

STEM CELLS AND TISSUES ENGINEERING

(TEUFL, STROBL)

KASPER 1: Biomaterials and scaffolding

Biomaterials overview:

- Natural
- Synthetic
- Composites
- Scaffolding
- Functionalization

→ **Preferred features:** Biocompatible, biodegradable, bioactive

Natural: Collagen (ECM), fibrin, silk, polysaccharides, decellularized tissue (bone, arteries)

Synthetic: Polymers, hydrogels, composites, ceramics (porosites, osteoinductive)

|| Consider: cell migration and cell adhesion

NATURAL BIOMATERIALS

→ Molecules / polymers of ECM (Proteins, Polysaccharides, Glycoproteins and Proteoglycans)

- Transplants are no real biomaterials
- But from biological materials scaffolds can be derived → semi natural / synthetic (decell. tissue)
- Through perfusion, immersion, physically (Pressure, temperature, supercritical CO₂), chemically (enzymes, pH, detergents, ...)

1. Collagen

- Most prominent protein in mammals (25%)
- More than 20 different collagens
- Triple helix structure
- Main component of mammalian ECM
- Flexibility and strength
- Responsible for tissue structure and stability
- Collagen Type I is the most important organic compound in bone

Matritypt: Pure collagen matrix

Matriderm: Collagen-elastin-matrix

2. Fibrin

- Activated glue of plasmatic blood clotting
- Precursor Fibrinogen, polymerized to fibrous network of insoluble protein (FXIII etc)

→ **Fibrin glue / sealant (physiological glue)**

- Fixing skin transplants
- Used since 1980s
- FDA approval 1998 (viral contaminations)

- Primary cells can be embedded into Fibrin gel, cultivated and reimplanted
- Also used as drug delivery system

3. Polysaccharides

- Widespread in nature
- Alginate, chitin, glycogen, hyaluronic acid, ...
- Polysaccharides do not provoke immune responses
- Mostly biodegradable

3.a Alginate

- Water soluble salts of alginic acid produced by brown algae and some bacteria
- Monomers of α -L-Guluronic acid and β -D-Mannuronic acid
- Heteropolymers build linear chains and homopolymeric regions build a sheet like structure
- Alginate can be transformed into hydrogels and used for cell entrapment

3.b Chitosan

Polyaminosaccharide which is produced by deacetylation of Chitin (enzymatic or chemical)

Chitin: Main component of exoskeleton of insects

3.c Hyaluronic acid

Hyaluronic acid is a water binding heteropolysaccharide

Stores up to 10.000fold of its own weight

Backbone of proteoglycans in ECM

Prominent in bone ECM

Used for cell embedding

Cartilage TE

3.d Silk

Silk from silk moth Bombyx mori

Furthermore silk can be generated from spiders (e.g. Nephila)

Silk from Bombyx mori has been used for surgical application for a long time (e.g. sutures)

Sericin causes immunological reactions/problems and must be completely eliminated

Spider silk does not contain Sericin!

Silk is used for e.g. tendon and nerve TE

SYNTHETIC BIOMATERIALS

→ Materials produced by different procedures / techniques but with similar features / characteristics as natural ones

- Polymers
- Composites
- Ceramics
- Hydrogels

BIOMATERIALS -SCAFFOLDING

For TE the biomaterials / scaffolds must be porous to ensure efficient cell seeding, nutrient supply / metabolic waste elimination, oxygen transport etc.

Most widely used methods for scaffolding:

- Electrospinning/extrusion
- Freeze drying
- Salt leaching / gas foaming
- Phase separation /solvent casting
- Sintering / PU foam template / compression moulding
- Gel-cast / sol-gel process (surfactant)
- Rapid prototyping / solid free form fabrication SFF
- Laser based techniques, ...

Electrospinning

- High voltage applied to polymer solution
- Loading of droplets
- Due to electrostatic repulsion droplets are expanded
- When electrostatic force is stronger than surface tension a beam is spouted out of the drop
- The „beam“ is accelerated through the electrical field toward the collector

Salt leaching

- Compounds (e.g. PLGA) are dissolved in organic solvent and mixed with salt
- Solvent is removed
- Result is solid matrix with incorporated salt crystals
- Polymer/salt mixture is transferred, salt will dissolve resulting in porous structure
- High porosity, limited interconnectivity

Gas foaming

- Gas foaming and salt leaching can be combined
- Salt is added before gas foaming process is initiated and dissolved from resulting structure → highly porous structures
- For production of highly branched porous structures
- Polymer is subjected to CO₂ atmosphere under high pressure (gas saturation of polymer)
- Pressure is decreased to ambient conditions → due to thermodynamic instability bound CO₂ is released from polymer → leaving / creating pores

COMPOSITES

Composite materials are engineered or naturally occurring materials made from two or more constituent materials with significantly different physical or chemical properties which remain separate and distinct within the finished structure

Mechanische Vermischung von Polymer und Keramik

- Simple method for production is combination of polymeric and ceramic / inorganic compound by mechanical mixing
- Nano hydroxy apatit / polymer composite with high porosity can be produced by thermally induced phase separation (TIPS)
- Solvent-cast technology
- Compression of hydroxy apatit / polymer mixture at high temperatures

Most important feature: Mechanical load at phase boundaries is passed through the materials through the combination of different compounds with different characteristics

- Specialized materials can be constructed with tailor-made features (not possible with single material!) → Ceramic provides mechanical stability, polymeric portion elasticity / compression / biomimetic features

Widely used polymers: PGA/PLLA (polyglycolic acid/poly-L-lactid acid), PCL (Polycaprolacton)

- Polymers are degradable

Widely used ceramics: Hydroxyapatite, Tricalciumphosphate

|| Bone is one example for a natural composite consisting of collagen (polymer) and hydroxyapatite (ceramic)

PEG Polyethylenglycol

- Polyethylenglycol (PEG)-based hydrogels
- PEG is liquid or solid depending on chain length, chemically inert / non-toxic polymer
- PEG-end groups can be functionalized to bind cells which then migrate into the gels
- PEG can thus serve as cell or drug delivery vehicle (controlled release)
- PEG hydrogels can also be loaded with organic compounds (e.g. proteins, proteoglycans, albumin)

Hydrogels

- Liquid or solid depending on chain length
- Chemically inert
- PEG-end groups can be functionalized
- Drug-delivery (stealth properties)
- Hydrogels loaded with organic compounds
- Immobilization by covalent binding: amino groups can bind to epoxy groups, which again serve as spacer

SURFACE MODIFICATION: ADSORPTION OR COV. BINDING

- For improvement of cell adhesion: Binding of e.g. peptides (RGD) or proteins (Poly-L-lysine)
- For stimulation of cell differentiation: Binding of growth factors
- ...

IMMOBILIZATION BY ADSORPTION

No chemical bond, only little conformational changes

Advantage: Bioactivity is preserved

Disadvantages:

- Adsorbate at surface only by Van-der-Waals forces, small changes in environment (temperature, pressure, solvent) can cause desorption
- Reduction of flexibility of molecules / biological activity through surface interaction / multi layer adsorption

IMMOBILIZATION BY COVALENT BONDING

- Covalent binding of molecules can be realized by reactive side groups of ligands
- Functional groups influence bioactivity
- **Important:** Reaction conditions must not have any influence of ligands (no modifications!)
- If bioactivity is decreased / lost, spacer molecules can be applied

|| Spacers are special molecules (e.g. polymers) to increase space to surface and support full flexibility of biomolecules

Two functional groups: One for binding to surface, other for biomolecule binding (affinity to ligand)

- Through spacer distance to surface is increased / defined and bioactive binding site is free for interaction with cells
- By using different spacers and ligands the bioactive characteristics can be controlled

|| Proteins bind by covalent bonds through amino group to epoxy group on the surface
Epoxy groups serves as spacer

- PLGA scaffold with amino groups can be synthesized by gas foaming or salt leaching
- HA is activated by treatment with EDC (1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide)
- Combination of materials by reaction of functional groups

RELEASE SYSTEMS

Microspheres:

Time of release and kinetics can be controlled by polymer selection / crosslinking etc.

