University Medical Centre Groningen

# Tissue Engineering by use of (smart) biomaterials: Towards Regenerative Medicine


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# Why is Tissue Engineering important?

Millions of surgical procedures require substitudes:

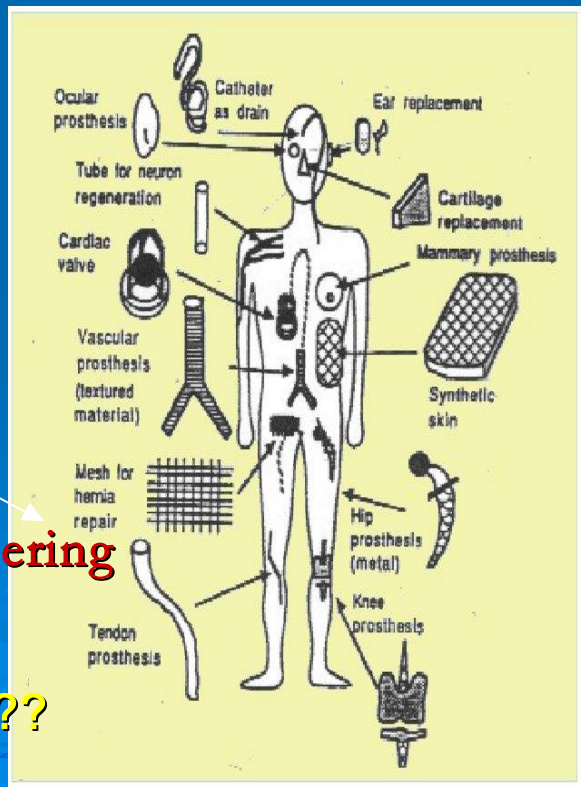
- **Autografts** -> 300.000 coronary bypasses/year (20% fail)
- **Allografts** -> 11-15 days rejection ???
- **Xenografts** -> Non-human: livers, kidneys, hearts

(rejection, infection, transgenic viruses)

- Ageing of the population
- Sports injury
- Device failures


Biocompatibility??

Tissue engineering



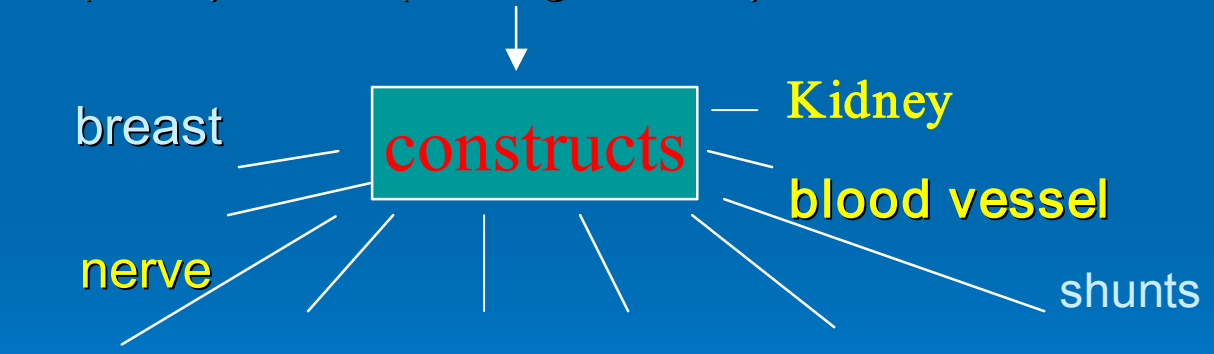

The bionic man; our future??

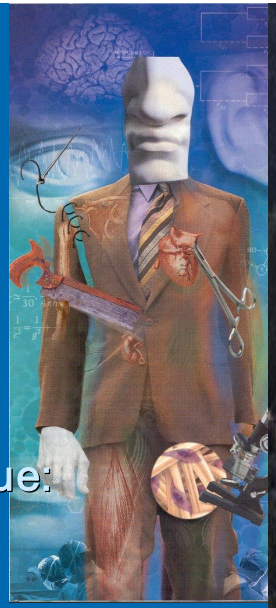
# Scenario's in TE:

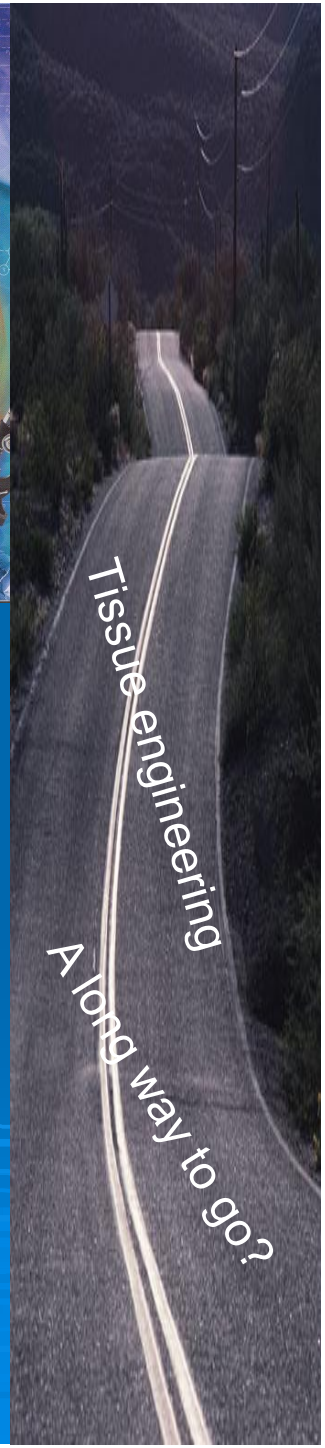
- **In vivo engineering:**
- Injection of active molecules (e.g. growth factors)
- Application of (stem)cells in autologous or donor tissue: +/- (injectable) degradable scaffolds

- **In vitro engineering of tissue:**

(stem)cells + (biodegradable) scaffolds





# From Bed to Bench and Back

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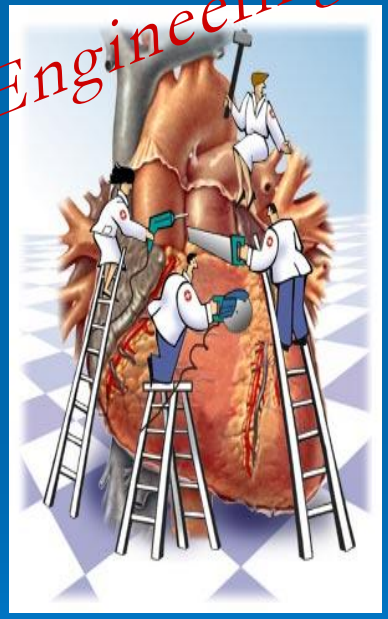
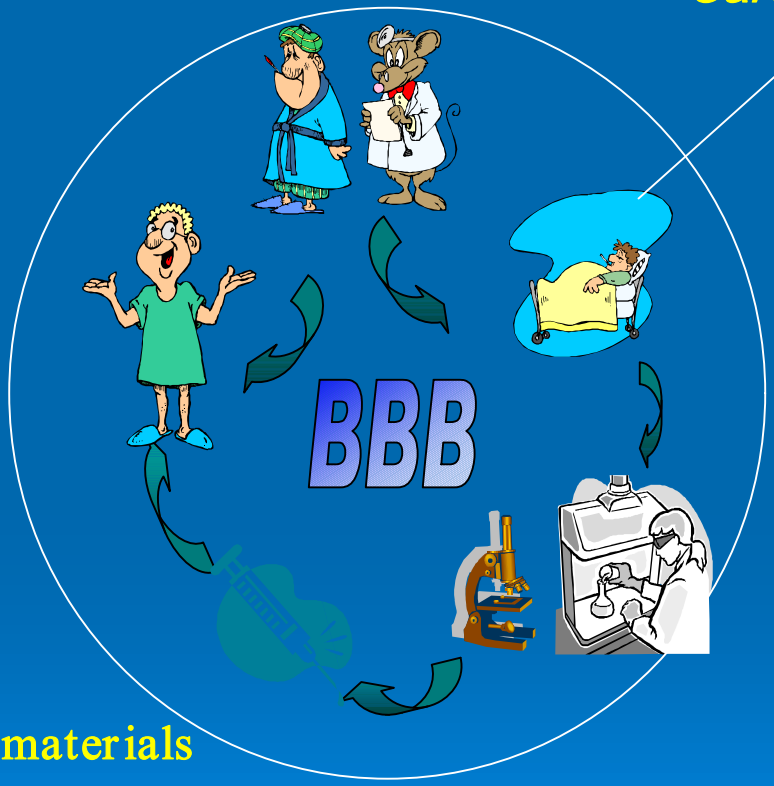
## Regenerative Medicine:

Multidisciplinary translational research

*Cardiovascular*

*Engineering*







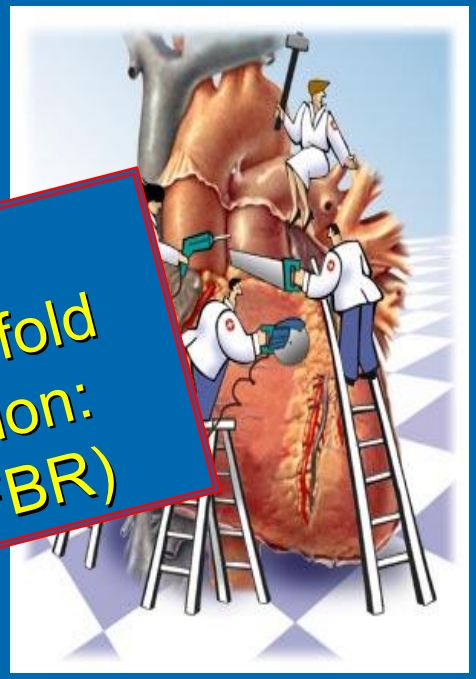
Application of (smart) biomaterials

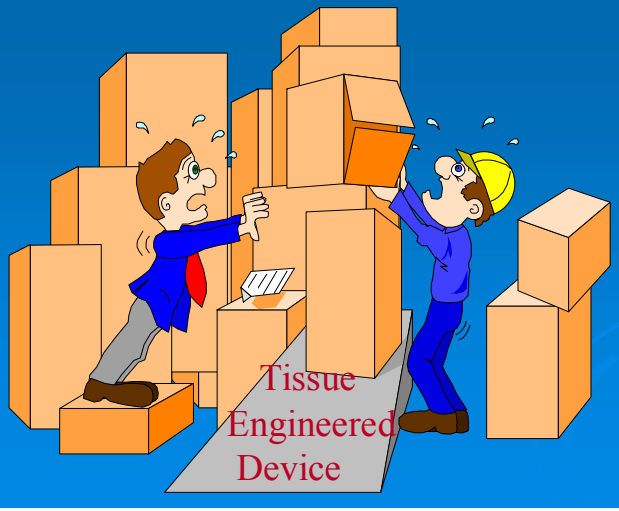
Pathology of tissues??

# Tissue Engineering / Regenerative Medicine

- Combined action of:
  - scaffolds
    - natural: collagen, fibrin etc
    - synthetic: polymers
  - cells
    - stem/progenitor cells
    - inflammat
    - etc
  - factors
    - growth fac
    - modifying e

Implantation of any type of scaffold causes an inflammatory reaction: the Foreign Body Reaction (FBR)



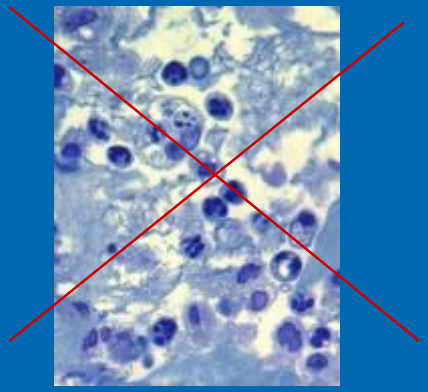



Which disciplines do you need?

- Polymer Technology
- Cell / Molecular Biology
- Biochemical Technology
- Medicine and Pathology
- Surgery (Animal labs and Clinic)

# Knowledge of the (biodegradable) material with respect to the foreign body reaction (FBR)

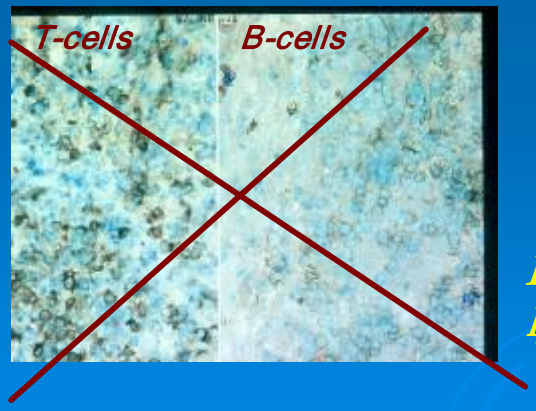
- Chemically inert



Invasion of granulocytes,
And cell/tissue damage

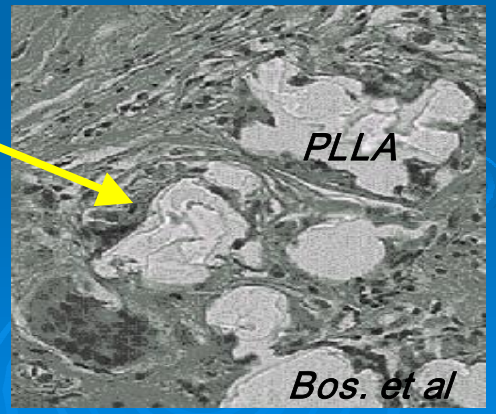
- Low levels of LPS
- Non-Cytotoxic
- Not compliment activating

