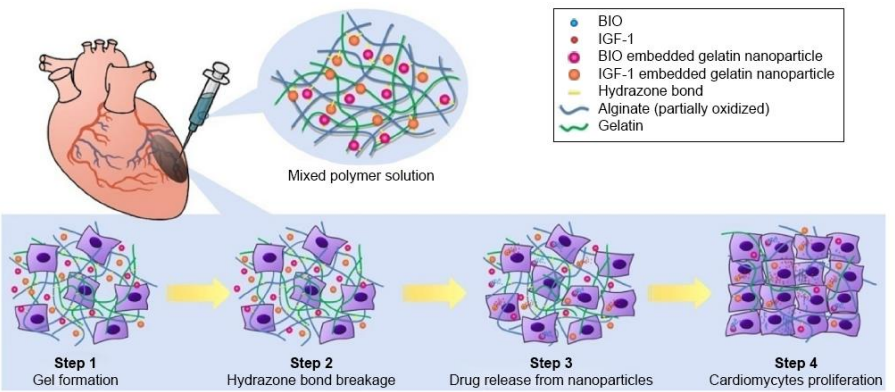
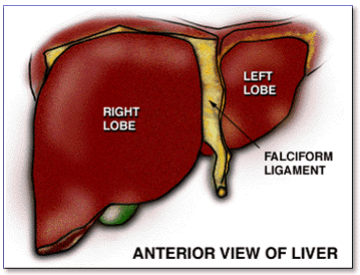


Figure 7 Scheme showing the mechanism of BIO release within the hydrogel after injection into the MI area.
Abbreviations: BIO, 6-bromoindirubin-3-oxime; IGF-1, insulin-like growth factor 1; MI, myocardial infarction.

The liver

The anterior surface of the liver is triangular in shape, made of two lobes. The right lobe is the larger of the two.



Ligaments connect the upper surface of the liver to the diaphragm and the abdominal wall and the under surface to the stomach and duodenum

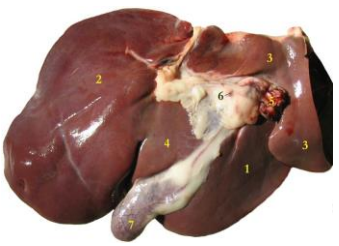
The liver

weighs between 1.7 - 3.0 kilograms

The liver is among the few internal human organs capable of natural regeneration of lost tissue; as little as 25% of remaining liver can regenerate into a whole liver again.
App. 67% of a liver can grow back in one week.

hepatocytes -unipotential stem cells

oval cells can differentiate into either hepatocytes or cholangiocytes (bile ducts).



Various functions of the liver

carried out by hepatocytes

-Bile production & secretion (emulsifying fats)
-several roles in carbohydrate metabolism

Gluconeogenesis -the synthesis of glucose from certain amino acids, lactate or glycerol)

Glycogenolysis (the breakdown of glycogen into glucose) (???)

Glycogenesis (the formation of glycogen from glucose)

Various functions of the liver

The breakdown of insulin and other hormones

protein metabolism.

The liver performs several roles in lipid metabolism

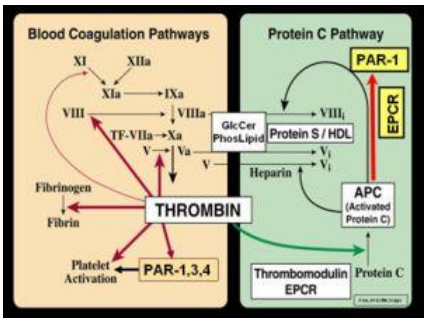
Cholesterol synthesis

The production of triglycerides

Various functions of the liver

produces coagulation factors I (fibrinogen),

II (prothrombin), V, VII, IX, X and XI,
as well as protein C, protein S and antithrombin



breaks down hemoglobin

Its metabolites are
added to bile as pigment
(bilirubin and biliverdin

breaks down toxic substances

Various functions of the liver

drug metabolism

converts ammonia to urea

stores a multitude of substances

glucose in the form of glycogen,
vitamin B12, iron, and copper

In fetus -the main site of red blood cell production

Various functions of the liver

responsible for immunological effects-

The **reticuloendothelial system** (**RES**),

consists of the phagocytic cells primarily

monocytes and macrophages.