Hydrogel embedding:

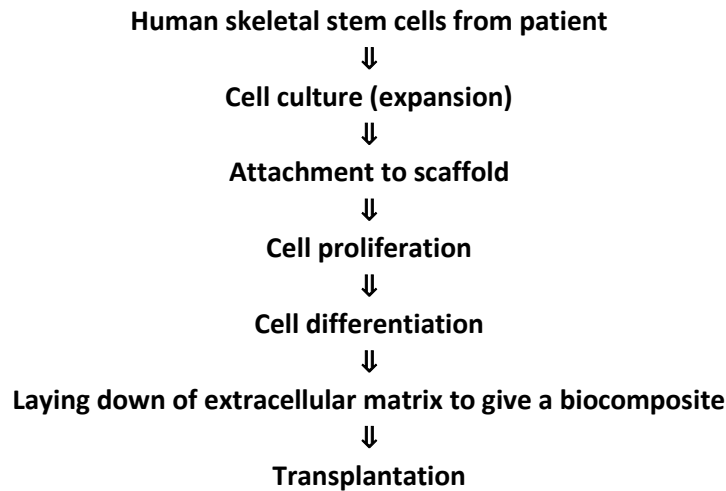
- Fibrinogen hydrogel beads
- Encapsulation MSC
- MSC encapsulated in hydrogel crosslinked via photoinitiation

“KASPER 2”: Scaffolds for Tissue Engineering, etc.

|| Need to shift from replacement of tissues to regeneration of tissues to their original state and function

An off-the-shelf or tailor made artificial alternative to transplants:

- An artificial implant that guides and stimulates in situ regeneration
- A tissue / construct grown in the lab



THE IDEAL TE SCAFFOLD

- Act as template for tissue growth in 3D
- Have an interconnected macroporous network for vascularisation, tissue ingrowth and nutrient delivery
- Bond to the host tissue without the formation of scar tissue
- Resorb at the same rate as the tissue is repaired
- Influence the genes in the cells of the tissue to enable efficient cell differentiation and proliferation
- Be easily and cheaply produced to ISO/FDA/CE standards (must be easily sterilised)
- Produce a construct with mechanical properties similar to the host tissue

CLASSIFICATION OF SCAFFOLD MATERIALS

Bioinert: No toxic response from the body on implantation

- Usually results in fibrous encapsulation (scar tissue formation)

Bioresorbable: Undergoes degradation in the body

- Dissolution products are harmless and can be secreted naturally

Bioactive: Produces a biological response from the body that results in a bond between the material and the host tissue

POLYMERS

Material made up of many units

Thermoset: Cross-linked polymer which is generally polymerised in its final shape

Thermoplastic: Not cross-linked, can be moulded to shape by extrusion type processes in the molten state

2 METHODS OF POLYMER SYNTHESIS

Addition Polymers: Produced by free radical addition reactions from unsaturated monomers, i.e. monomers containing C=C bonds, e.g. polyethylene and poly(methylmethacrylate)

Condensation Polymers: Formed by reacting two monomers together in a reaction in which a small molecule is eliminated. Condensation polymers may be hydrolysed in the body, e.g. polyamides and polyesters

POLYMER PROPERTIES

- Degree of polymerisation (N): The number of monomer units in the polymer chain (100-1000)
- Molar mass: The molecular weight of a polymer molecule
- Most polymers have a distribution of chain lengths, i.e. they are polydisperse

BIORESORBABLE POLYMERS

- A bioresorbable material is designed to degrade within the body after performing its function
- Useful materials often degrade to give normal metabolites of the body
- Examples include: Polylactide, polyglycolide, poly(-3-hydroxybutyrate), polyhyaluronic acid esters
- Biodegradable / hydrolysable polymers are the basis of many scaffolds for tissue engineering

DEGRADATION BY HYDROLYSIS

- Polymers, produced by a condensation route, especially polyesters, are prone to hydrolysis (a reaction with water involving the splitting of a bond and the addition of H^+ and OH^- from the water)
- The rate of hydrolysis is dependent on the water absorption and is often limited by the diffusion of water through the polymer
- Diffusion of water in polymers is often related to their solubility parameter, T_g , MW and degree of crystallinity

POLYGLYCOLIDE AND POLYLACTIDE

- Both polymers are polyesters and possess an ester group in the polymer backbone that can be hydrolysed
- The degradation products of these two polymers are glycolic acid and lactic acid respectively, both occur naturally in the body
- Degradation is pH dependent; esters are hydrolysed at a faster rate under acid and alkaline conditions; acid is produced on hydrolysis of an ester, so pH falls during hydrolysis, accelerating the degradation process

Polyglycolide

- High MW PGA is a hard tough crystalline, $T_m \sim 228^\circ C$, $T_g \sim 37^\circ C$
- The polymer can be spun to produce fibres when the molar mass is between 2×10^4 and 1.45×10^5
- The strength of PGA in the fibre direction is increased when spun into fibres, because of the preferred molecular orientation of the polymer chains \rightarrow sutures
- High MW PGA is made by condensation polymerisation

Poly lactide

- Replacing H by CH_3 leads to a more hydrophobic polyester and results in lower water uptake and lower hydrolysis rates
- The degree of crystallinity influences strength, fracture roughness and degradation behaviour, the crystalline regions do not take up much water and are much more resistant to degradation
- This can result in crystallites giving rise to particulate debris following degradation

DEGRADATION OF POLYLACTIDES

- Degradation is often autocatalytic
- Degradation of thick sections can occur faster than thin sections due to the build up of a localised low pH accompanying degradation within the section
- This can result in the rapid release of lactic acid and polylactide oligomers resulting in a toxic response
- This is overcome by using basic fillers which neutralise the acidic carboxyl groups produced on hydrolysis
- Applications for polylactides include bone plugs, screws and fracture fixation plates
- Applications are limited as a result of rapid reduction in strength in vivo

PROCESSING POLYMERS → POROUS SCAFFOLDS

Phase separation:

- Low pore diameter, difficult to control pore size

Fibre bonding:

- Lack of mechanical strength of bonds

Porogen leaching / salt leaching:

- Closed pores
- Freeze drying
- High-pressure CO₂
- Rapid prototyping / solid freeform fabrication

RAPID PROTOTYPING / SOLID FREEFORM FABRICATION

- Ink-jet printing
 - Stereolithography
 - Solid freeform fabrication
 - Selective laser sintering
-
- ✓ Pore network defined by CAD file
 - ✓ Pore network can be tailored to the CT scan of a patient's defect
 - ✓ A pore size gradient can be obtained
-
- X Mechanical properties poor?
 - X Not all materials can be used in the techniques yet
 - X Expensive equipment

HYDROGELS AS BIOMATERIALS FOR TE

- Hydrogels are insoluble water-swollen networks
- They encapsulate cells but have such high water content that nutrients reach the cells by diffusion

Advantages	Disadvantages
Wide range of chemistries available Typically biocompatible High water content Potentially injectable for minimally invasive implantation	Poor mechanical properties Difficult to sterilise

It is important to note that most cells do not exist in crystalline matrices or free solution, but in hydrogels

Mechanical properties of Hydrogels

- Hydrogels are typically weak compared to other polymers due to the high water content
- Mechanical properties altered by cross-linking density of the polymer, polymerisation conditions during network formation and hydrogel swelling
- Tensile testing and dynamic mechanical analysis (in liquid) measure the rubber elastic and viscoelastic behaviors, respectively

Degradation properties of Hydrogels

- Mechanisms include hydrolysis, enzymatic cleavage and dissolution
- Degradation controls properties such as hydrogel mesh size which is important in the release of entrapped molecules and the diffusion of extracellular matrix components produced by encapsulated cells

Natural Polymers	Synthetic Polymers
Fibrin	Poly(ethylene glycol)
Collagen and Gelatin	Poly(acrylic acid)
Hyaluronic Acid	Poly(vinyl alcohol)
Alginate	Polypeptides
Agarose	Polyphosphazene
Chitosan	Poly(hydroxyethyl methacrylate)
Dextran	Poly(NIPAAm)
Chondroitin sulfate	and many combinations of the above
Poly(L-lysine)	

COLLAGENS AS BIOMATERIALS FOR TE

- Collagen can be processed to create conventional porous scaffolds or as a hydrogel to encapsulate cells
- Cross-linked with various techniques (e.g. glutaraldehyde, carbodiimide, photooxidation) to improve the physical properties (mechanics and degradation)
- Controllable resorbability in vivo
- Sterilisation by gamma irradiation, ethylene oxide treatment, or electron beam irradiation

HYALURONIC ACID AS BIOMATERIAL FOR TE

- The most common naturally occurring hydrogel is based on hyaluronic acid
- Commercially available
- Being a polysaccharide its structure is not determined by the genetic code and it does not elicit an immune response
- Its molar mass and molar mass distribution determine its mechanical properties
- Being a naturally occurring polysaccharide, hyaluronic acid is completely biodegradable in vivo: suitable for controlled drug delivery and implanting living cells in a hydrogel matrix

ALGINATE AS BIOMATERIAL FOR TE

- A natural polysaccharide (from seaweed) composed of α-Dmannuronic acid and β-L-guluronic acid
- Ionic cross-linking occurs in the presence of various divalent cations (e.g., Ca²⁺, Mg²⁺) by cross-linking the carboxylate groups of the guluronate groups on the polymer backbone