- Not immunogenic



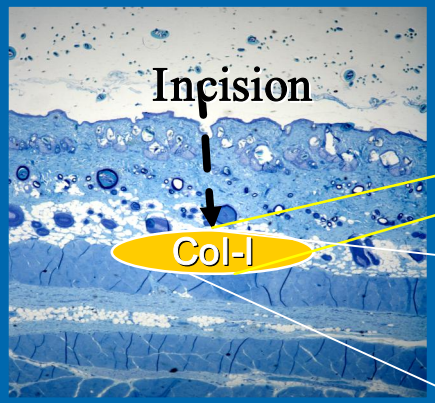

*Infiltration of lymphocytes*

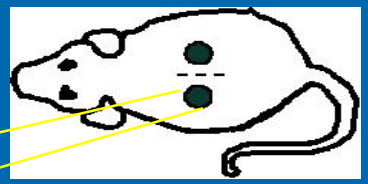
Secondary FBR

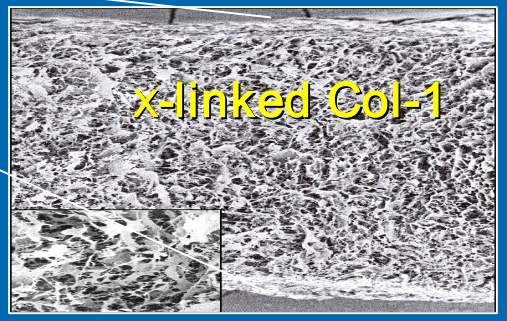



*May cause swelling = unwanted*

# In TE: important to assess the (acute) tissue reactions and biocompatibility of a biomaterial

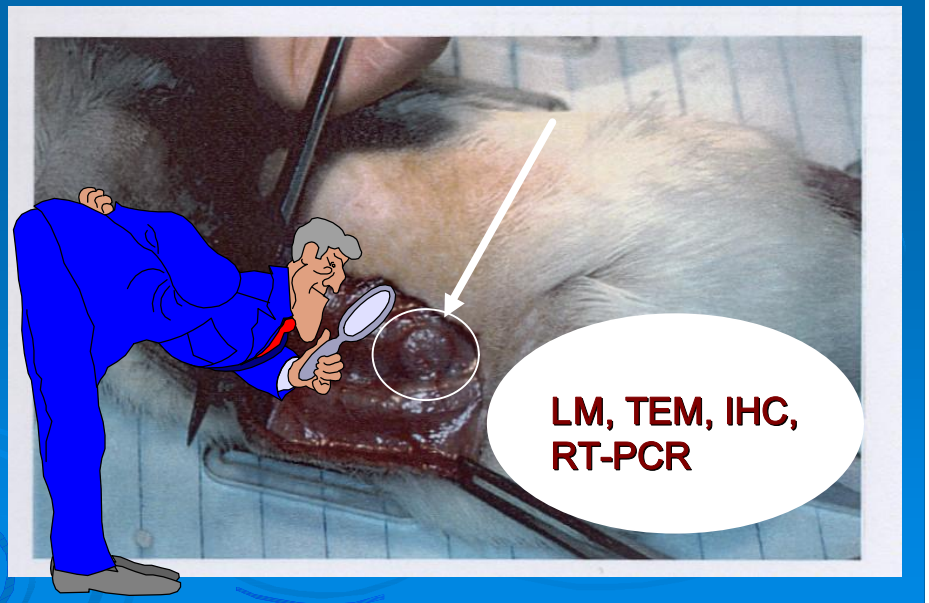


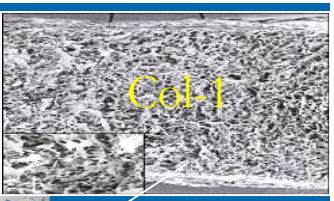


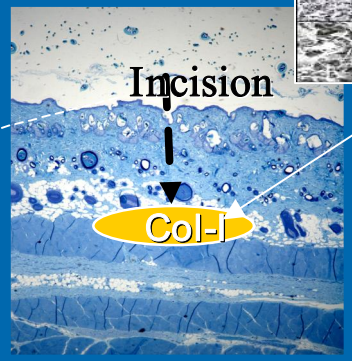

*Sub-Q model*

Explantation of the material with surrounding tissue



# FBR: A-specific inflammatory response




Induction:

Incision

*Tissue injury*

platelet aggregation

activation of surrounding tissue

fibro, MΦ

cytokine release

fibrinogen

thrombin

fibrin

CR3

CR3

P1-fragment

C3b

IgG

Biomaterial

*nature?*

C3b

C3Bb

B b

C5a

CR3

Biomaterial independent

Biomaterial dependent

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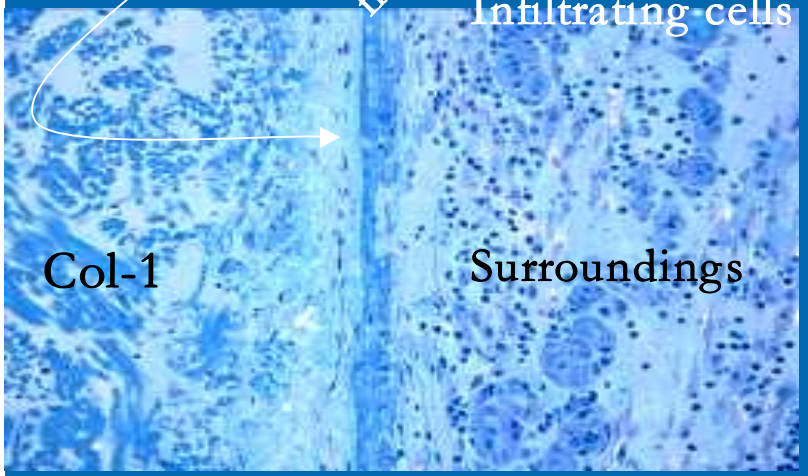
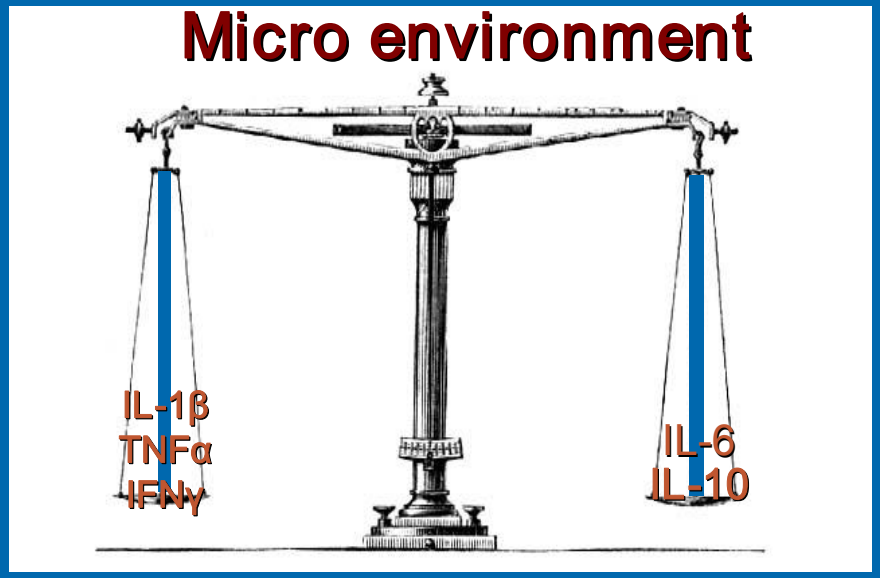
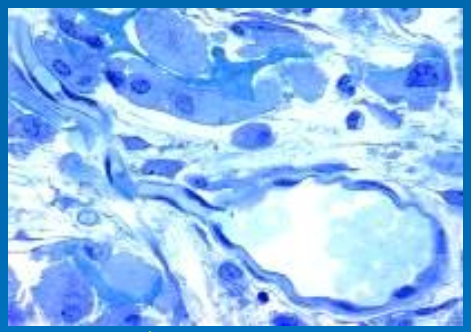
D.T. Luttikhuizen, et al. Tissue Engineering, 2006

# Phase in the Foreign Body Reaction

Onset

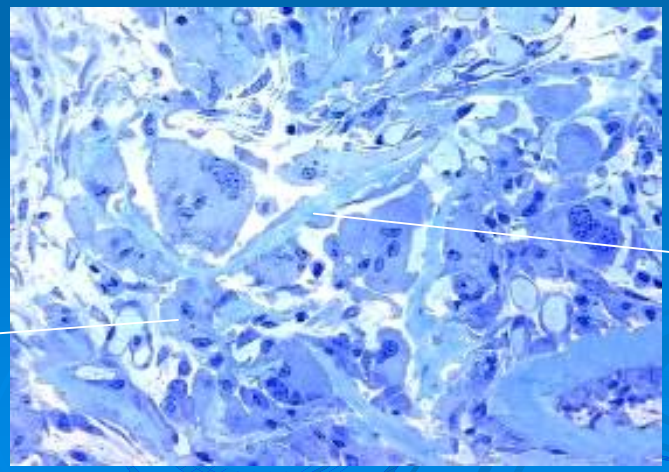


Angiogenesis

Progression



**Resolution phase:**

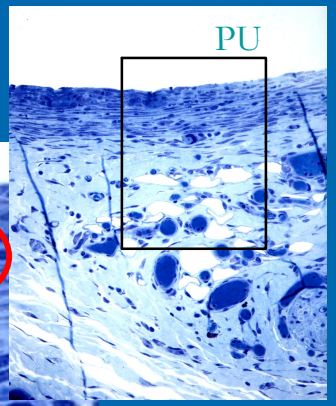
-Tissue remodeling/repair

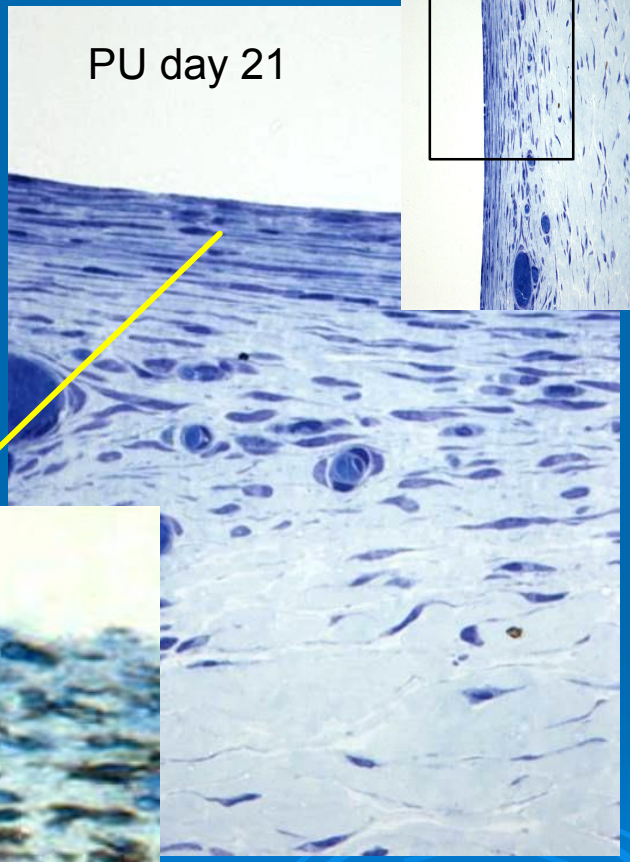
-Degradation of material or incapsulation

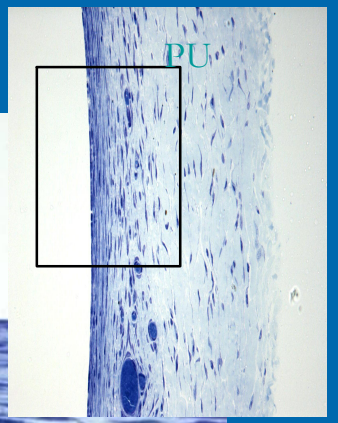
# Onset -> Resolution?

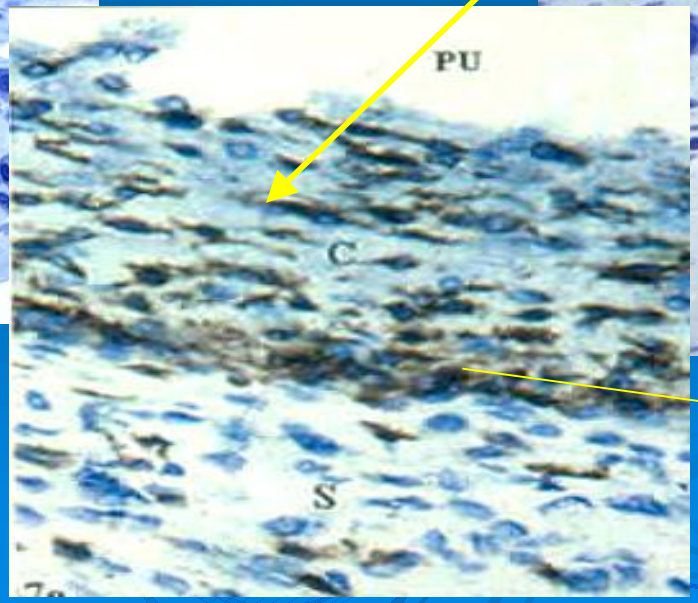
## Non-degradable PU disks:

*Capsule formation; but still macrophages present……*

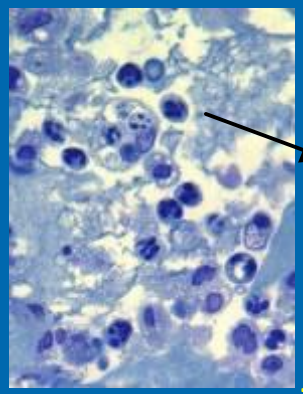




*ED1 staining*

*Q: TGFb, TNFa release:*
*ECM formation / breakdown?*

# Onset:

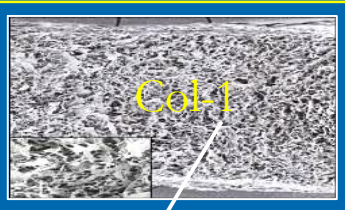

# Extravasation of leukocytes

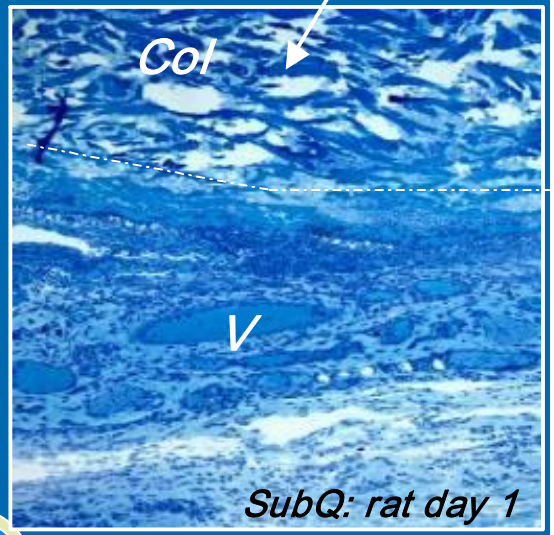


Tissue damage

Blood vessels

Angiogenesis




TNFα, IL-1β

TNFα, IL-1β

extravasation

VEGF, PDGF

$\downarrow[O_2]$

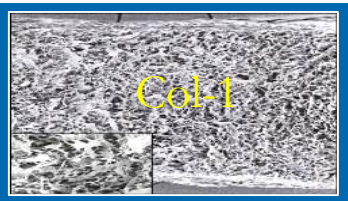
Biomaterial

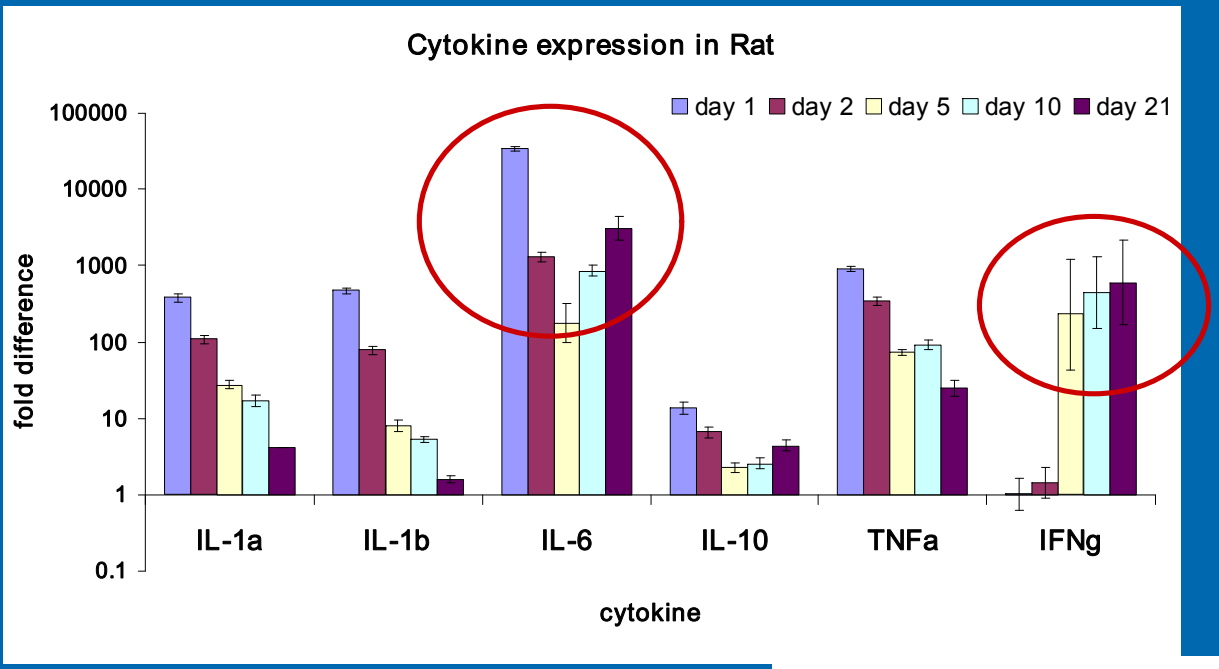
E-selectin

Integrin

ICAM and VCAM

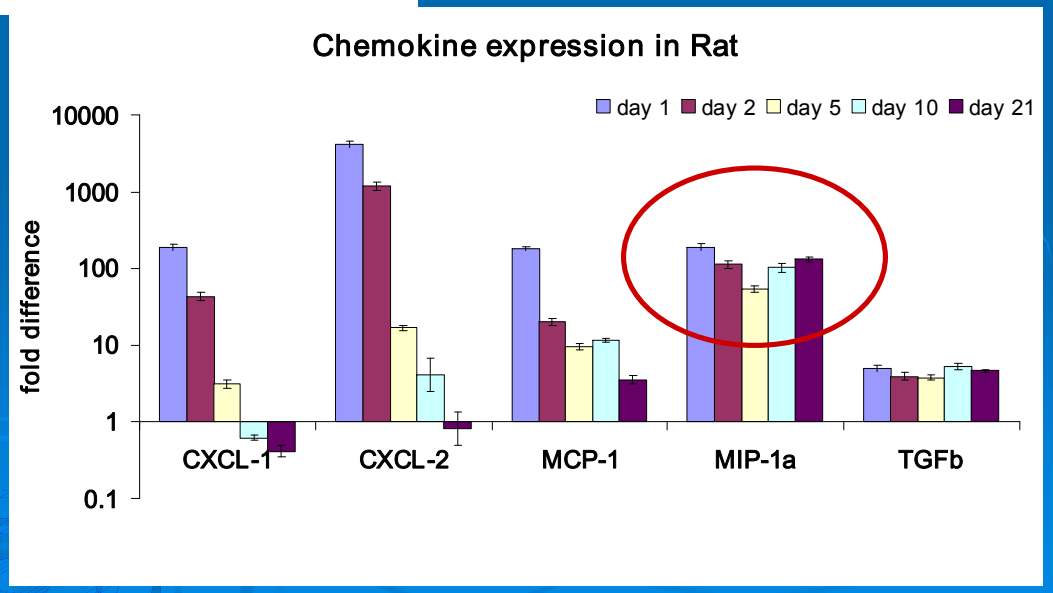
# Cyto/Chemokine expression (to X-linked collagen) in the rat



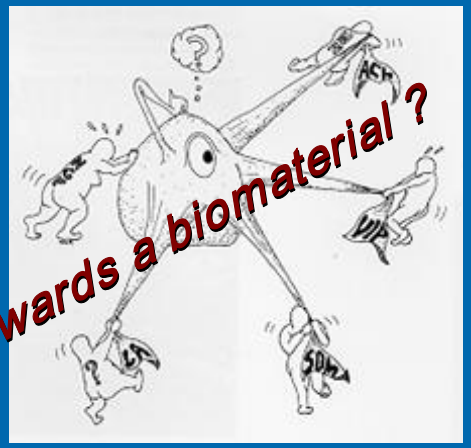

Important knowledge for biomaterial based TE

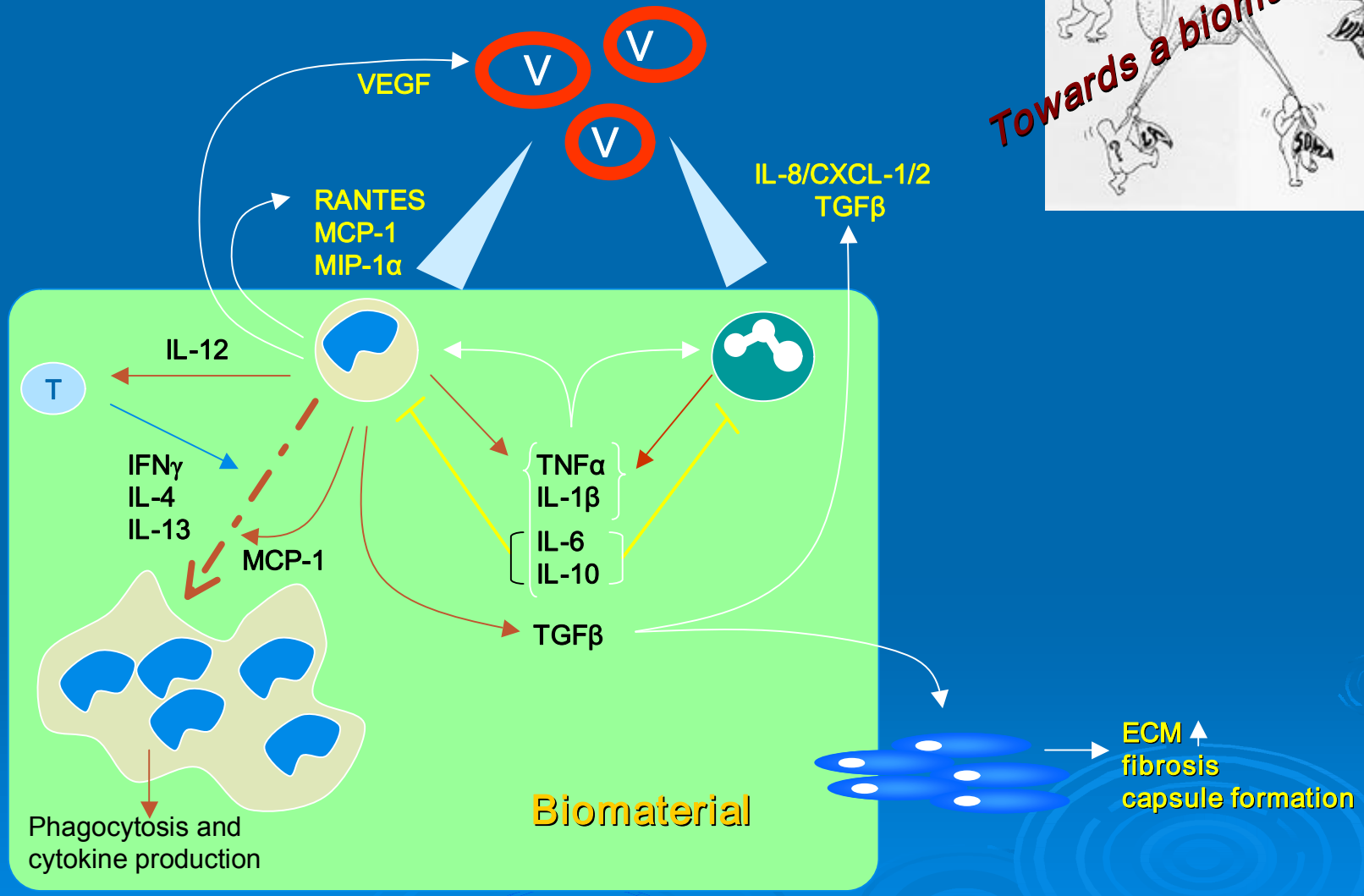
- Increased expression of genes with the onset of the FBR



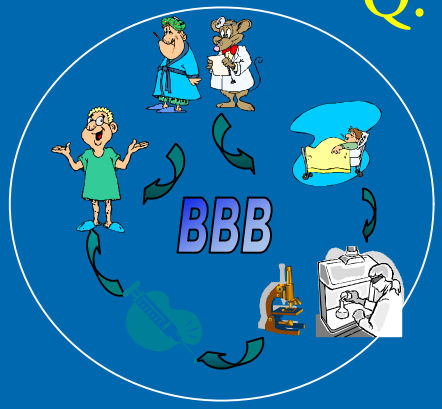

UMCG: D.T. Luttikhuizen, et al. J Tissue Eng Regen Med. 2007

# Progression of the FBR is orchestrated by cytokines and growth factors?

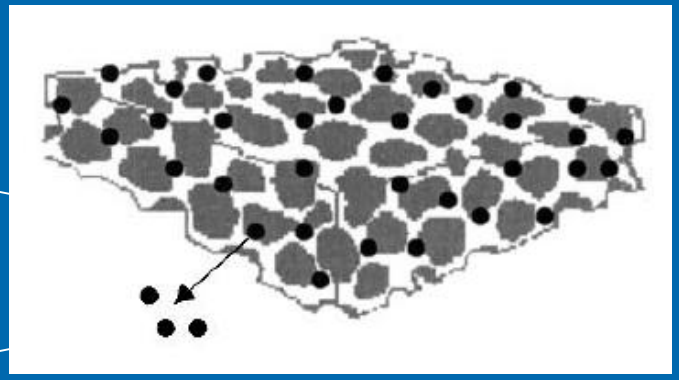


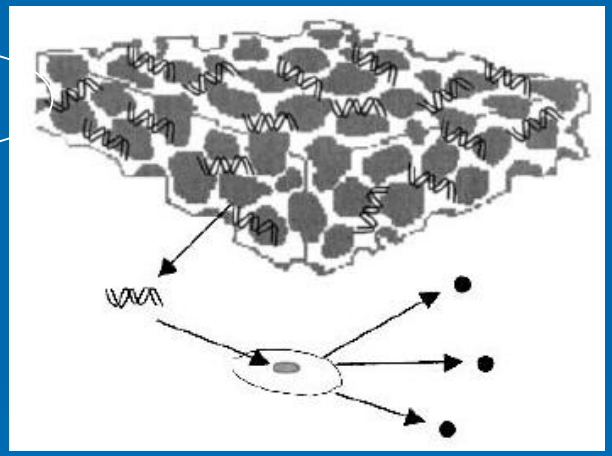
# Q: Possibilities to Orchestrate Regeneration of Tissues by use of 'smart' biomaterials?