These cells accumulate in lymph nodes and the spleen.

The Kupffer cells of the liver

MULTIFUNCTIONAL & VITAL organ

A few numbers

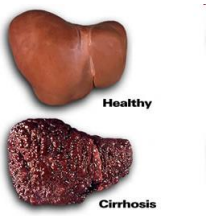
In 2003 in US :

4000 donor livers available

20.000 patients on waiting lists

30.000 die from liver failure

90% mortality rate for patients with fulminant hepatic failure



Liver failure – temporary support

There are two types of liver assist devices:

Artificial liver – use non-living components to cleanse the blood or plasma of its toxins.

Removal is based on physical/chemical gradients and adsorption

Bioartificial liver (BAL) – devices contain a cell-housing bioreactor ,the role of which is to replace the primary and most important liverfunctions

B. Carpentier et. al. 2009

Non-biological approaches to liver failure

-Hemodialysis (removing waste products such as potassium and urea, as well as free water from the blood)

-Hemoperfusion (large volumes of the patient's blood are passed over an adsorbent substance in order to remove toxic substances from the blood)

-Plasmapheresis

Extracorporeal biological approaches to liver failure

Whole-liver perfusion- treatment is to expose the liver to high doses of chemotherapy
The blood supply to the liver is completely isolated from the systemic circulation so that the body is not exposed to the high dose of drugs

Liver slice perfusion

Cross hemodialysis -performed under general anesthesia, the circulation of patients with hepatic failure is directly connected to that of healthy human donors

A problematic organ…..

Complex function

Purely mechanical device – out of question….