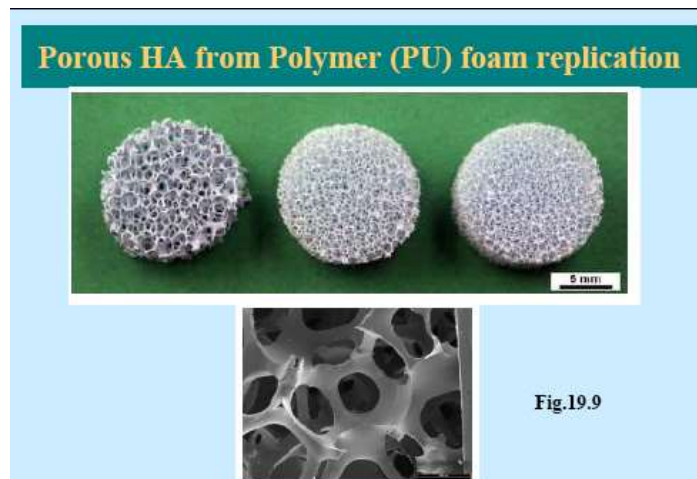
CERAMICS

CERAMICS PROCESSING

- Not produced from the molten state
- Usually produced via a powder (sintering route)
- Can be also produced by various deposition techniques (e. g. CVD, EPD, etc.) and by colloidal and sol-gel processing
- Powder technology and sintering

SYNTHETIC HA

- Made by continuous precipitation from solution using calcium hydroxide as a precursor
- Osteoconductive class B bioactive material
- Forms a bond to bone
- Not resorbable



Advantages of polymer foam replication:

- ✓ Open cell structures can be made in relatively simple process
- ✓ Supporting structures (e.g. glass fibres) can be introduced to the foam

Disadvantages of polymer foam replication:

- X 2-step processing technique (time consuming)
- X After polymer is burnt out, the ceramics struts are hollow, therefore mechanical properties are low

GEL CASTING

- Powder slurry + binder + cross-linking agent
- Foaming with aid of surfactant
- Sintering and burn out of organics

BIOACTIVE GLASSES

- Consist of a silica (O-Si-O) network structure (+Ca, P, Na, etc. network modifiers)
- Form an HCA layer on contact with body fluid
- Class A bioactive materials
- Osteopductive
- Bond to soft tissue and hard tissue
- Stimulate genes in osteoblasts (Xynos 2001)
- Brittle in tension
- 2 types

1 Melt-derived bioactive glasses

- Oxide compositional components melted & mixed in a furnace
- Usually quaternary e.g. Bioglass® (46.1% SiO₂, 24.4% Na₂O, 26.9% CaO and 2.6% P₂O₅, in mol)
- Fully dense, i.e. no pores
- Can cast to any shape / powder size
- Difficult to produce porous scaffolds
- Commercially available as Perioglas® and Novabone® (USBiomaterials)

2 Sol-gel derived bioactive glasses

- Alkoxide components mixed in solution in fume hood (sol)
- Polymer reactions form silica network (gel)
- Various compositions
- Mesoporous texture
- Enhanced bioactivity and resorbability
- Silver can be added for wound healing
- Just got EU approval (CE mark)

COMPOSITES

|| Aim is to combine the stiffness of a ceramic (+ bioactivity?) with the toughness (+ resorbability?) of a polymer to tailor the properties of a scaffold to that of the host tissue

Bone is a composite:

- Hydroxyapatite crystals on collagen fibrils
- Gives combination of toughness of collagen (polymer) and compressive strength of a HA (ceramic)

HAPEX

- HA + HDPE [Bonfield 1981]
- 40% HA in HDPE matrix
- Made by blending and extrusion at 200°C
- Not bioresorbable
- Licensed to Smith and Nephew

TO OBTAIN A BIOCOMPOSITE FOR IMPLANTATION?

- Optimisation of scaffold from atomic to macro scale with respect to cell response
- Delivery of the critical concentration of ions / signals for correct genetic stimulation
- Optimisation of culture conditions in vitro
 - Type and quantity of media
 - Growth factors
 - Perfusion systems: Bioreactor

SUMMARY

- There are many criteria for an ideal scaffold
- It is important to mimic the structure of the tissue as closely as possible when designing a tissue engineering scaffold
- It is important to select materials specific to the application
- An ideal scaffold material should be tailorable to the exact needs of individual patients
- Cells will be affected by material composition, curvature, surface chemistry and surface roughness
- Culture conditions must also be optimised

KASPER 3: Growth factors in bone TE

L-ASCORBIC ACID

Function: Radical quencher, reducing agent, antioxidant and cofactor

Mechanism: Serves as a cofactor in the hydroxylation of proline and lysine residues during the biosynthesis of collagens. Recent studies show mitogenic effects by upregulation of different genes. In cell culture the derivative L-ascorbic acid 2-phosphate is used because L-ascorbic acid is unstable under standard conditions.

DEXAMETHASONE

Type: Artificial glucocorticoid

Function: Anti-inflammatory and immunosuppressant effects which are 25 times higher than naturally occurring corticoids like cortisol. It is widely used in medical treatment of different diseases.

Mechanism: Although the exact mechanism of dexamethasone remains unclear it is supposed to induce transcriptional effects. It is still an essential requirement for in vitro osteogenic differentiation.

FGF2 (FIBROBLAST GROWTH FACTORS)

Size: 60 – 288 AA, 7 – 38 kDa (FGF2 155 AA, 18 kDa)

Family: FGF-family

Types: FGF 1- 15, 16 - 23

Function: Regulation of cell growth and differentiation, embryonic development, wound healing, angiogenesis and regeneration of nerves and cartilage.

Mechanism: FGFs are binding on four different FGF-receptors (FGFR) causing intracellular signal cascades. FGF2 especially has mitogenic effects causing proliferation, migration and differentiation and most cause angiogenesis. FGFs are highly secreted in damaged tissue and under hypoxic conditions.

IGF (INSULIN-LIKE GROWTH FACTOR)

Size: IGF-I 70 AA, ~ 7.6 kDa, IGF-II 67 AA, ~ 7.4 kDa

Family: IGF

Isoforms: IGF-I, IGF-II

Function: IGF-II plays an important role in fetal development and childhood growth whereas IGF-I causes anabolic effects in adults.

Mechanism: Synthesis of collagen I and inhibition of expression of collagenases and apoptotic process via different pathways where PKB pathway is most potent.

TGF- β (TRANSFORMING GROWTH FACTOR BETA)

Size: Homodimer of 390 AA, ~ 25 kDa

Family: TGF- β -superfamily

Isoforms: TGF- β 1, 2, 3

Function: Control of cell proliferation, differentiation, growth, apoptosis and other functions.

Mechanism: Induces the transcription of Cbfa1/Osf2 (Runx2). The resulting transcription factor Runx2 regulates lots of genes related to osteogenic differentiation.

BMP (BONE MORPHOGENETIC PROTEIN)

Size: Homo and heterodimers between ~400-525 AA, ~30-38 kDa

Family: TGF- β -superfamily

Types: BMP 1-15 (not all of them osteogenic)

Function: Development of heart, central nervous system, cartilage (during embryonic development) and post-natal bone development.

Mechanism: Mobilization of the SMAD family proteins which regulate lots of intracellular functions.

BMP 2, 4, 7

- BMPs are divided into three subclasses with regards to size and structure
- BMPs with highest osteoinductive potential are BMP 2, 4 and 7
- BMP 5, 6, 8 and 3 also show lower osteoinductive effects

BMP	Known functions
BMP2	Acts as a disulfide-linked homodimer and induces bone and cartilage formation. It is a candidate as a retinoid mediator. Plays a key role in osteoblast differentiation.
BMP4	formation of teeth, limbs and bone from mesoderm. It also plays a role in fracture repair, epidermis formation
BMP7	Plays a key role in osteoblast differentiation. It also induces the production of SMAD1. Also key in renal development and repair.
BMP3	Induces bone formation.
BMP5	Performs functions in cartilage development.
BMP6	Plays a role in joint integrity in adults. Controls iron homeostasis via regulation of hepcidin.
BMP8	Involved in bone and cartilage development.

VEGF vascular endothelial growth factor

Size: VEGF-A: 121 - 206 AA, ~ 34 - 42 kDa

Family: Cysteine knot growth factor family

Function: Key signal protein stimulating vasculogenesis and angiogenesis in embryonic development, after injury and in forming collateral circulation. Inducing mitosis and migration of endothelial cells.

Mechanism: Binding of VEGF to different VEGF-receptors (VEGFR) leads to intracellular signaling through different pathways.