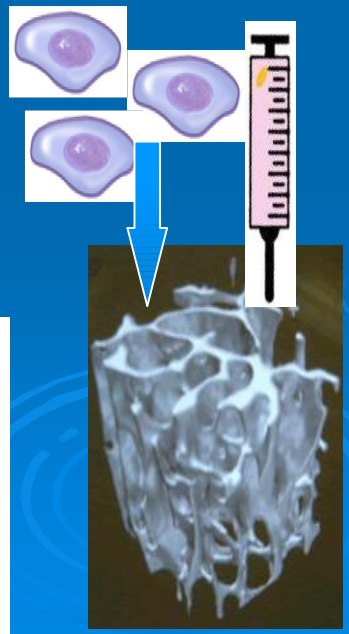



factor

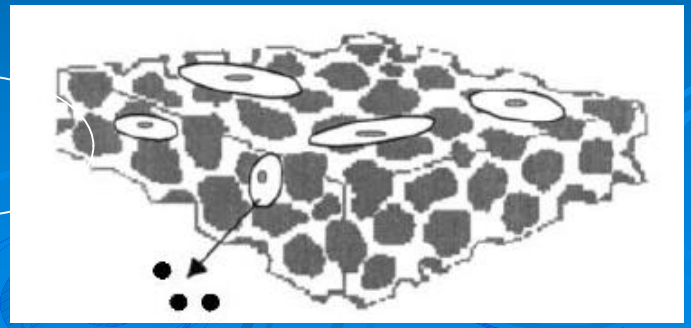


gene



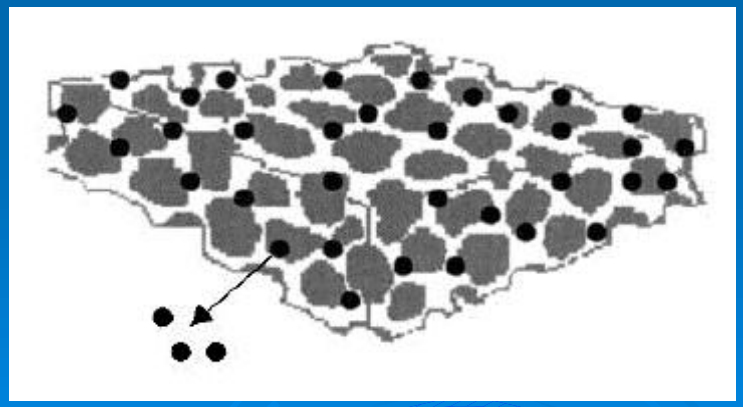


cells



# Towards Tissue Engineered devices……

- e.g. Cytokine loading for controlled release in tissue repair

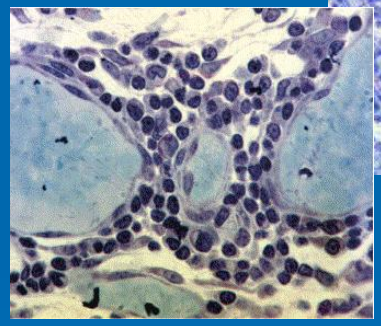
factors

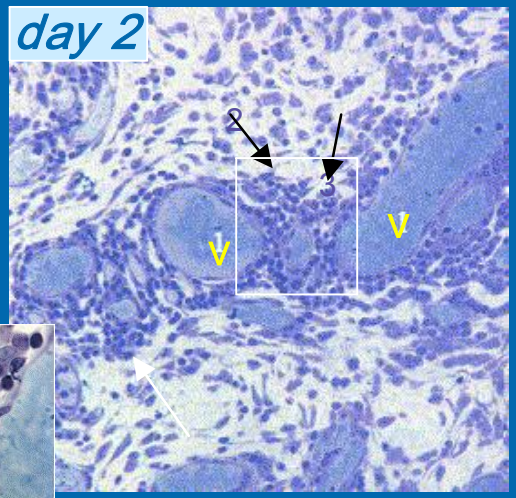
Example

# Cytokine loading:
# IL2-loaded Dextran-microspheres (MS)

*IL2 injection Sub Q in rats*

*day 2*

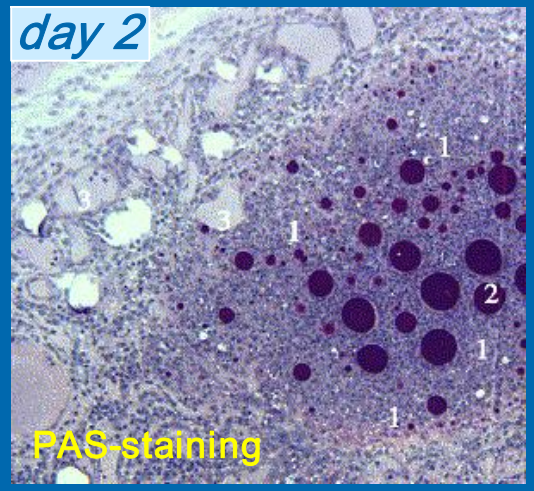




- High vascularisation
- Many lymphocytes

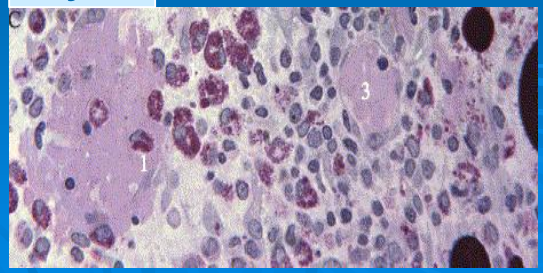
*MS*

*day 2*

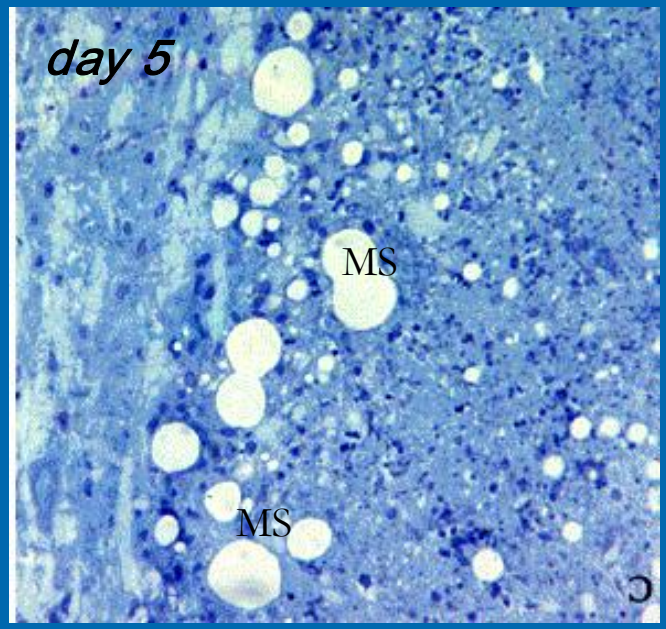



- *Macrophage infiltration*
- *< 1% lymphocytes !*

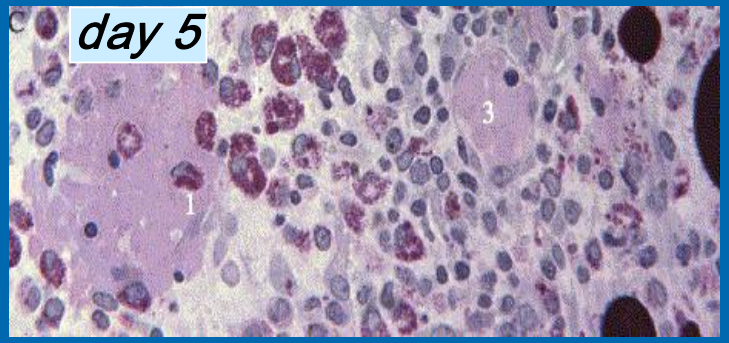
*day 5*



IL2 - loaded MS




MS



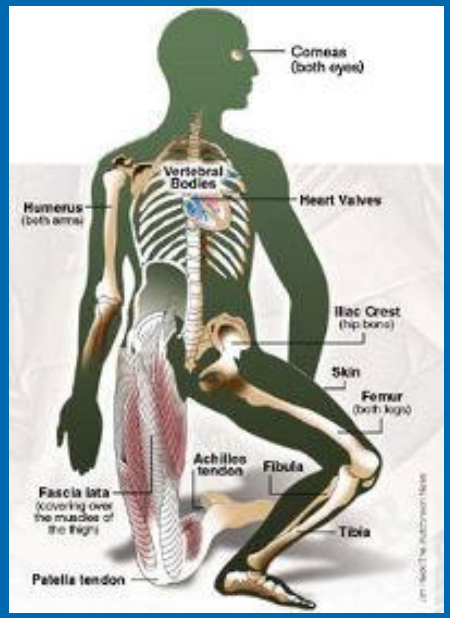

Biocompatable?

- *massive necrose*

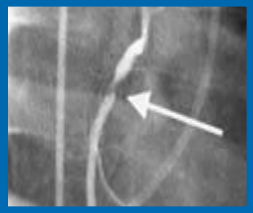
- *Related to microenvironment ?*
- *Possibilities for aimed cancer therapies.....*

J.W. Koten et. al. Cytokine 2003.

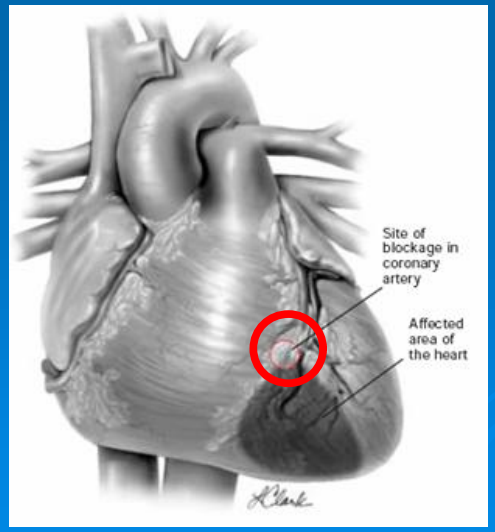
# Pathology and repair of tissues




Q: local inflammatory cascade?
Impaired angiogenesis?
Homing of SC impaired?
Induction of fibrosis?

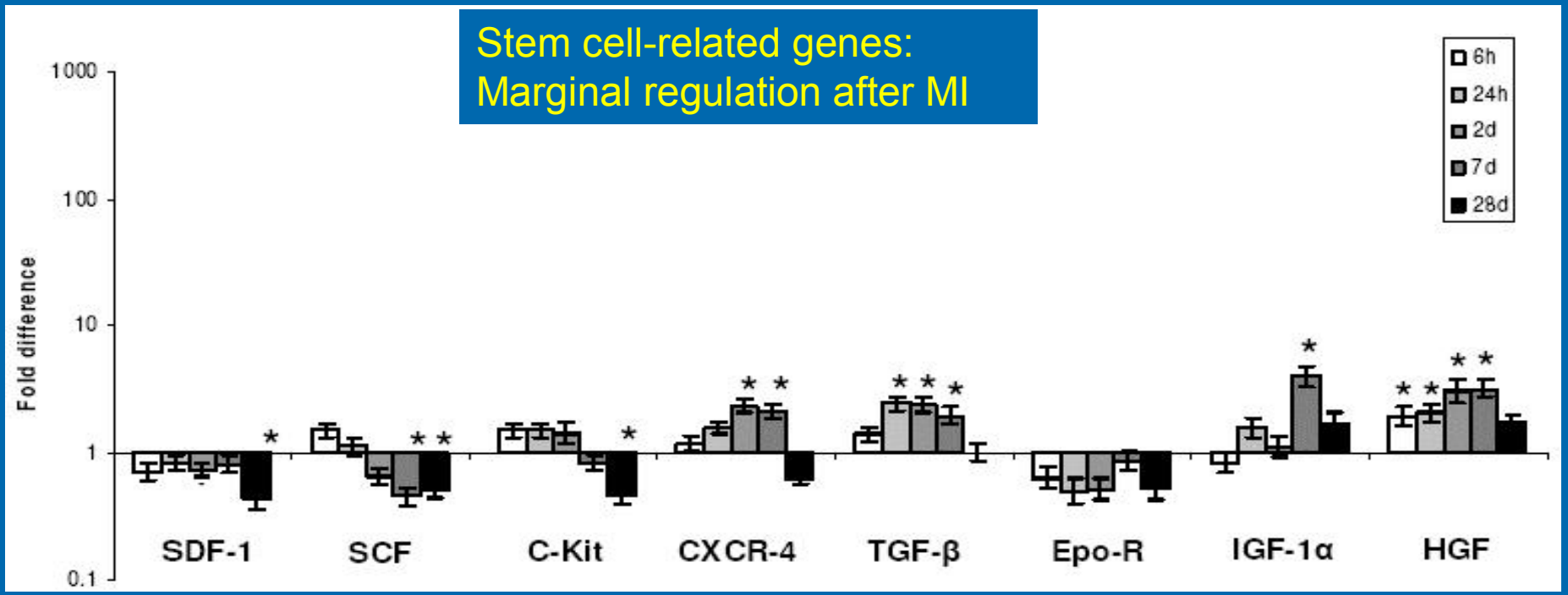


Q in Reg Med




Vandervelde *et al.* Cardiovasc Res. 2007

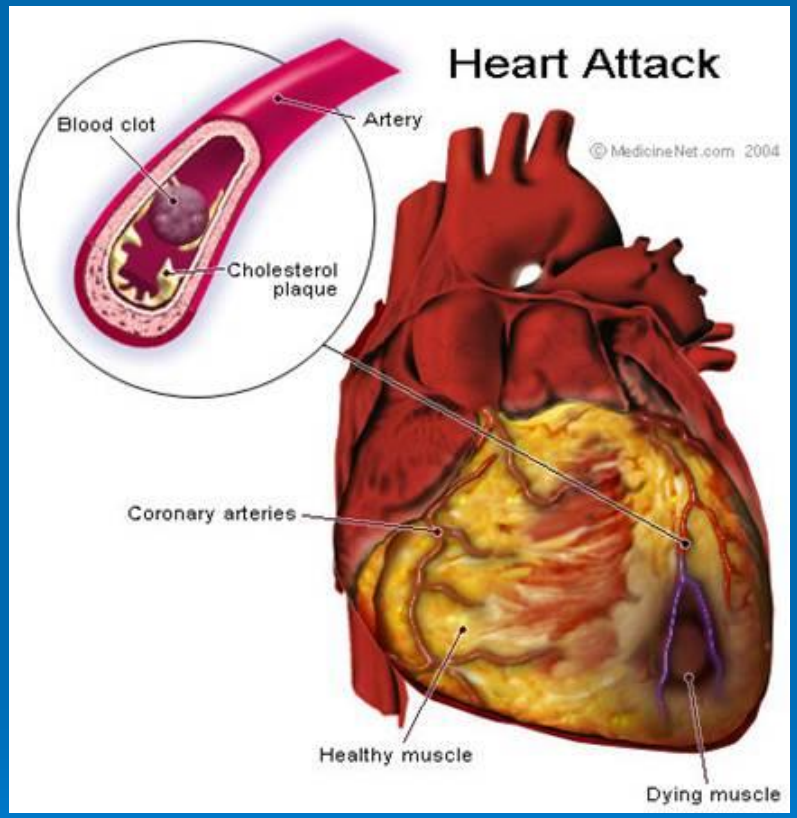
## Stem cell-related genes: Marginal regulation after MI