Growth of the organ in vitro – too difficult

↓

Bioartificial liver systems

Bioartificial liver systems

Oxidative detoxification

biotransformation

excretion

synthesis

BAL systems – the principle

*continuous withdraw of patient's whole venous blood

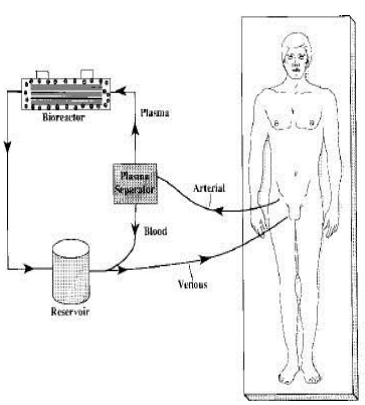
*maintenance of temperature

*oxygenation to arterial levels

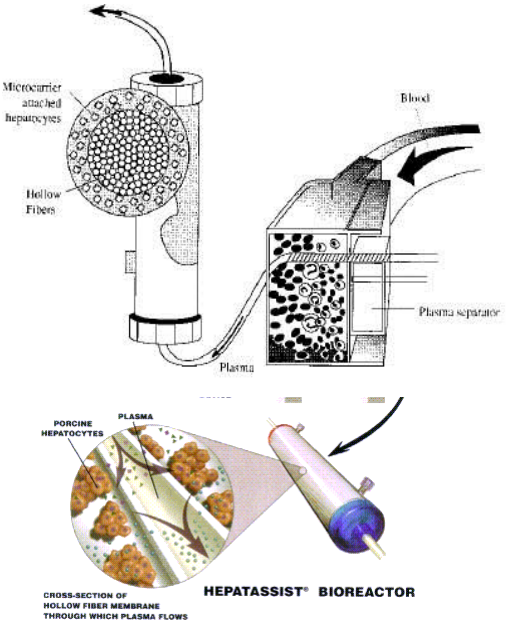
*adjustment pH to 7.2

*perfusion using bioreactor charged with liver cells

*reintroduction to the patient



BAL systems



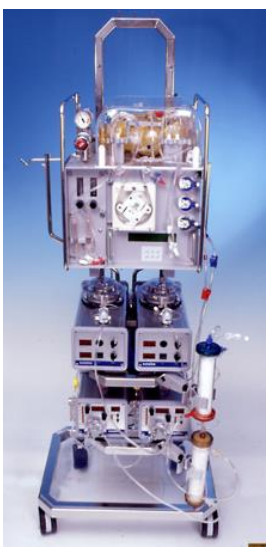
Flow through the artificial liver:

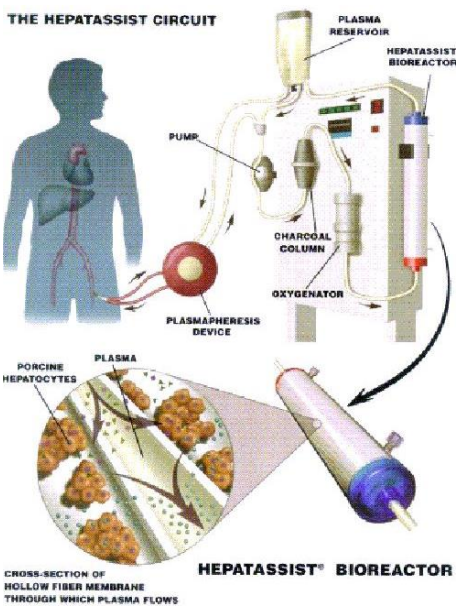
1. Separation of plasma from blood by filtration through membrane

2. Removal of toxins as plasma passes through functional hepatocytes

3. Reconstitution of the Plasma with the blood

4. reinfusion to patient





Advantages of hollow fiber approach

*The separate chamber design allows replacement with fresh hepatocytes.

*separating the hepatocytes from the bloodstream with a semi permeable membrane, the potential for immune responses to cell surface alloantigens or xenoantigens is theoretically reduced.

Nonetheless, the potential exists for soluble antigens or antigenic peptides to pass through the semi permeable barrier and initiate an immune response.

Current Work in BAL's

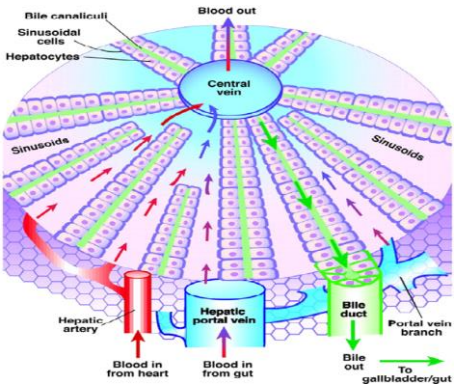
- Molecular Absorbent Recycling System (MARS®)
  - Teraklin, uses human albumin
- Extracorporeal Liver Assist Device (ELAD®)
  - Vitagen, uses immortalized human hepatocytes
- HepatAssist 2000 system
  - Circe Biomedical, uses porcine hepatocytes
- Bioartificial Liver Support System (BLSS®)
  - Excorp Medical, Inc., uses primary porcine hepatocytes
- LIVERX2000 system
  - Algenix, Inc., uses porcine hepatocytes
- Modular Extracorporeal Liver System (MELS®)
  - Charite Virchow Clinic-Berlin, uses human hepatocytes

Cell types

-Human primary hepatocytes (shortage)
-Porcine primary hepatocytes
- Liver cancer cells
- stem cells

Issue: in liver have distinct epithelial polarity
cell-cell communication structures, different functions

Loose these characteristics *in vitro*



Cell culture

TABLE 3. Hepatocyte culture techniques and their statuses of application to BAL systems