KASPER 4: Regenerative Medicine

- Biopharmaceutical products (proteins, antibodies etc.)
- Cell therapy
- Gene therapy
- Cloning
- Tissue Engineering

Model animal: Axolotl (amphibian), Wikipedia:

The feature of the salamander that attracts most attention is its healing ability: the axolotl does not heal by scarring and is capable of the regeneration of entire lost appendages in a period of months, and, in certain cases, more vital structures. Some have indeed been found restoring the less vital parts of their brains. They can also readily accept transplants from other individuals, including eyes and parts of the brain—restoring these alien organs to full functionality. In some cases, axolotls have been known to repair a damaged limb, as well as regenerating an additional one, ending up with an extra appendage that makes them attractive to pet owners as a novelty. In metamorphosed individuals, however, the ability to regenerate is greatly diminished. The axolotl is therefore used as a model for the development of limbs in vertebrates.

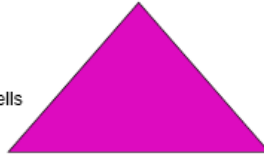
Historical aspects

- Until 1515 dissectioning of dead bodies was forbidden!
- Around 1600 the microscope was invented and A. van Leeuwenhoek developed methods for blood, sperm and egg cell analysis
- As early as 17th century the first attempts were made for replacement of human skin by animal derived skin
- End of 18th century early experiments for keeping organs outside the body vital / alive were performed
- Around 1840 scientists discovered that human body consists of single cells - about 200 different cell types
- In 19th century medical / technical equipment was developed such as stethoscope, anesthesia, laughing gas, morphine...
- 1900 K. Landsteiner (Vienna) discovered blood group system A, B, O: 1930 receives Nobel prize for his work
- 1902 first technically successful kidney transplantation in dog (H. Ullmann, Vienna)
- 1950 first successful human kidney transplantation in human. After a few months organ was destroyed by the immune system / rejection
- 1967 Chr. Barnard (Capetown) performs first heart transplantation; patient dies after 18 days of infection
- 1970 Discovery of Cyclosporin. Substance was first used 1978 after transplantation for immunosuppression (since then several "followers")
- Many produced by biotechnological production processes!

Today: Transplantation of bone marrow, pancreas, kidney, heart, liver, lung, arms / hands / legs, face, uterus, ...

Tissue Engineering
human (autologous/allogeneic)
cells, biomaterials, bioreactors,
bioprocess engineering,
biomechanical stimulation, cell
culture media and supplements ...

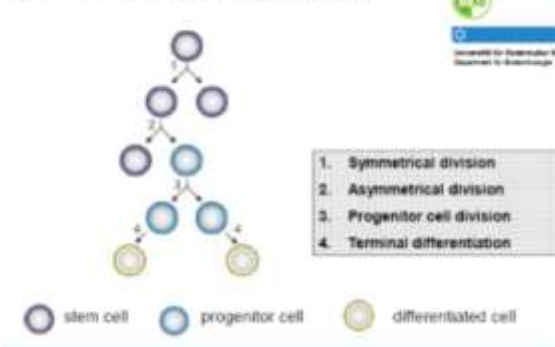
Cell therapy
tissue derived cells, stem cells
(embryonic / adult),
expansion, differentiation,
aging...



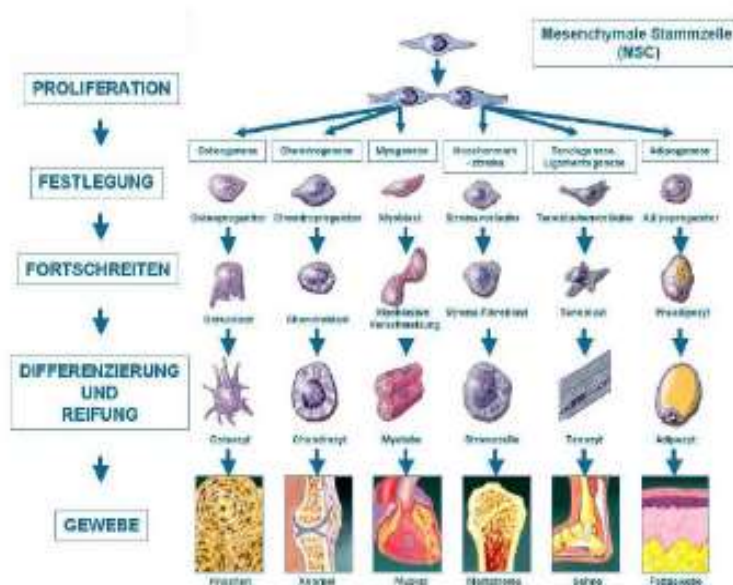
Gene therapy
vector, genome, DNA/RNA, plasmid,
gene transfer, transfection/
transduction, viral/ non-viral...

Ethics, regulatory/legal/economical issues ...

Stem cell division and differentiation



Stem Cell Differentiation



Frischzellkur?

- In 1950th and 60th reports came out on “rejuvenation treatments” that were applied by using fetal or juvenile cells of animal origin (Xenotransplantation)
- First treatment reported 1931 by Swiss surgeon Paul Niehans (1882-1971)
- Patients are supposed to have been Pope Pius XII (1876-1958) and Pablo Picasso
- 1997 it was prohibited; 2000 main court decided that it is within the responsibility of Federal States (Bundesländer) – Situation in Austria?

CELL THERAPY

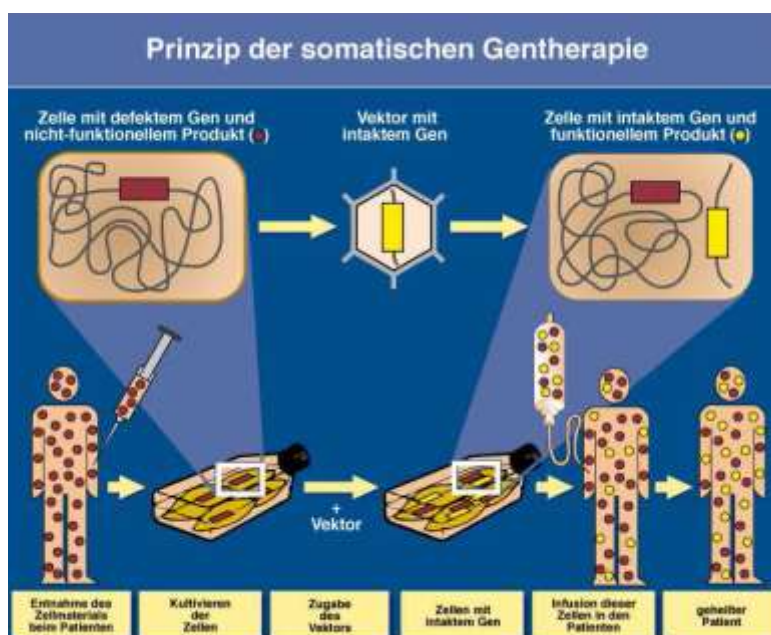
Nowadays, most products derived from placenta of sheep, canine, bovine, pigs

- Living cells
 - Lyophilisate
 - Ultrafiltrate of different organs and tissues
 - Homeopathic formulations
 - Cosmetics etc.
-
- Transplantation of stem cells from bone marrow or blood for leukemia treatment after chemotherapy (HSCT - hematopoietic stem cell therapy)
 - **ACT / MACI: Autologous Chondrocyte Transplantation**
 - Treatment of cartilage defects (e.g. meniskus, disks)
 - Advantage: No species difference, risk of transmission of zoonosis is minimized (infectious diseases transmitted from animal to human)
 - No rejection

Application of stem cells in CT:

Information through international Societies (e.g. ISCT, ISSCR) and / or <http://clinicaltrials.gov>

Fields of therapeutic interest: Infarction, joint / cartilage / bone defects, multiple sclerosis, diabetes, eye diseases, autoimmune diseases, Parkinson's, ...



CLONING

- II A clone is a genetically identical living organism from the DNA of the original organism.
- II Clones have the same genes, but this does not mean they are phenotypically identical!

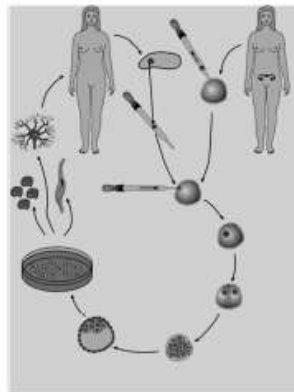
HWANG CASE

- 2004/2005 Korean scientists at Seoul National University (W.S. Hwang) report about success in the cloning of pigs and dogs. Furthermore Hwangs team yields stem cells from human embryos and report on successful generation of stem cell lines (Science express 2004 und Science 2005)
- 2006: confirmation that his data were invented (fraud)!