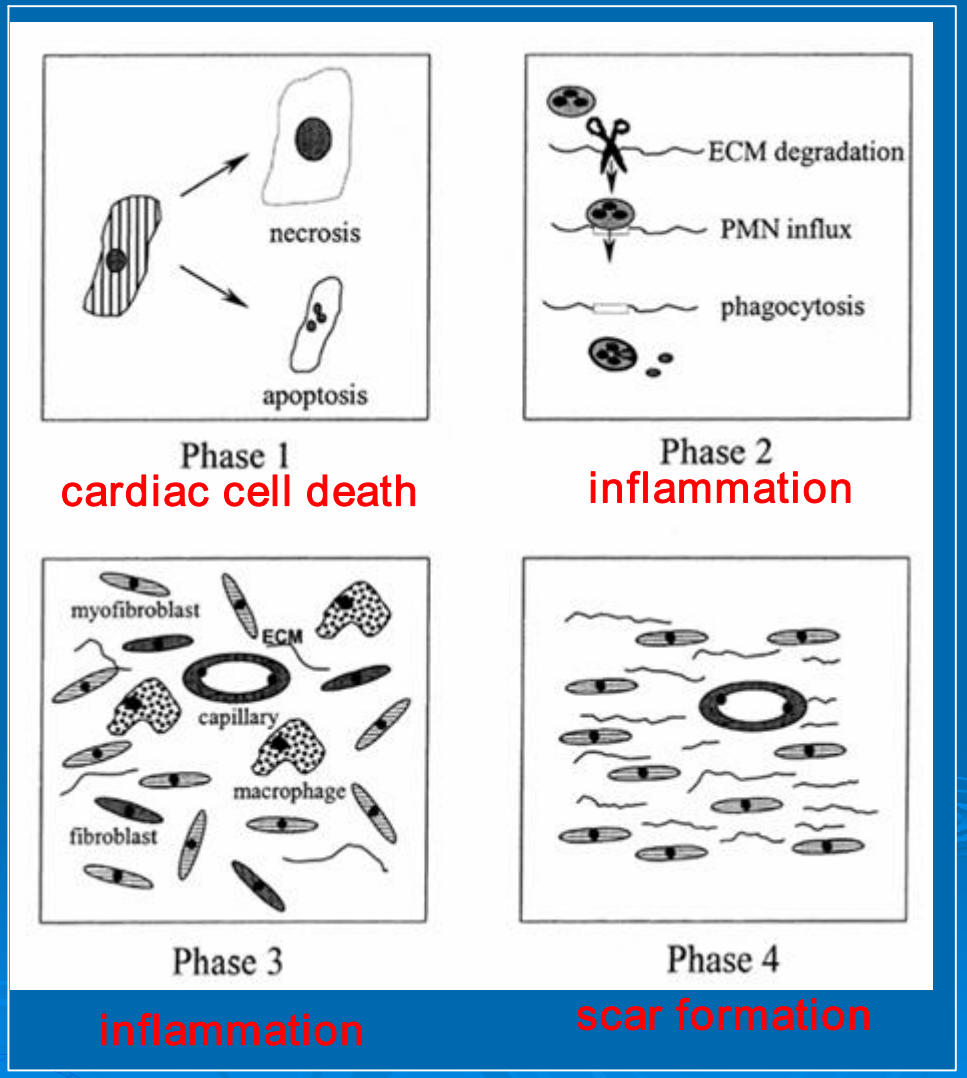



Conclusion:
The inflammatory microenviroenment in the myocardial infarct discourages stem cell action and favours inflammation


Vandervelde et al (2005) JMCC **39**:363
Vandervelde *et al* (2006) Cardiovasc Pathol. **15**:83
Van der Velde *et al* (2007) Cardiovasc. Res. **73**:783