Hepatic function and treatment time	Culture method	Current statuses of application to BAL systems			Performance of BAL system
		In vitro research	Preclinical study	Clinical trial	
Long-term and enhanced functions	Liver-like structure	?			High
	3-D coculture	Lee *et al.* (65)		e	
	2-D coculture	Washizu *et al.* (66)			
	Collagen sandwich		j		
	Spheroids or organoids		g, h, i	d	
	Microencapsulation		h		
	Porous matrix		i	c, d	
	Extracellular matrix			b, f	
Short-term and poor functions	Microcarrier			a	Low

a, HepatAssist; b, BLSS; c, RFB; d, AMC-BAL; e, LSS or MELS; f, LIVEx2000; g, LLS-BAL; h, AHS-BAL; i, PUF-BAL; j, FMB-BAL.

Park et al. 2005

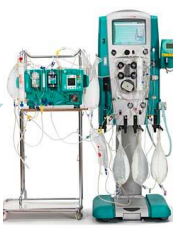
What have we got and what do we expect???

TABLE 4. Characteristics of present and next-generation BAL systems

	Present BAL system	Next-generation BAL system
Hepatocyte source	Pig or hepatoma	Human hepatocytes from stem cells or humanized pig
Culture methods (bioreactor)	Simple and conventional culture techniques	Liver-like structures with optimum cell–cell interactions, microarchitectured 3-D coculture, etc.
Treatment time	Less than 24 h	Weeks to months
Liver function	Several vital functions	Entire liver functions including bile excretion
Target disease	Acute liver failure, mainly fulminant hepatic failure	Chronic as well as acute liver failure

Park et al. 2005

Current Work in BAL's



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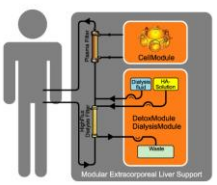
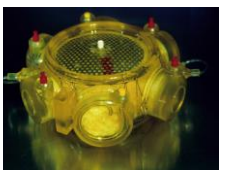
  - Charite Virchow Clinic-Berlin, uses human hepatocytes





LifeLiver

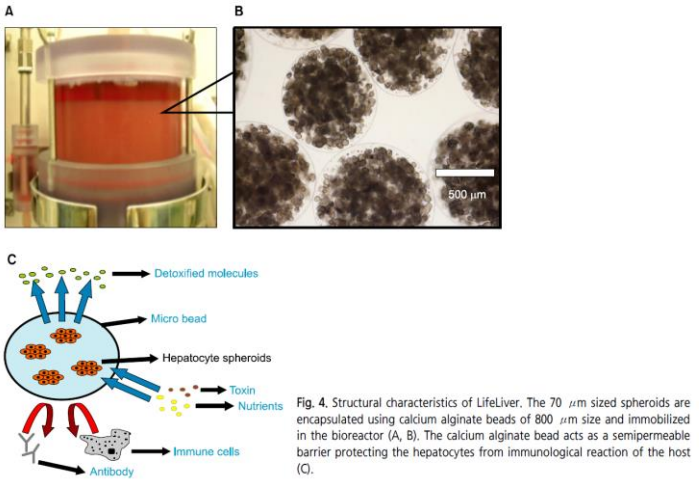
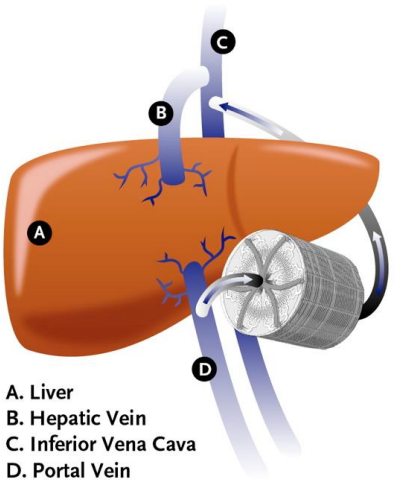


Fig. 4. Structural characteristics of LifeLiver. The 70 μm sized spheroids are encapsulated using calcium alginate beads of 800 μm size and immobilized in the bioreactor (A, B). The calcium alginate bead acts as a semipermeable barrier protecting the hepatocytes from immunological reaction of the host (C).