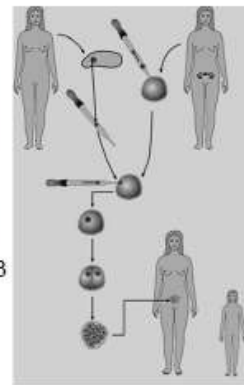
Therapeutic vs. Reproductive Cloning



Universität für Bodenkultur Wien
Department für Biotechnologie



"Dolly" 1996 – 2003



KASPER 5: Bioreactors for Tissue Engineering

BIOREACTOR BASICS

Static Cultivation

- Cultivation of adherent cells on plastic surfaces
- Petridishes, multiplates, flasks and „cell factory“
- Inhomogenous gas and nutrient supply
- No removal of toxic metabolites
- Extended cultivation periods difficult

e.g. Roux Bottle, Spinner Bottle (hohlfasermembran um den Rührer für erhöhten Sauerstoffeintrag)

Cell Types

Adherent cells

- Anchorage dependent
- Flat, elongated
- Growth on surfaces in a monolayer

Suspension cells

- Non anchorage dependent
- Spherical
- Growth in suspension

Bioreactor systems

Uniformity



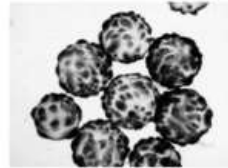
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- **Homogeneous bioreactor systems**

→ Suspension cells + culture medium

- **Heterogeneous bioreactor systems**

→ Adherent cells + carrier + culture medium



e.g. Microcarrier
(100-300 μm diameter;
glass/dextran/polystyrene)

Bioreactor Systems



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Classical stirred tank reactor



Rotating bed
bioreactor



Wave reactor

BIOREACTOR DESIGN

Important aspects:

Bioreactors for different purposes:

- Cell proliferation / expansion

- Generation of 3D tissue constructs / cell differentiation

- Direct organ support devices / extracorporeal devices, storage

- Control of environmental conditions (oxygen tension, pH, temperature, shear stress etc.)

- Aseptic operation (feeding, sampling)

- Automated processing steps

Critical Issues:

- Mass transfer problems (e.g. oxygen and nutrient supply, removal of toxic metabolites)

- Size of engineered constructs is limited, as they are only supplied by diffusion (cell layers 100-200 μm)

CULTIVATION MODI

- Batch
- Fed batch (toxic metabolites stay inside, reactor volume limits)
- Perfusion (optimal)
- Continuous

Perfusion system

- Always optimal conditions
- Building of reservoirs in the body
- Translation to cell culture conditions: from the present nutrients only the necessary amount is consumed → waste of valuable components!

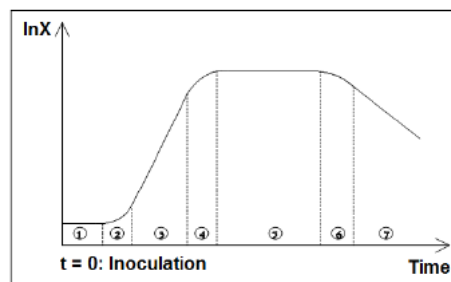
Fed Batch system

- Continuous supply of nutrients is ensured
- Low technical complexity
- Simple process control
- Toxic metabolites accumulate in the system
- Reactor volume limits cultivation time



Cell growth under real conditions

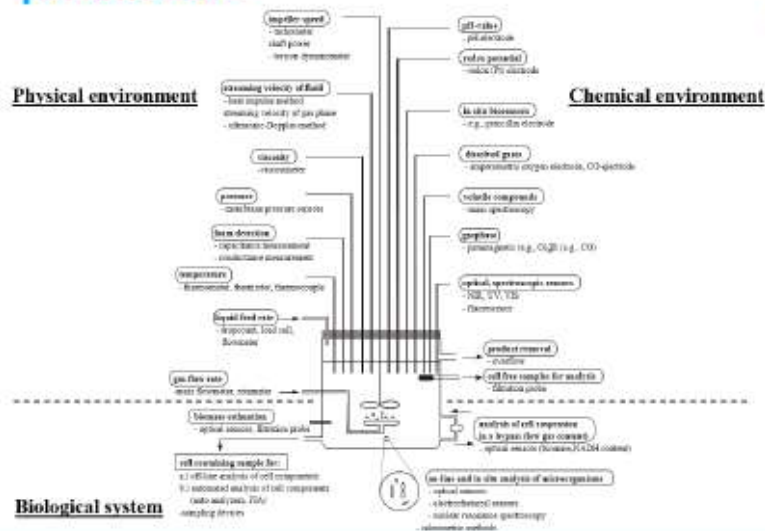
- Batch reactor (STR)
- Simple organism
- Substrate limitation



Physical, chemical and biological parameters



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- Oxygen supply
- CO₂ - concentration
- pH-value
- Temperature
- Osmolarity
- Cell concentration / -viability
- Substrate concentration (glucose, glutamine)
- Waste products (lactate, ammonium)

1) AERATION

a Surface aeration: Widely used for laboratory scale applications

Limitations

- Oxygen transfer rate is related to the liquid surface area (gas-liquid interface)
- Increasing culture scale (maintaining constant geometric properties)
- **Volume increase with the third power, whereas the surface area increases with its square**

Recent option: Wave bioreactor (generation of waves increases oxygen transfer by augmenting interfacial area and the transfer coefficient kLa)

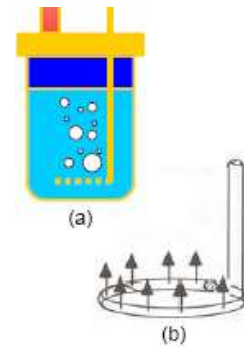
b Aeration through a membrane device or gas-permeable tubing

- Membrane device
- Gas-permeable-tubing + surface aeration
- Widely used for laboratory scale applications
- Bubble free aeration
- **Limitations:** see 1) surface aeration



c Bubble aeration

- Widely used in animal cell culture
- The sparger is generally positioned below the impeller, to promote a homogeneous distribution of bubbles inside the culture vessel



Problems in bubble aerated cultures:

- Animal cells have a low resistance to mechanical stress
 - Shear stress and cell damage due to bubble bursting and foam formation
- Addition of surfactants like Pluronic® F68 to decrease cell adhesion on ascending bubbles!

2) CELL CULTURE MEDIA

Supply of cells with sufficient nutrients in order to sustain cell metabolism (adaption of medium composition dependent on the cell type) *

Media contain:

- Nutrients for important cell functions and for the metabolism
- Amino acids for protein synthesis
- Vitamins and trace elements for growth and differentiation
- Salt etc. for example for cell anchorage (Mg^{2+} , Ca^{2+})

Cell Culture Media (2)

RPMI-1640 Medium

Dulbecco's Modified Eagle's Medium

Inorganic Salts (g/liter)

CaCl ₂ (anhydrous)	0.20000
Fe(NO ₃) ₃ ·9H ₂ O	0.00010
MgSO ₄ (anhydrous)	0.09770
KCl	0.40000
NaHCO ₃	1.50000
NaCl	6.40000
NaH ₂ PO ₄ ·H ₂ O	0.12500

Amino Acids (g/liter)

L-Arginine-HCl	0.08400
L-Cystine-2HCl	0.05260
L-Glutamine	0.58400
Glycine	0.03000
L-Histidine-HCl·H ₂ O	0.04200
L-Isoleucine	0.10500
L-Leucine	0.10500
L-Lysine-HCl	0.14500
L-Methionine	0.03000
L-Phenylalanine	0.06500
L-Serine	0.04200
L-Threonine	0.09500
L-Tryptophan	0.01600
L-Tyrosine-2Na·2H ₂ O	0.10379
L-Valine	0.09400

Vitamins (g/liter)

Choline Chloride	0.00400
Folic Acid	0.00400
myo-Inositol	0.00720
Nicotinamide	0.00400
D-Pantothenic Acid (hemicalcium)	0.00400
Pyridoxine-HCl	0.00400
Riboflavin	0.00040
Thiamine-HCl	0.00400

Other (g/liter)

D-Glucose	4.50000
Phenol Red, Sodium Salt	0.01500
Sodium Pyruvate	0.11000

Inorganic Salts (g/liter)

Ca(NO ₃) ₂ ·4H ₂ O	0.10000
MgSO ₄ (anhydrous)	0.04884
KCl	0.40000
NaHCO ₃	1.50000
NaCl	6.00000
Na ₂ HPO ₄ (anhydrous)	0.80000

Amino Acids (g/liter)