# Myocardial Wound Healing after Infarction

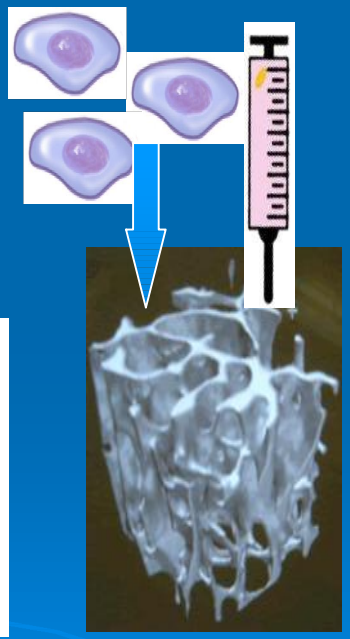


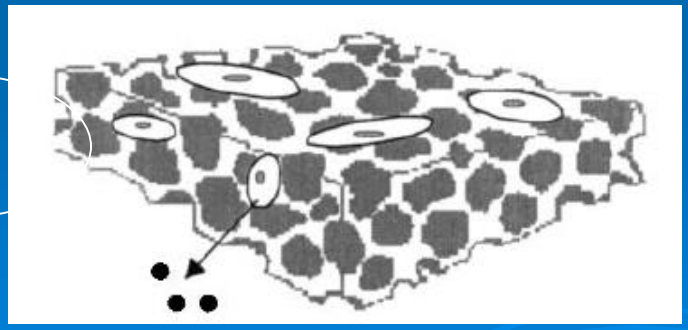
Towards Tissue Engineered devices……

- Cell injection to induce/ improve angiogenesis

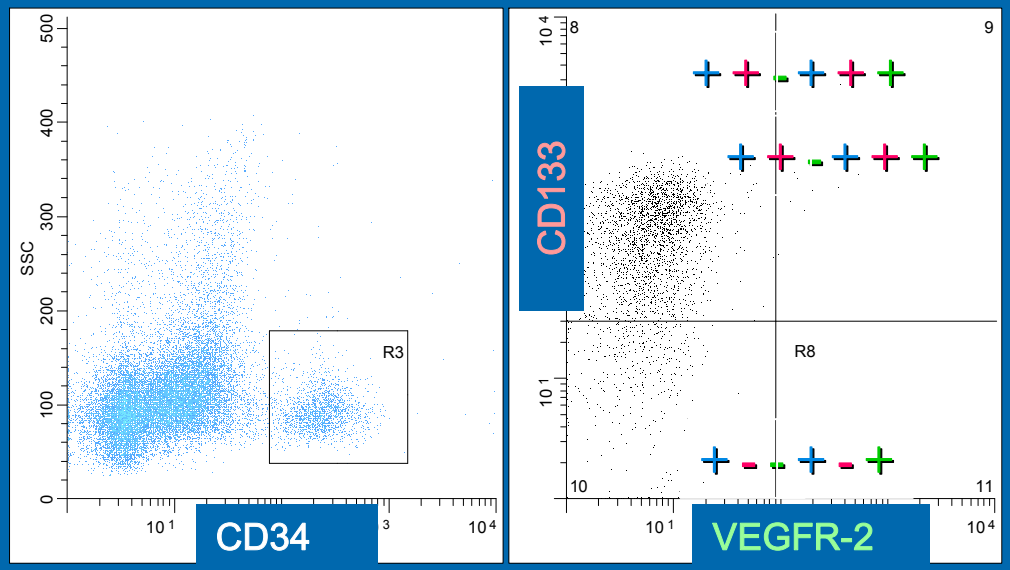
Tissue Engineering

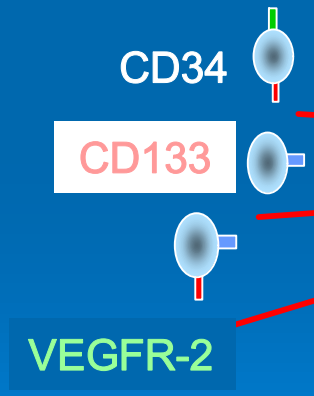


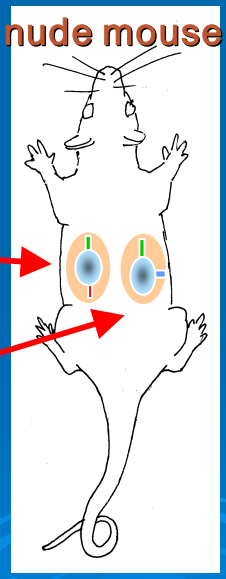
cells



# Human EPC-subsets injected in matrigel



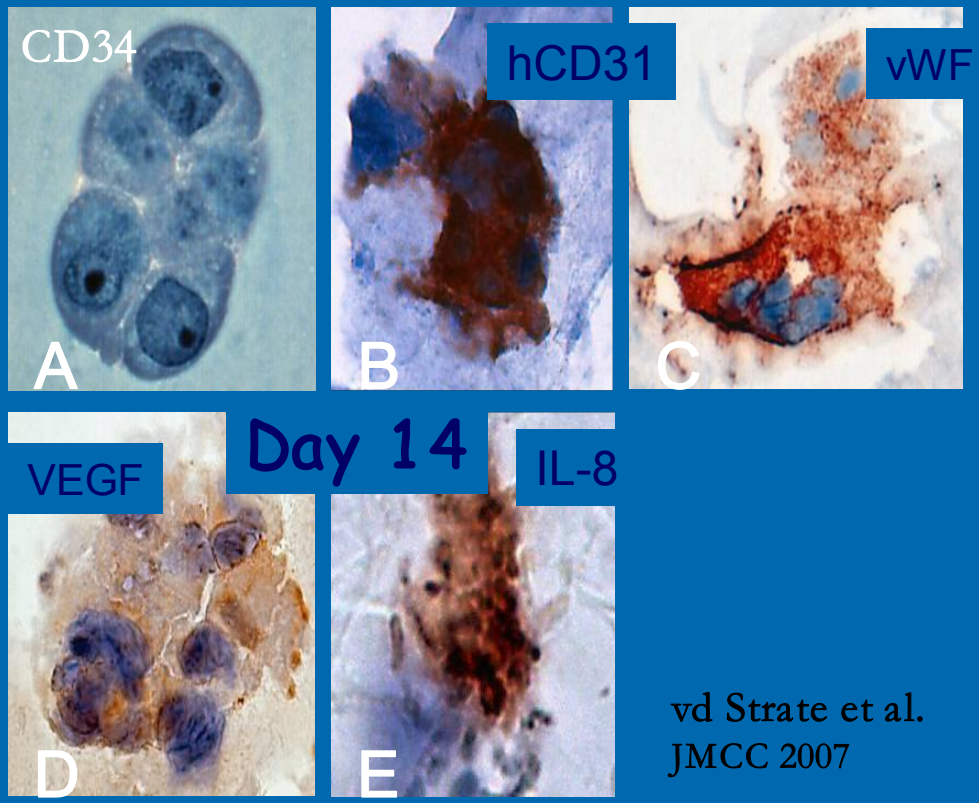



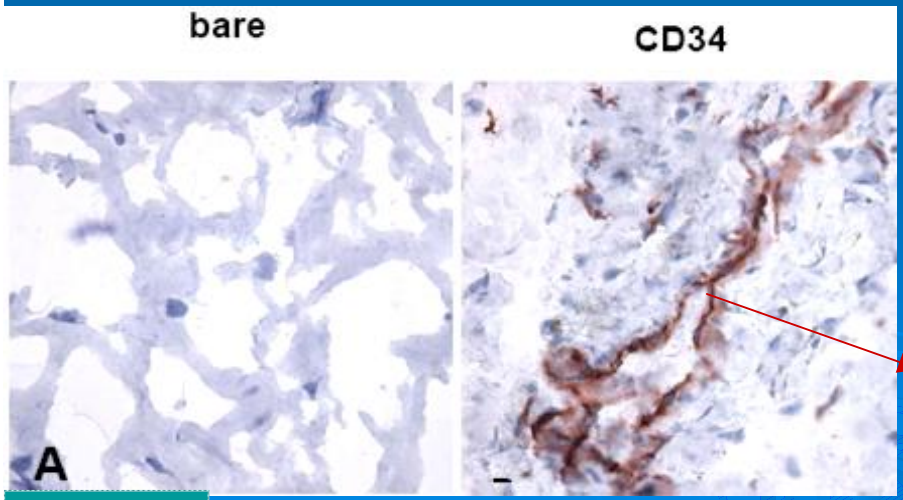

Patent # 0021399002004
Medtronic-UMCG

# Only CD34+ EPC showed angiogenesis and act in a paracrine way

After 14 days




vd Strate et al.
JMCC 2007




*Popa et. al, J. mol Cell Cardiol. 2007*

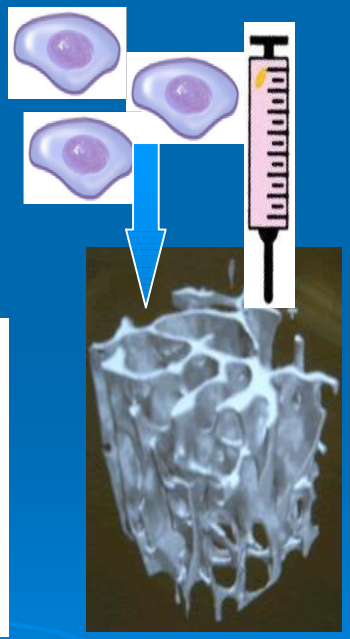
Marginal contribution
of Hu CD34+ cells

*Possibilities in cell therapy?*
*(ischemic insults)*

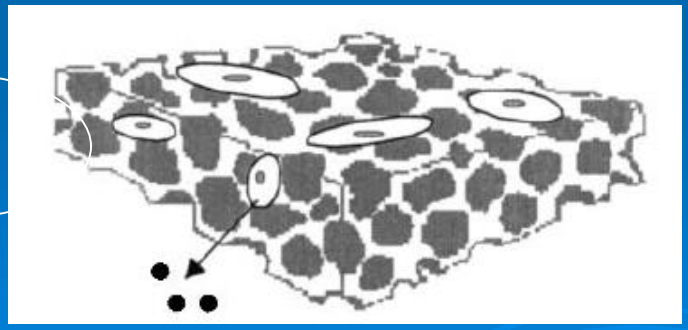
Towards Tissue Engineered devices……

- Engineering of
  e.g. cardiac tissue

Tissue Engineering



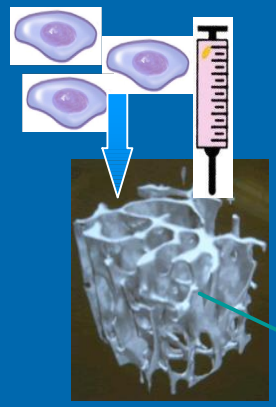
cells

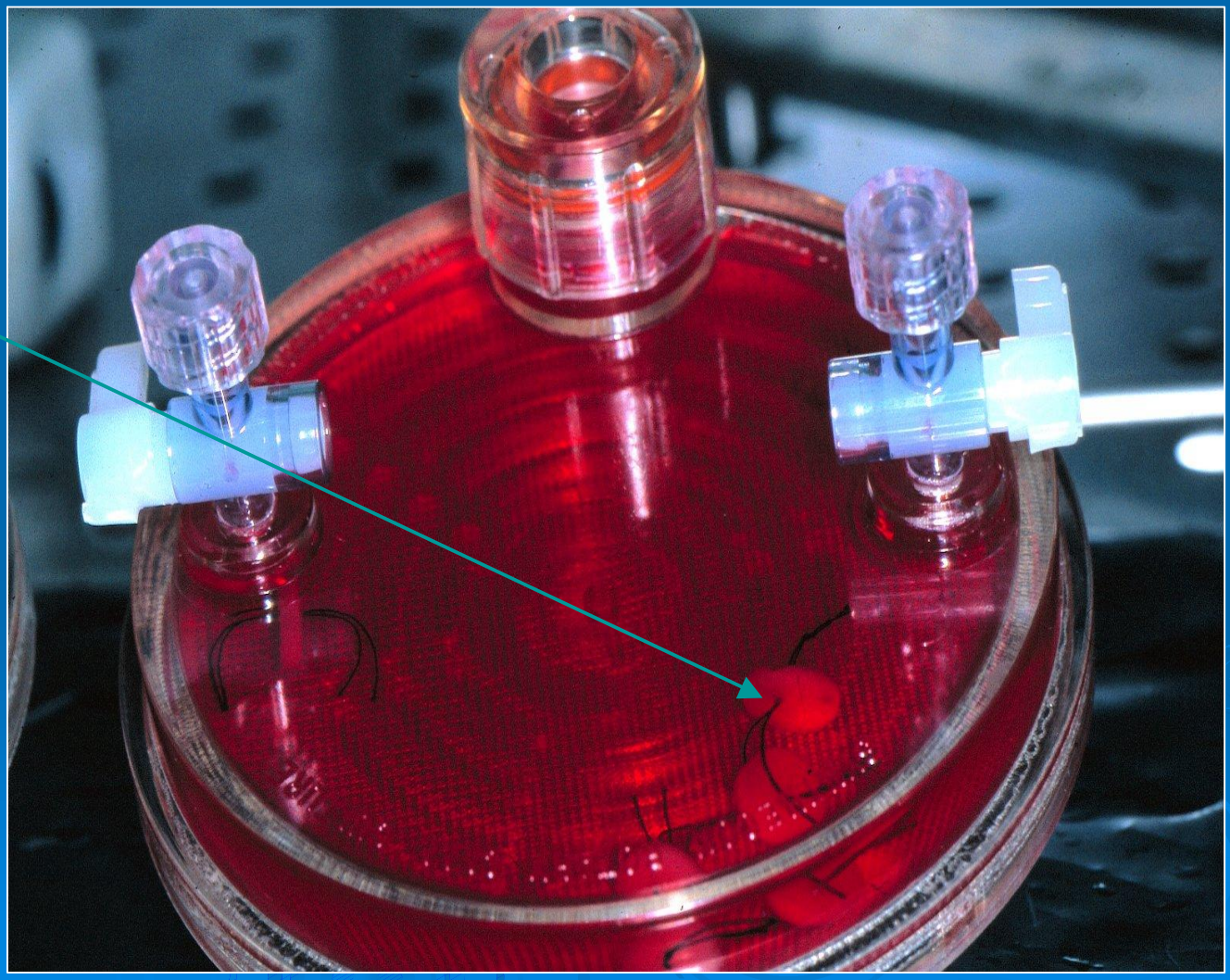


# Bioreactor technology

## High Aspect Rotary Vessel (HARV)

Cells



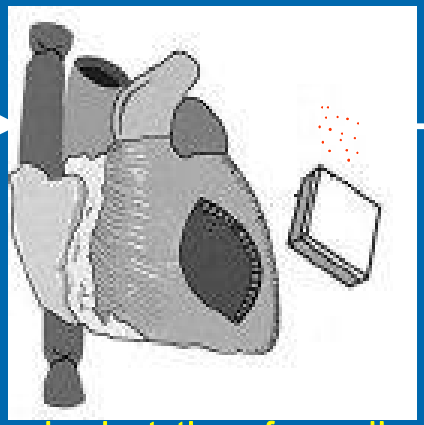


# Myocardial Repair by Tissue Engineering

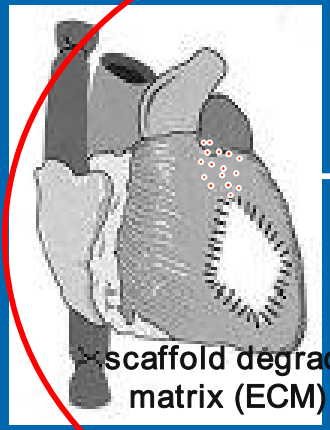
cells

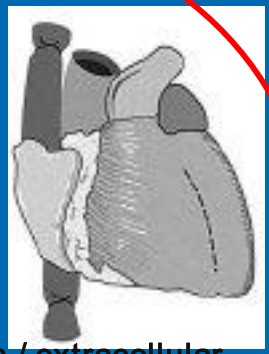
+

degradable scaffold

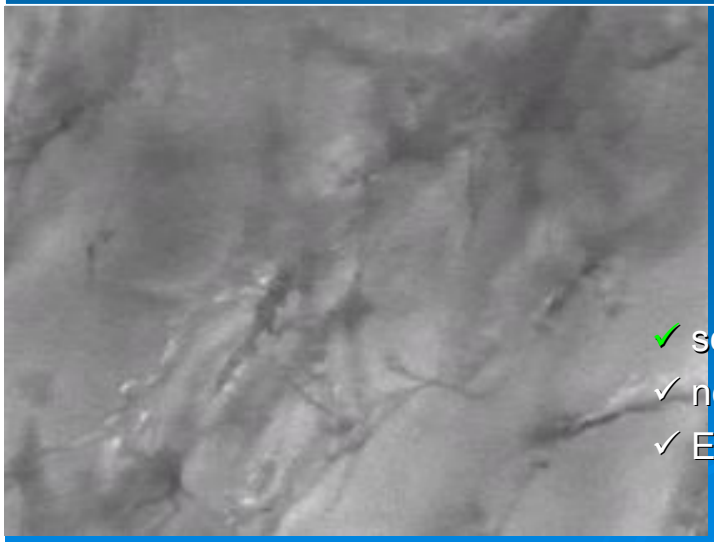


implantation of a cardiac scaffold on / in infarct area





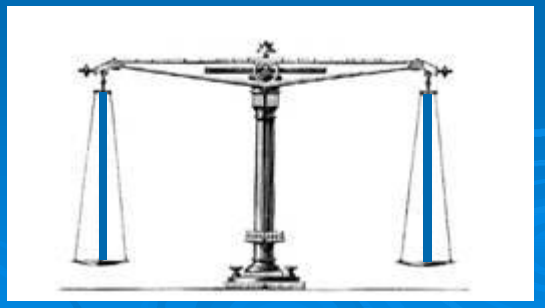
scaffold degradation / extracellular matrix (ECM) formation by cells



- ✓ scaffold degradation
- ✓ neovascularisation
- ✓ ECM formation

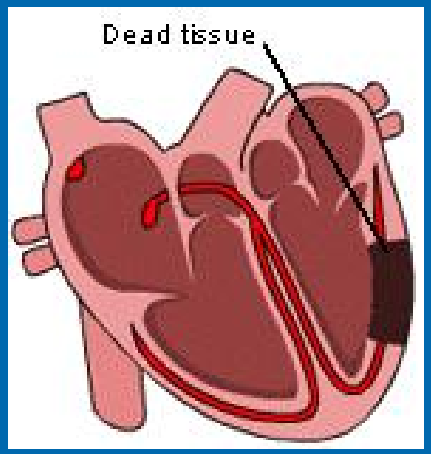
Van Luyn et al. Biomaterials, 2002

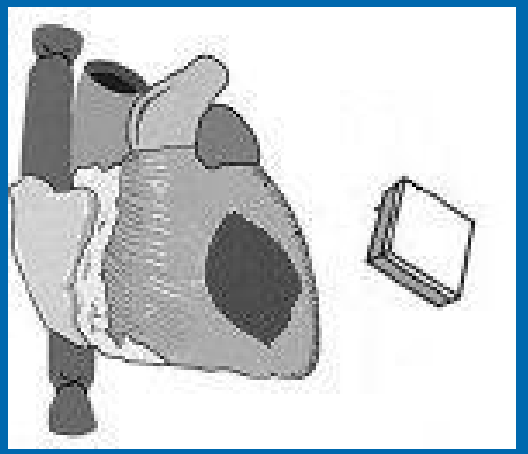
## Foreign Body Reaction (FBR)



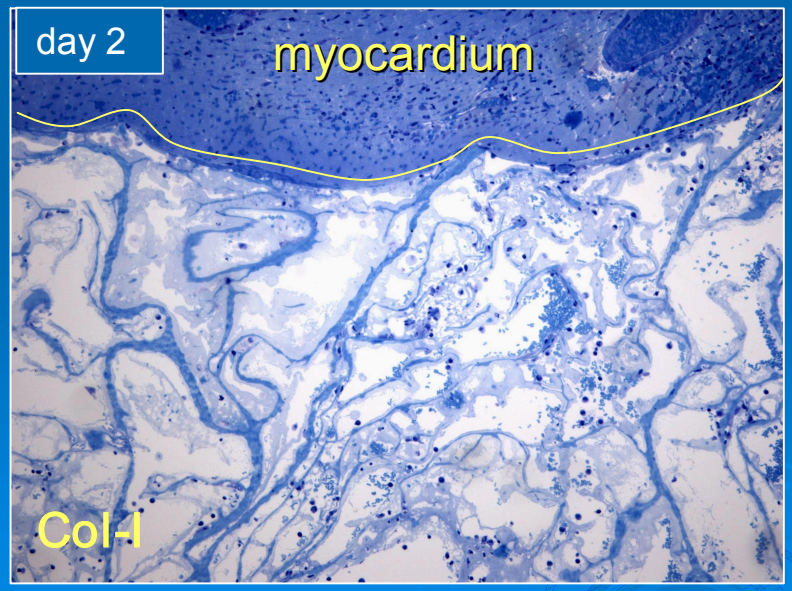
- ✓ side effects
- ✓ implant failure

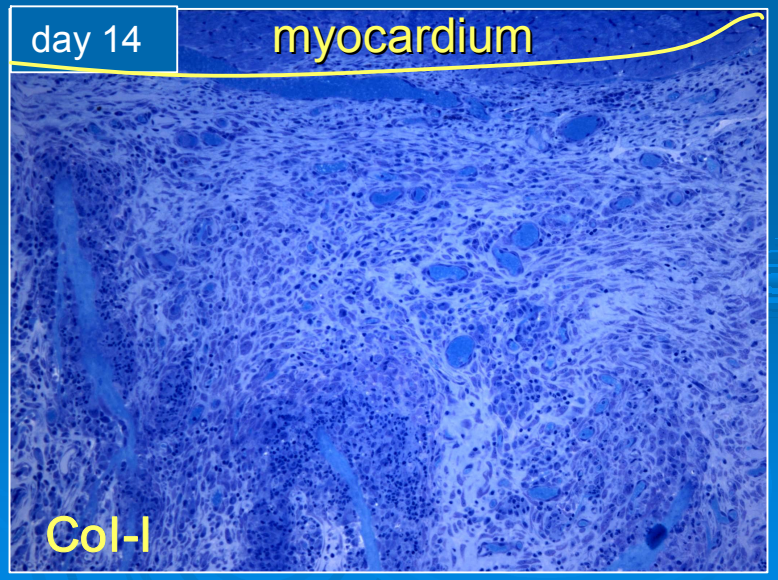
# Myocardial Cryoinjury and Biomaterial (Col-I) Application in Mice



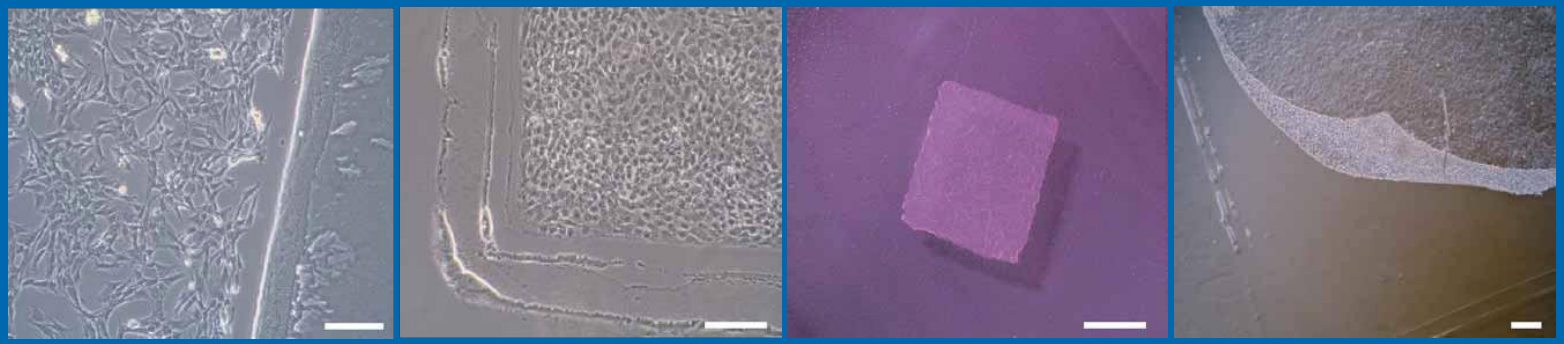
*Degradation of the col-1 on the heart !*



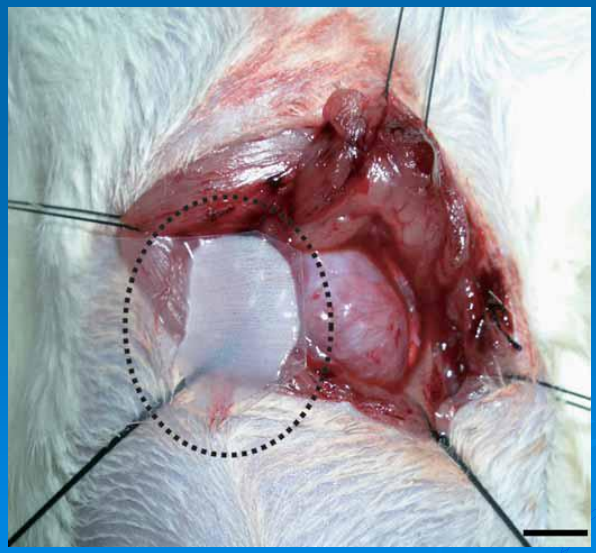




Van Amerongen, Biomaterials. 2006 .2247-57

# Use of Thermo-responsive Coatings in Tissue Engineering



(a) MSCs 2 days after seeding on a temperature responsive dish.
(b) Cultured MSCs expanded to confluence within the square area of the dish by day 3.
(c) The monolayered MSCs detached easily from the culture dish at 20 1C.
(d) The completely detached monolayered MSCs were identified as a 12 mm square sheet.