Bio Engines Implantable Device

- Designed to take place of a liver or a portion of the liver
- Polymer grid-like mesh used as artificial vasculature resembling that of an actual liver
- Patterned silicon wafers serve as molds for polymer sheets
- Currently being tested on pigs
- Clotting issues



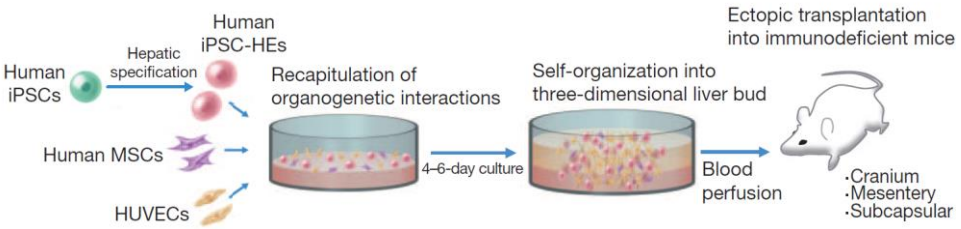
A. Liver
B. Hepatic Vein
C. Inferior Vena Cava
D. Portal Vein

LETTER

doi:10.1038/nature12271

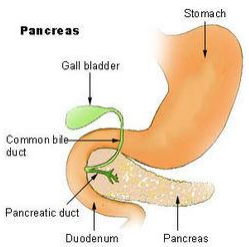
Vascularized and functional human liver from an iPSC-derived organ bud transplant

Takanori Takebe[1,2], Keisuke Sekine[1], Masahiro Enomura[1], Hiroyuki Koike[1], Masaki Kimura[1], Takunori Ogaeri[1], Ran-Ran Zhang[1], Yasuharu Ueno[1], Yun-Wen Zheng[1], Naoto Koike[1,3], Shinsuke Aoyama[4], Yasuhisa Adachi[4] & Hideki Taniguchi[1,2]

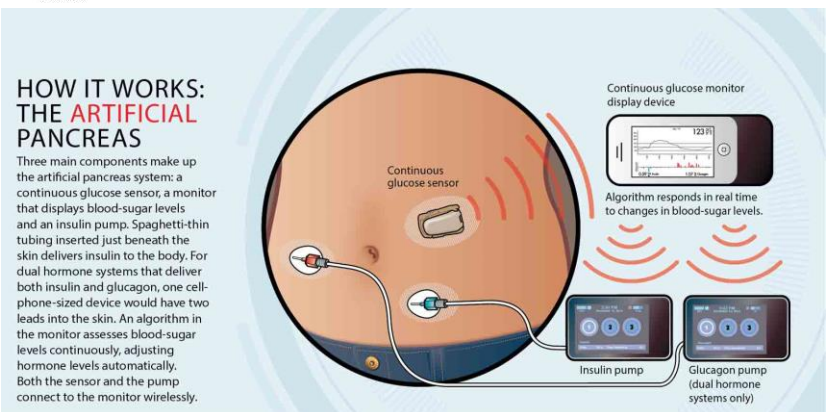


Nature 2013

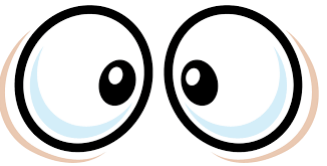
Also on-going research on artificial pancreas



It is both exocrine (secreting pancreatic juice containing digestive enzymes) and endocrine (producing several important hormones, including insulin, glucagon, and somatostatin)



Interesting to watch:



https://www.youtube.com/watch?v=kpNR7Atvn-A

https://www.youtube.com/watch?v=7SfRgg9botI

https://www.youtube.com/watch?v=sCEWiFwWbXg

https://www.youtube.com/watch?v=eyHVlU1dNoE