L-Arginine (free base)	0.20000
L-Asparagine-H ₂ O	0.05682
L-Aspartic Acid	0.02000
L-Cystine-2HCl	0.06520
L-Glutamic Acid	0.02000
L-Glutamine	0.30000
Glycine	0.01000
L-Histidine (free base)	0.01500
Hydroxy-L-Proline	0.02000
L-Isoleucine	0.05000
L-Leucine	0.05000
L-Lysine-HCl	0.04000
L-Methionine	0.01500
L-Phenylalanine	0.01500
L-Proline	0.02000
L-Serine	0.03000
L-Threonine	0.02000
L-Tryptophan	0.00500
L-Tyrosine-2Na·2H ₂ O	0.02883
L-Valine	0.02000

Vitamins (g/liter)

D-Biotin	0.00020
Choline Chloride	0.00300
Folic Acid	0.00100
myo-Inositol	0.03500
Nicotinamide	0.00100
p-Amino Benzoic Acid	0.00100
D-Pantothenic Acid (hemicalcium)	0.00025
Pyridoxine-HCl	0.00100
Riboflavin	0.00020
Thiamine-HCl	0.00100
Vitamin B-12	0.000008

Other (g/liter)

D-Glucose	4.50000
Glutathione (reduced)	0.00100
HEPES	2.38300
Phenol Red, Sodium Salt	0.00500
Sodium Pyruvate	0.11000

for adherent cells

for suspension cells



for adherent cells



for suspension cells

Media Additives

Glutamine (essential amino acid) **glucose** (glycolysis) → Main energy suppliers

Proteins

- Albumin: Transport of lipids, minerals and globulins
- Transferrin: Iron binding (transport proteins)
- Insulin: Supports the uptake of glucose and amino acids
- Fibronectin: glyco protein, for cell anchorage
- Anti-foam: Pluronic F 68, antifoam C, etc.

* **Penicillin / Streptomycin / Neomycin:** Antibiotics against bacteria

* **Amphotericin:** Fungicide against fungi

Serum: Growth factors, hormones, adhesion molecules, proteins, etc.

Examples: Fetal calf serum (FCS), new born calf serum (NCS), horse- and human serum etc.

Problems: Potential of infection, variability of the serum content

Serum substitutes:

- Serum free/protein free
- Natural origin (e.g. soya, pea, grain)
- Synthetic (e.g. Syn Q)

Advantages of chemically-defined media:

- Working under defined and controlled conditions
- Avoiding contaminations
- Avoiding biological variations

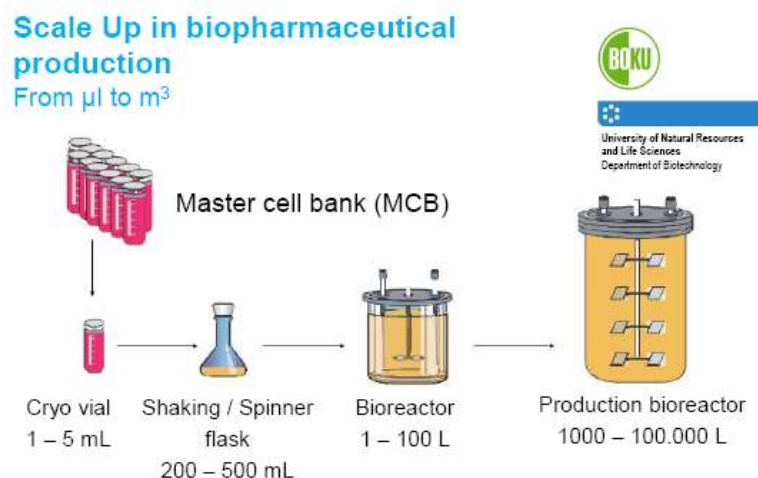
* Anything that does not occur naturally in the human body should not be part of a medium!

* Medium for adherent cells (DMEM) e.g. contains 4.5 g/L sugar, which would resemble a highly diabetic person! Cannot be used for TE!

* Many companies do not specify the media content → Problem for research!

3) SCALE UP IN TISSUE ENGINEERING

... does not work as in biopharmaceutical production!



BIOREACTORS FOR TISSUE ENGINEERING

Bioreactors for Cell Expansion

Cell Factory

- Tissue cell culture plastic
- Disposable
- 40 layer, area 25.000 cm²
- Surface modification possible
- Gas flow system
- No process monitoring or regulation



UniVessel® SU Satorius Stedim

- Disposable Polycarbonate
- 2 L volume
- Easy to handle, less cost intensive
- Axial stirring
- Stirring speed max 212 rpm
- Septum for sampling and seeding
- Disposable patches for CO₂ and O₂ measurement
- Cultivation of adherent cells on microcarriers



Mobius CellReady

- Disposable
- 3 L volume
- Axial stirring
- Additional septum for sampling
- Easy harvesting
- Cultivation of adherent cells on microcarriers



Bag Bioreactors

BIOSTAT® CultiBag RM/ Wave® Bioreactor

- Disposable bag
- Waved induced medium mixing
- Continuous gas exchange in medium
- Elasticity of bag due to pharmaceutically approved ethyl vinyl acetat (EVA) surface modification
- 1-600 L / 1-125 L
- Possibility of disposable pH and pO₂ sensors
- Heating plate
- Batch, fed-batch or perfusion mode
- Control tower for monitoring and regulation
- Microcarrier for cultivation of adherent cells



Synthecon Rotary Cell Culture System

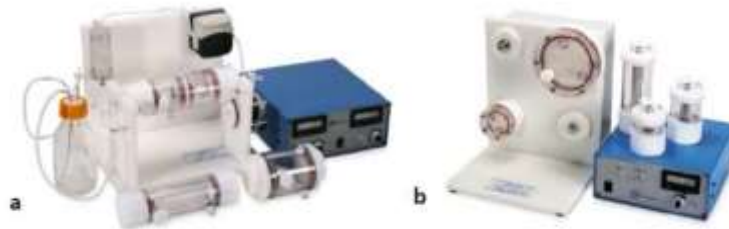
Rotary Cell Culture System™

Synthecon Incorporated (Houston, USA)

a) Modell for perfusion modus: Reusable Perfused Culture System

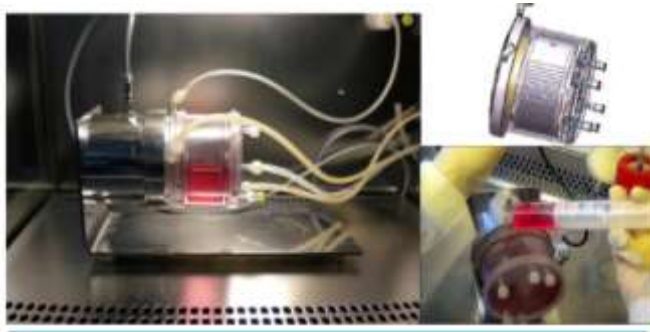
b) Modell for batch modus: Reusable Batch Culture System

Cells are in „zero gravity“, initiated by NASA, whole reactor rotates



Zellwerk ZRP® GMP Breeder

Only the inner parts rotate



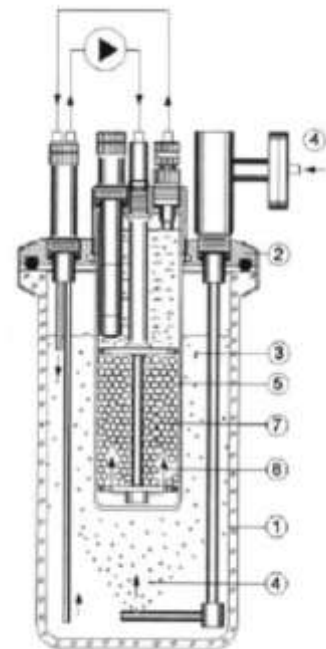
Medorex Fixed Bed Bioreactor

Architecture:

- Medium conditioning (1)
- Bioreactor lid (2)
- Medium (3) pumped in fixed bed by peristaltic pump
- Gas supply (4)
- Fixed bed (5)
- Macroporous carrier (7)
- Medium supply (8)

- ✓ Regulation of flow for shear stress regulation
- ✓ Online monitoring possible
- ✓ Variable set up

Problem: If cells settle down or differentiate it's hard to get them off the bed



Quantum Cell Expansion System

- Functionally closed, automated culture system
- Disposable hollow fiber bioreactor
- Ex-vivo expansion of pre-cultured cells
- After 5 days $3,36 \times 10^8$ MSC (seeding 2×10^7)
- Clinical scale, FDA approved as medical device



BioBLU 5c Single-use Vessels

- Improvement over the standard shaker flasks
- Single-use vessels (up to 40 L) for cell expansion
- Medium monitored and controlled throughout the expansion process (e.g. oxygen, pH, temperature, glucose)