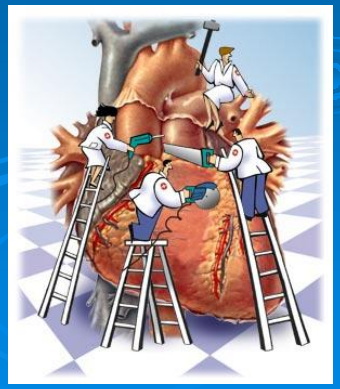


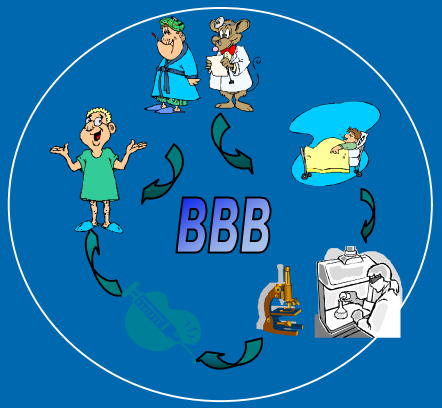
(b) Monolayered MSCs (in the dotted circle) transferred to the infarcted heart.

Miyahara Y et al., Nature Med, 2006, 12 (4); 459-65

# Scaffold application on the heart

- No adverse effects noted *i.e.* similar regenerative profile as without biomaterial (not shown)
- On the heart: neutrophil mediated, enzymatically driven breakdown of Col-1: not observed after Sub-Q implantation!
- Potential application for slow-release of regeneration promoting factors



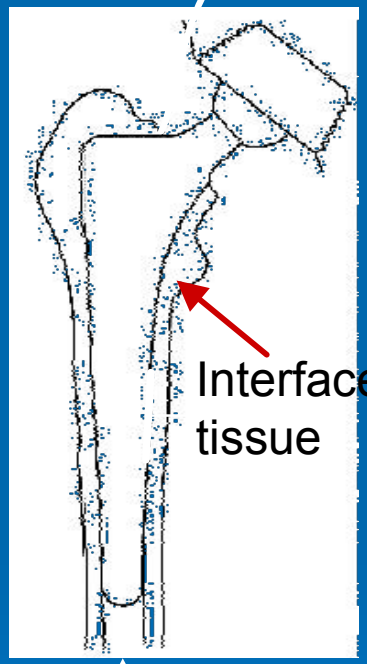



# Hip implant loosening

RuG

umcg

# Hypothesis of "(a)septic" hip implant loosening




High Local Fluid Pressure/Particles

Macrophages

Cytokines/growthfactors

Bacterial biofilm (prim./sec.)

(Proliferation)/activation of bacteria/endotoxin

Osteoclast activation

Osteolysis

Loosening of the prosthesis

# Interface retrieval study (UMCG)

## Systemic response

## Luminex analyses of plasma samples

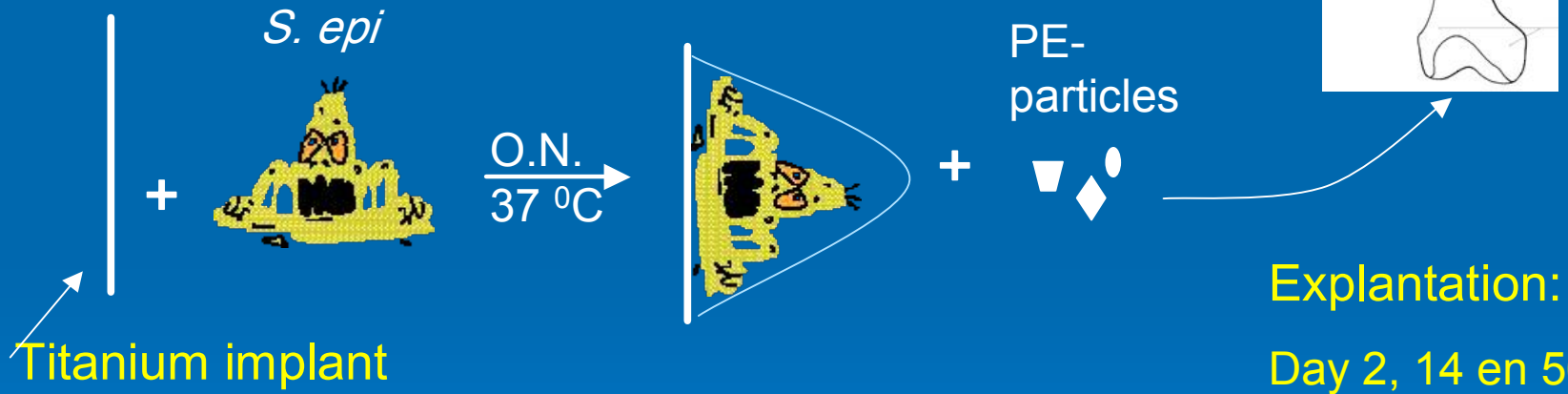
| | Pro inflammatory | | | | Anti inflammatory |
|---|---|---|---|---|---|
| concentration in pg/ml | IL-1β* | IL-6** | TNFα | IFNγ* | IL-10 |
| Loosened implants | 5,11 (±7,69) | 51,39 (±20,52) | 88,73 (±56,48) | 116,59 (±96,85) | 52,24 (±49,46) |
| Well-fixed implants | 5,01 (±13,3) | 25,98 (±7,64) | 51,88 (±15,88) | 37,03 (±27,56) | 17,49 (±5,17) |
| Healthy controls | 36,35 (±34,82) | 25,29 (±3,60) | 47,35 (±2,91) | 29,60 (±5,57) | 17,59 (±2,38) |
| | Compared with healthy volunteers Loose p=0.027 Fixed p=0.046 | Loose compared with: Fixed p=0.009 healthy p=0.006 | Loose compared with: Healthy p=0.076 | Loose compared with: Fixed p=0.05 | Loose compared with: Fixed p=0.089 Healthy p=0.088 |

| | Chemokines | | | Other |
|---|---|---|---|---|
| concentration in pg/ml | IL-8/CXCL8* | RANTES/CCL5 | MCP-1/CCL2 | GM-CSF |
| Loosened implants | 21,51 (±1,01) | 9516,69 (±7014,95) | 329,35 (±117,09) | 35,56 (±30,83) |
| Well-fixed implants | 19,96 (±0,84) | 4966,84 (±7237,93) | 237,11 (±73,86) | 10,16 (±14,93) |
| Healthy controls | 19,84 (±0,40) | 5200,87 (±5175,15) | 238,55 (±79,99) | 14,11 (±22,10) |
| | Loose compared with: Fixed p=0.001 healthy p=0.007 | | Loose compared with: Fixed p=0.09 | Loose compared with: Fixed p=0.069 |

# Rat model: Proof of Principle

Experimental groups:

1. Titanium + *S. epi* biofilm + PE particles
2. Titanium + PE-particles
3. Titanium + Biofilm
4. Titanium
5. PE-particles




Explantation:

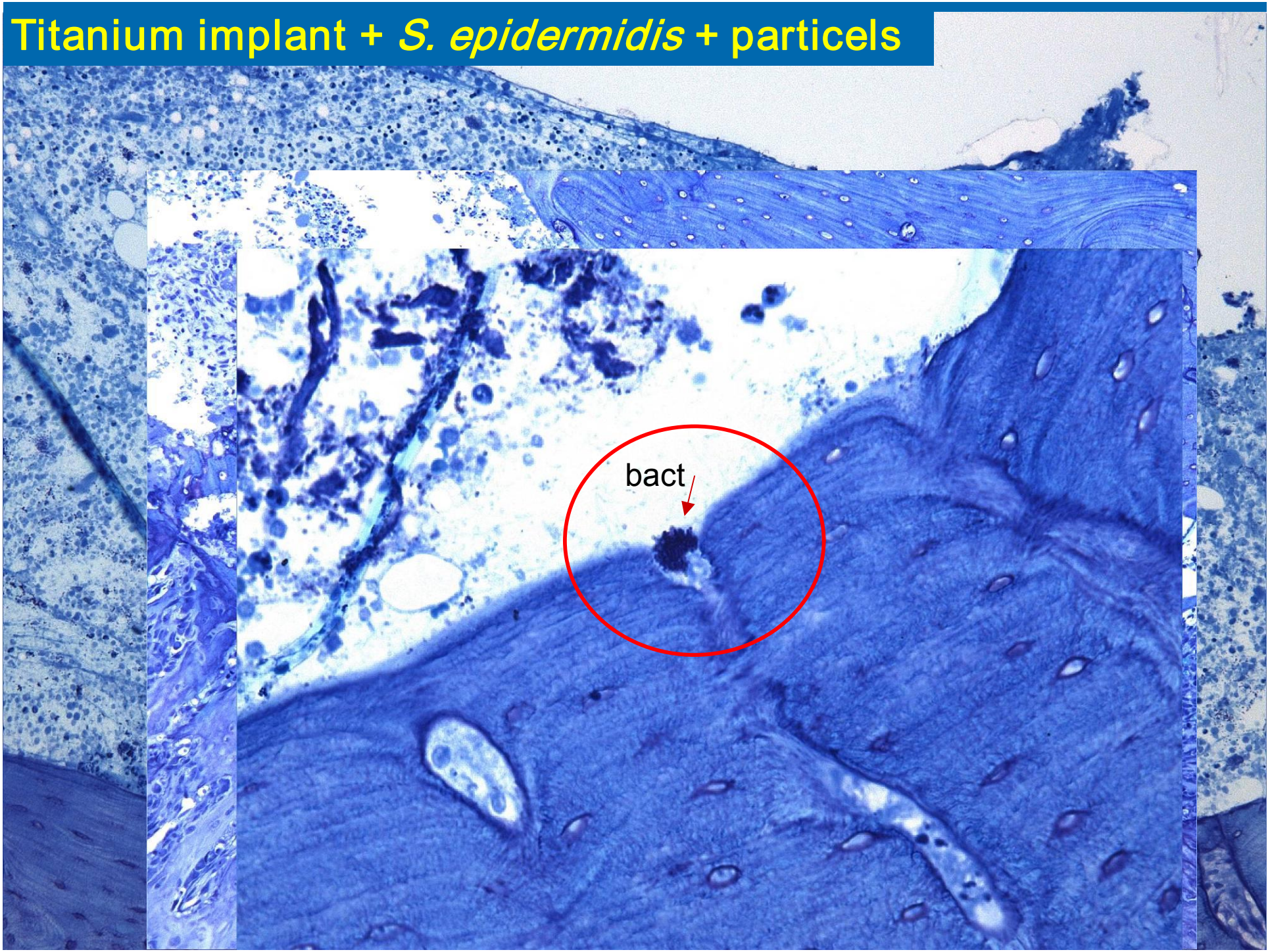
Day 2, 14 en 56

# Implants at day 2,  Group: T+B+PE

AP, T+B+PE impl rat 2

Lateraal, T+B+PE impl rat 1

# Titanium implant + *S. epidermidis* + particels



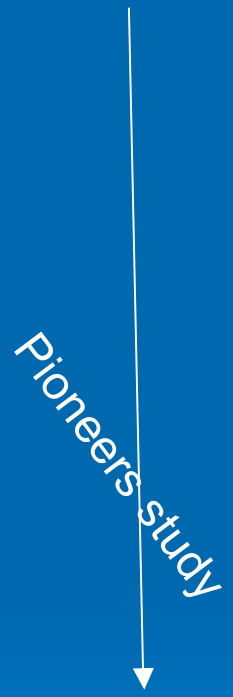
# The Artificial Kidney

(Towards an implantable kidney device)

## Why?

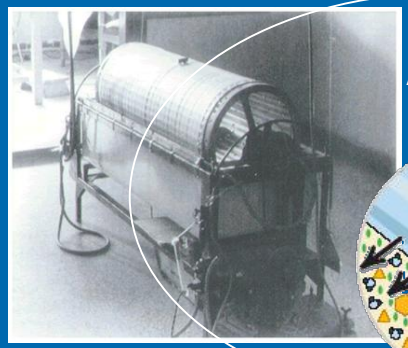
Disadvantages of conventional hemodialysis:

- incomplete urea clearance
- secondary hyperparathyroidism and osteodystrophy
  (due to incomplete vitamin D activation)
- amyloidosis (due to inability to metabolise low-molecular weight proteins)
- hypotension (due to dysregulation of body fluid volume)
- fatigue (due to reduced red blood cell count)

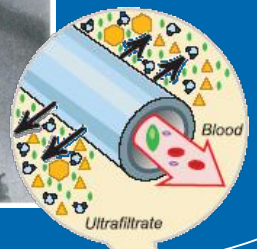


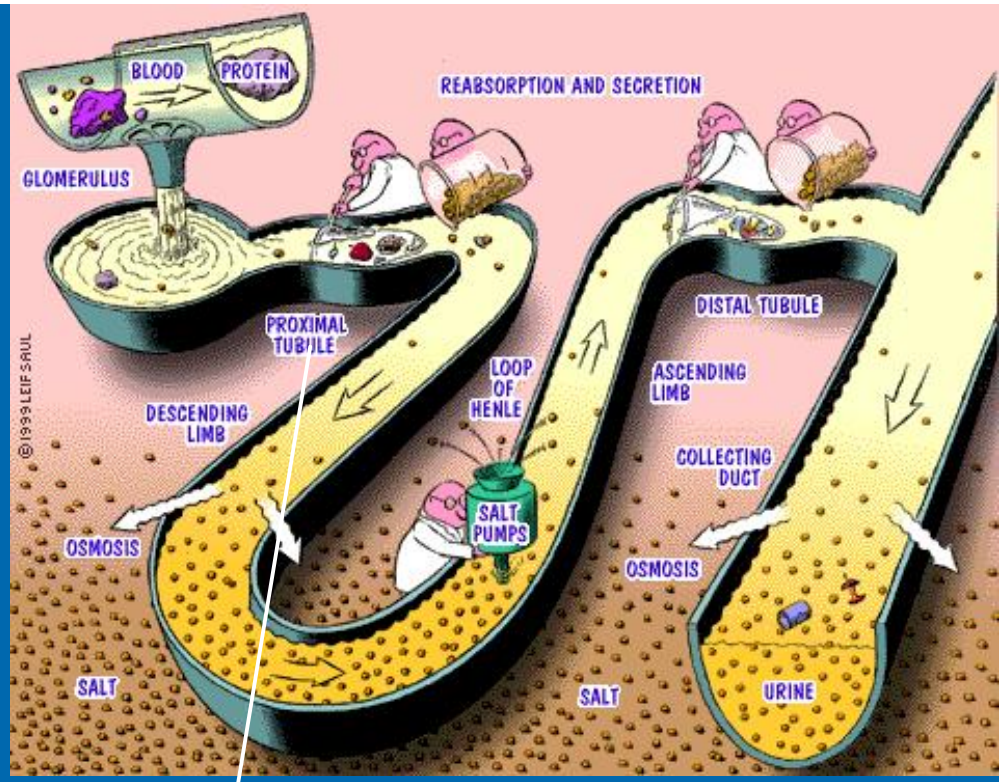

The artificial nephron replaces **tubular** function in addition to **glomerular** function!