Example: MSCs cultured on collagen microcarriers (working volume 3.75 L):

- Initial cell density: $\sim 30,000$ cells/mL
- After 16 days: ~ 0.4 million cells/mL with a total cell number of ~ 1.6 billion cells

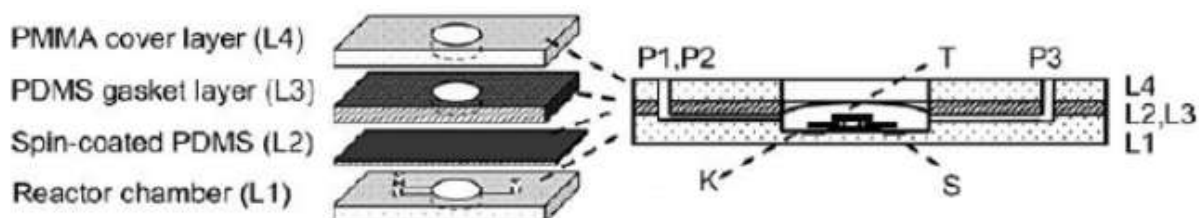


Perfusion Micro-Bioreactor-Assay

- Perfusion Micro-Bioreactor-Array (Lee et al.) for cell interaction analysis
 - a) Array-slide
 - b) Cultivation chamber
 - c) Mathematically simulated flow profile in a cultivation chamber
- Flow channel and cultivation chamber (4 cm^2)
- Gas supply
- Highest flow in entrance /exit of chamber, in chamber lowest flow

Membrane Micro-Bioreactors (diagnostics rather than production)

- Membrane-based micro-bioreactor (Zang et al.) for batch modus consisting of:
 - Cultivation chamber (T) with integrated stirrer (K) and optical Sensors (S)
 - Channels for medium / water (P1), base (P2) or acid (P3)
- Membrane-based static micro-bioreactor (Chowdhury et al.) consisting of:
 - Two medium chambers, separated by porous cell-seeded membrane



Commercial bioreactor systems

- Bose Electroforce
- Ebers Medical Technology
- Tissue Growth Technologies TGT

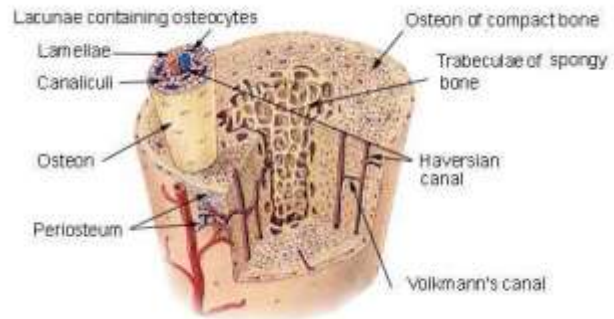
BIOREACTORS FOR TISSUE ENGINEERING

Bioreactors for 3D Tissue Constructs

BONE TISSUE ENGINEERING

- Complex tissue
- Special requires on scaffold material
- Perfusion
- Mechanical stimulation
- Growth factors
- Long cultivation periods (up to 4 weeks)
- **Biaxial Rotating (BXR) Bioreactor**

Compact Bone & Spongy (Cancellous Bone)



HEART VALVE TISSUE ENGINEERING

- Heart failure: Infarct, failure of valves
- Transplantation approaches
 - Synthetic heart valves
 - Xenogenic heart valves → Host versus graft!
- For pediatric application special implants required
- Cardiovascular failure due to weight loss / gain, pregnancy, training etc., change in mechanical environment
- Pulsatile flow, transvascular pressure and fluid flow
- High cell viability
- Clinical relevant construct size

CARDIOVASCULAR TISSUE ENGINEERING

Perfusion Bioreactor with directed oxygen gradient (Moore et al.)
 Pulsed flow-stretch bioreactor (Syedain et al.)

- Pulsatile electric field stimuli applied to cardiomyocytes
- Medium monitored and maintained at 37°C and 5 % CO₂
- Samples are sealed off from the environment with a teflon cone
- 3-D constructs placed in wells between electrodes
- Pressure has to be considered (bursting veins!)

EXTRACORPORAL SYSTEM

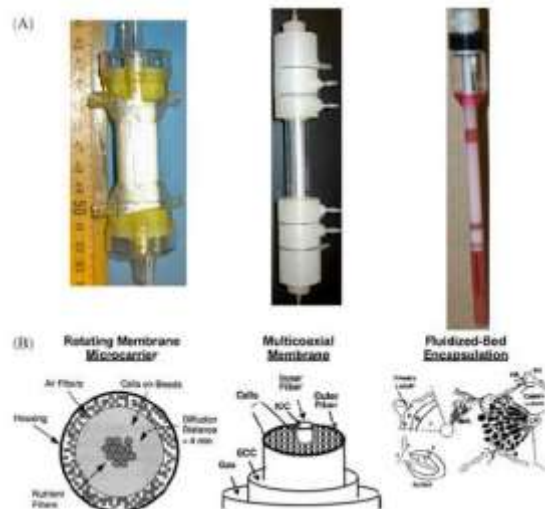
Bovine, sheep or porcine hepatocytes are cultured in the membrane chamber and can support patient by detoxification

MARS Molecular Adsorbent Recirculating System - filtration system for detoxification



LIVER TISSUE ENGINEERING

- Various bioreactor parameters for hepatocyte viability
- Diffusion distance, shear force, channelling, gelling or aggregation
- Three bioreactor-types
 - 1) Hollow fiber membrane bioreactor inoculated with microcarriers
 - 2) Membrane multicoaxial hollow fiber bioreactor
 - 3) Fluidized bioreactor consisting of alginate entrapped hepatocytes



LifePort® Kidney Transporter

- Organ Recovery Device
- Delayed graft function (DGF) and failure within the first year significantly post-transplant
- The use of LifePort in kidney transplantation is cost-effective compared with cold static storage
- Devices for Heart, Liver and pancreas in development
- Might be useful as model system for research, especially in the field of organ printing

GID Platform

GID 700

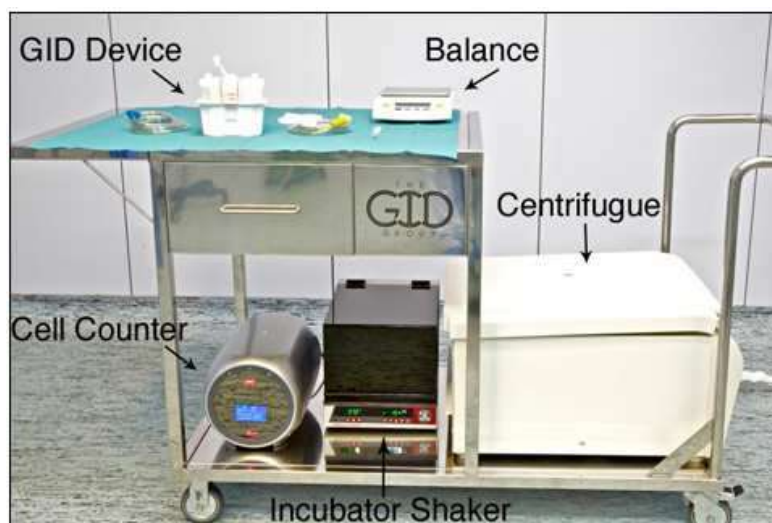
- Harvesting, rinsing and filtering of adipose grafts
- Free of oil, blood and compounds of the anaesthetic solution

GID SVF-1

- Isolation of stromal vascular fraction cells (including MSCs) from lipoaspirate
- Requires additional lab equipment and enzymes

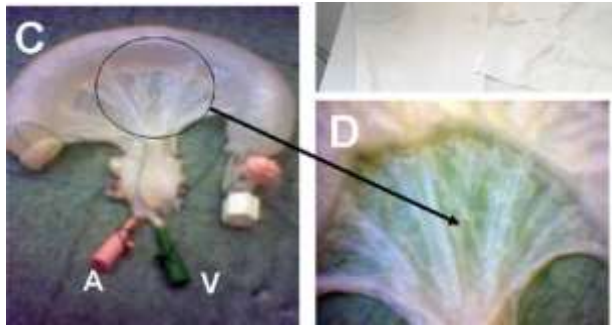
GID technology platform

- All-in-one package of laboratory equipment used to isolate cells in the surgical theatre
- Includes incubator-agitator, centrifuge, precision scale and cell counter on a mobile stainless steel cart



Vascularized Human Tissue Model

- Biological vascularised scaffold (BioVaSc®)
- Decellularized porcine small bowel segment
- Preserved tubular structures of the capillary network within collagen matrix
- Realized in human liver intestine, trachea and skin models
- Alternative to animal experiments



Artificial Trachea

- Artificial wind pipe
- Bone-marrow derived mesenchymal stem cells of the patient on polymer matrix
- Cultivation in rotating bioreactor for 5 days
- Implantation in June 2011 at Karolinska Institute in Stockholm
- First cell-seeded tissue engineered construct to be applied!!