# Development of a bio-artificial kidney device

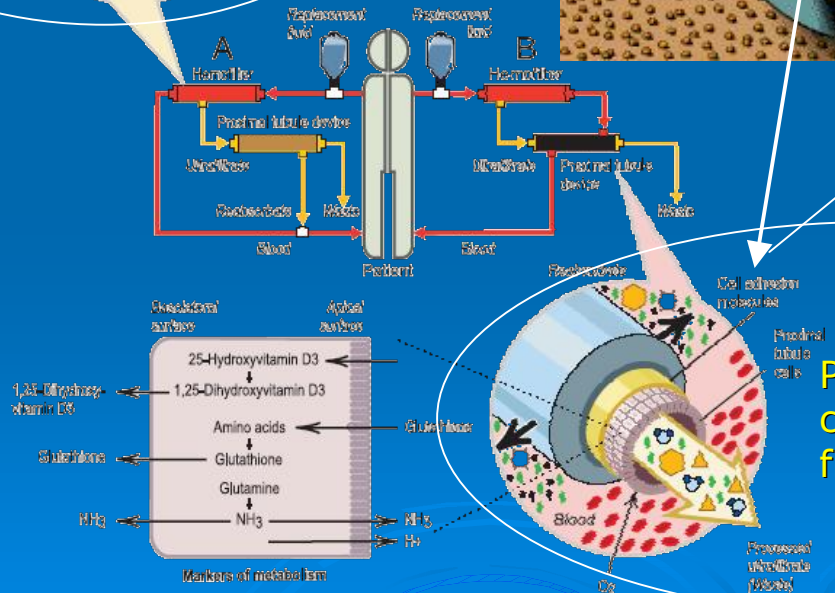


A conventional hemofilter




Secretion of organic ions, drug metabolites. Vitamine D glutathione etc.




Proximal porcine tubule: cells seeded on hollow fiber membranes

Figure 1. Schematic diagram of extracorporeal circuit employed by Humes et al[7] system that combines these functions, a synthetic hemofilter followed in series with a tissue-engineered proximal tubule device comprising a monolayer of porcine proximal tubule epithelial cells attached to the luminal surface of hollow fiber membranes.

Secretion of organic ions, drug metabolites etc.

Porous membrane (polysulfone)

Epethelial cells

Blood

Oxygen, nutritients

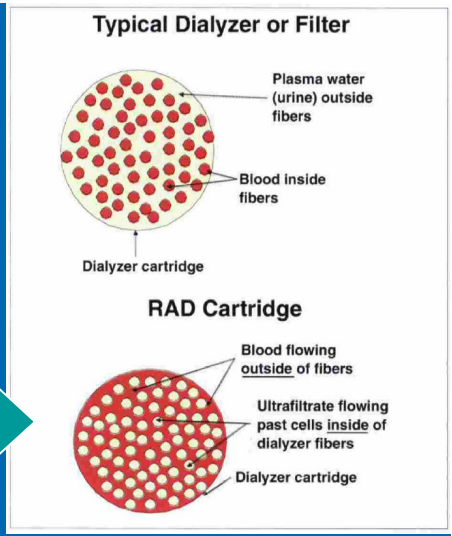
ultrafiltrate

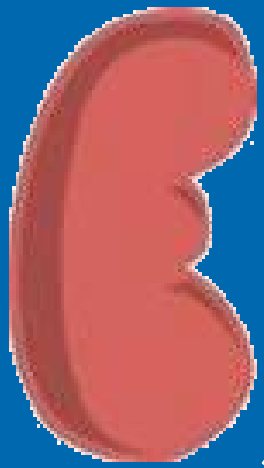
Secretion of vitamin D, glutathione

Biocompatibility?
-attachment
-differentiation
-proliferation
-immuno-barrier

Hemocompatibility?

Re-absorption of fluids, glucose, amino acids etc.





Donor kidney not suited
for transplantation

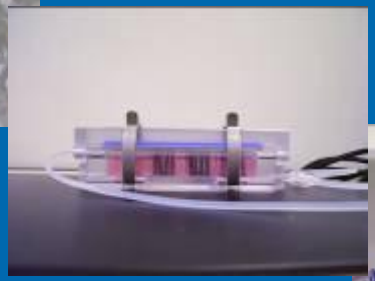
Isolation of tubular
epithelial cells

Cultivation:

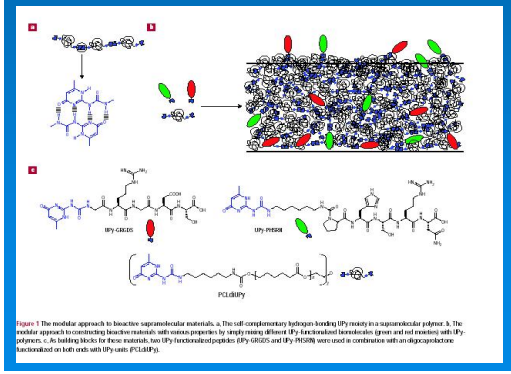
conditions?

Characterisation
and function !

# Organo-typical cultures

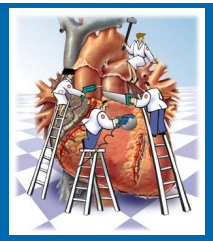


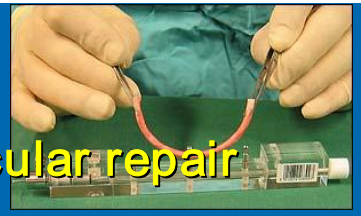

Coatings
Culture medium supplements
(Oxygen level)



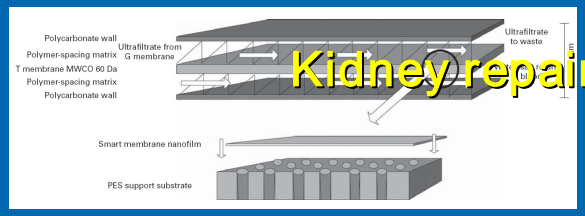
Use of bioactive
Membranes!

Dankers et al, BioMarcomol, 2011





Cardio/vascular repair

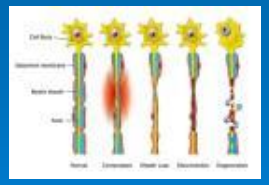



Kidney repair / device

Development of TE-shunts

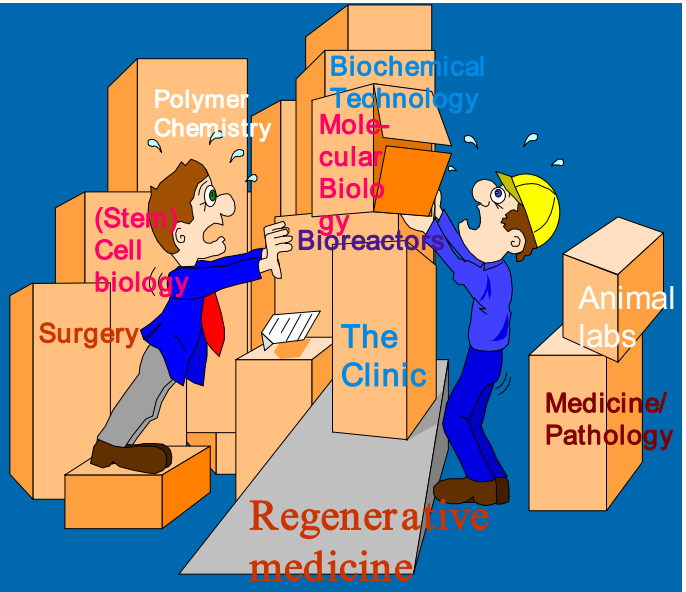


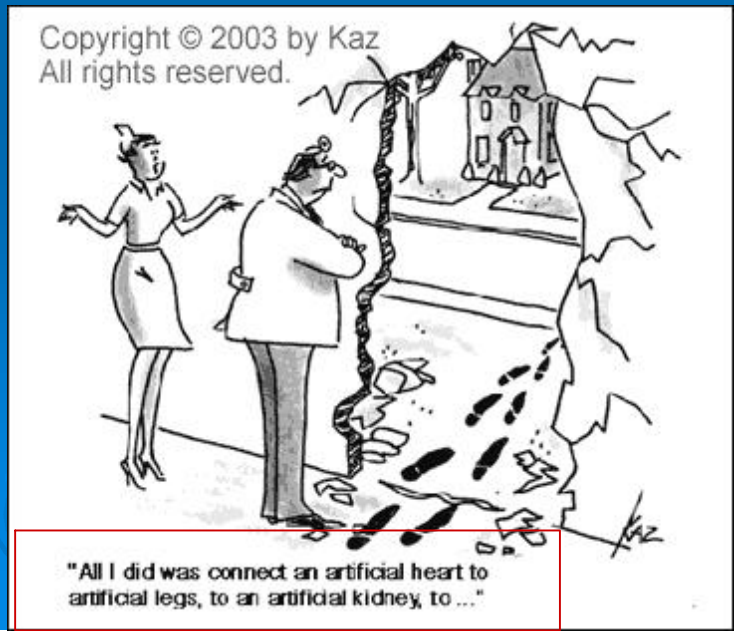
Cartilage/ bone repair / devices

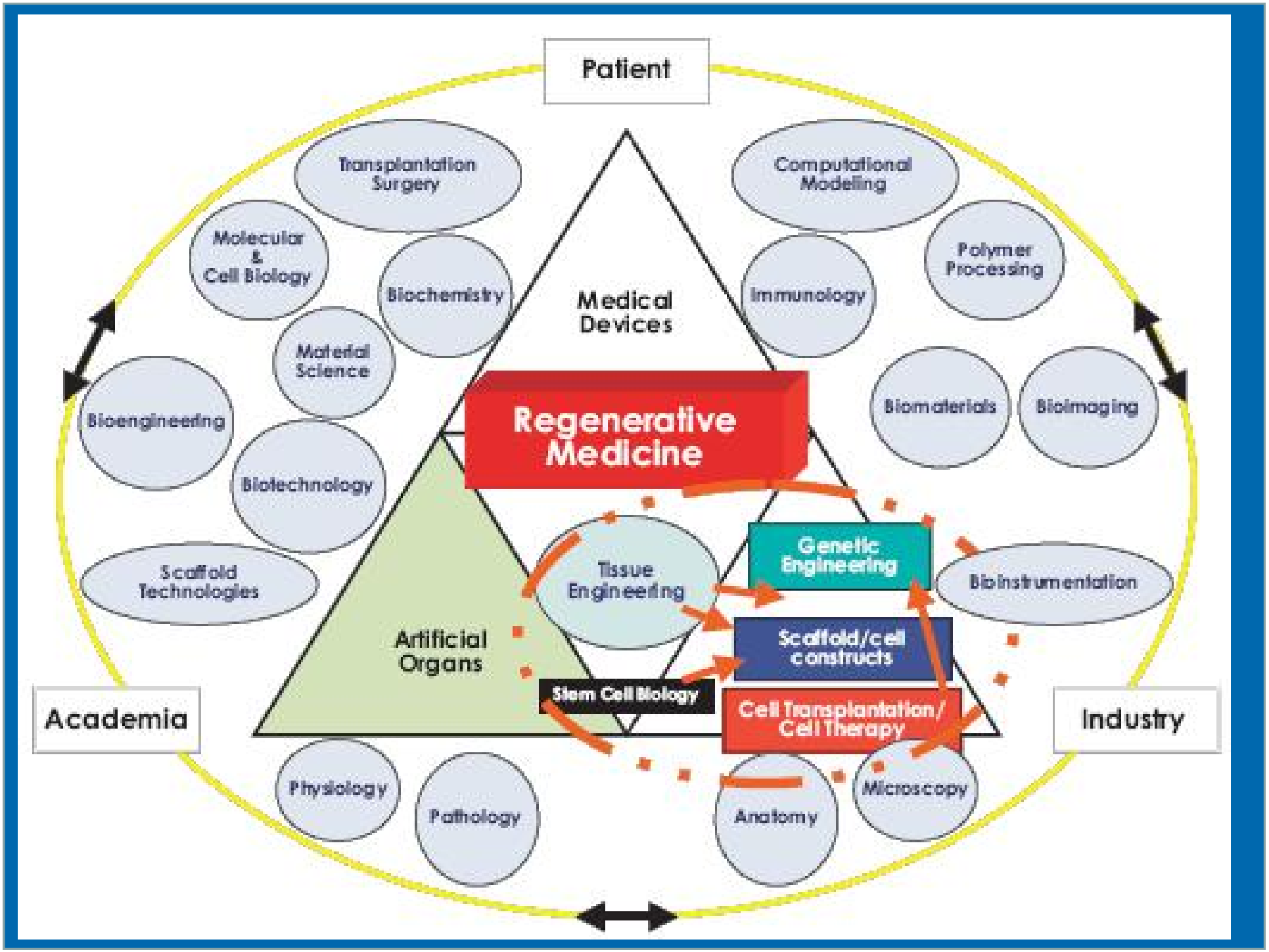


Nerve guide


Extrahepatic bile duct




Patient
Transplantation Surgery
Molecular & Cell Biology
Biochemistry
Material Science
Bioengineering
Biotechnology
Scaffold Technologies
Computational Modeling
Polymer Processing
Immunology
Biomaterials
Bioimaging
Medical Devices
Regenerative Medicine
Tissue Engineering
Genetic Engineering
Bioinstrumentation
Scaffold/cell constructs
Stem Cell Biology
Cell Transplantation / Cell Therapy
Artificial Organs
Academia
Industry
Physiology
Pathology
Anatomy
Microscopy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