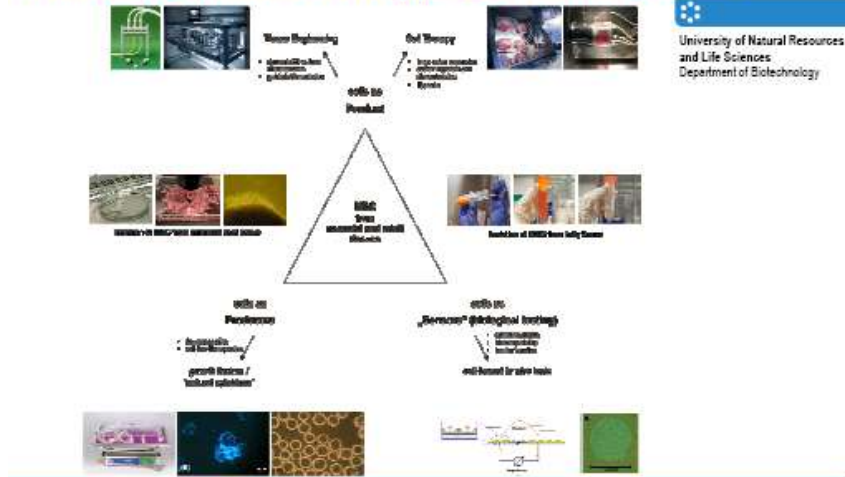
Tissue Factory

- Fraunhofer Institute, Stuttgart, Germany, Prof. Heike Walles
- Fully automated manufacture of 5.000 human skin equivalents per month
- 100 % quality control via non-invasive methods
- Widely GMP-compliant
- Biopharmaceutical / cosmetic testing

Human Mesenchymal Stem Cells Isolation, Expansion and Application Strategies



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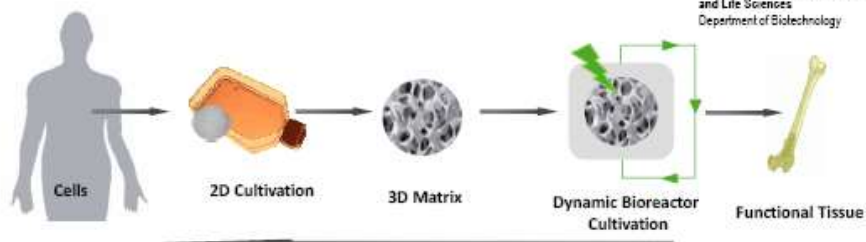


- Clinical studies in the USA and 179 countries
- Developed by U.S. National Institutes of Health (NIH) through the Library of Medicine (NLM) in collaboration with Food and Drug Administration (FDA)
- Contains over 120.000 trails
- Open database for patients that might want to participate in a study

Principle of Tissue Engineering



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Department of Biotechnology



- Cell biology
- Basic medical and veterinary science
- Transplantation science
- Biomaterials
- Biophysics and Biomechanics
- Biomedical engineering

The issue with oxygen

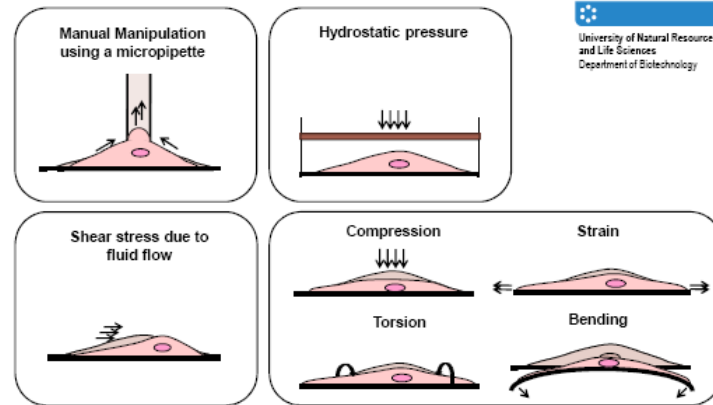
Standard cultivation: 21% O₂

Body: Brain 1-3%, lungs 16-18%, bone marrow: 1-3%, cartilage 1-3%

Mechanical stress on body cells:

- Compression
- Strain
- Torsion
- Bending
- Hydrostatic pressure
- Shear stress
- Manual manipulation (pipette)

Mechanotransduction



Bioreactor Systems in AG Kasper

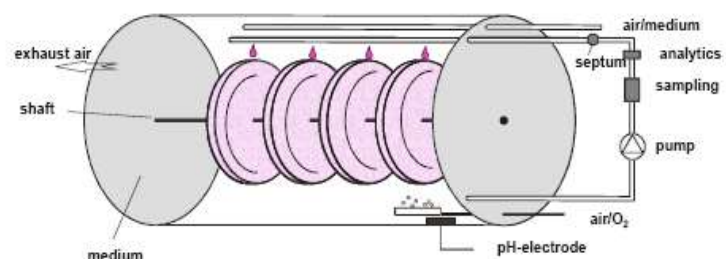
- Cell expansion
- Cell differentiation / TE
- Biocompatibility testing
- Systems for application of fluid flow
- Systems for mechanical stimulation
- Special lab installation for hypoxic conditions

Z[®] RP-System

- Complete system for:
- Production of
- Proteins
- Vaccines
- Cytokines
- Growth factors
- Stem cell expansion and differentiation

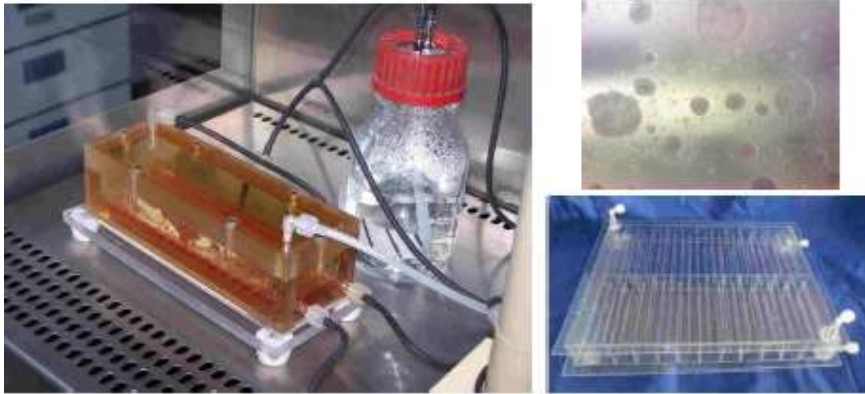


- Rotating inner parts
- Ceramic disks
- 50% breathing, 50% feeding
- Osteogenesis
- Flow as in stem cell niche
- Dynamic environment (increasingly calcified ECM)



Z[®]RP 50 M bioreactor system

Expansion of HSCs, NK cells, T lymphocytes



Bioreactors for stem cell differentiation (e.g. bone) → Stresskammer, mechanical stimulation and physiological training for generating functional tissues

GUEST LECTURER: Henschler, HSCs

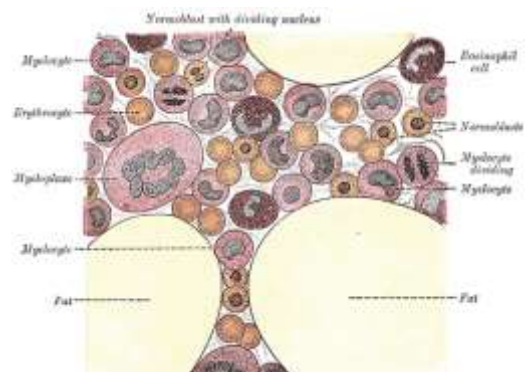
Part I: Hematopoietic Stem Cells – Basics to Clinical Applications

Daily demand of new blood cells:

Erythrocytes ca. 2×10^{11}	(200.000.000.000)
Neutrophiles ca. 1×10^{11}	(100.000.000.000)
Thrombocytes ca. 2×10^{11}	(200.000.000.000)

Hematopoietic cells / progenitors in bone marrow

Common ancestor of all blood cells in bone marrow?



→ **1959-1961:** McCulloch and Till: One origin of all blood cells in the bone marrow

For ingenious experiments that first identified a stem cell - the blood-forming stem cell - which set the stage for all current research on adult and embryonic stem cells.

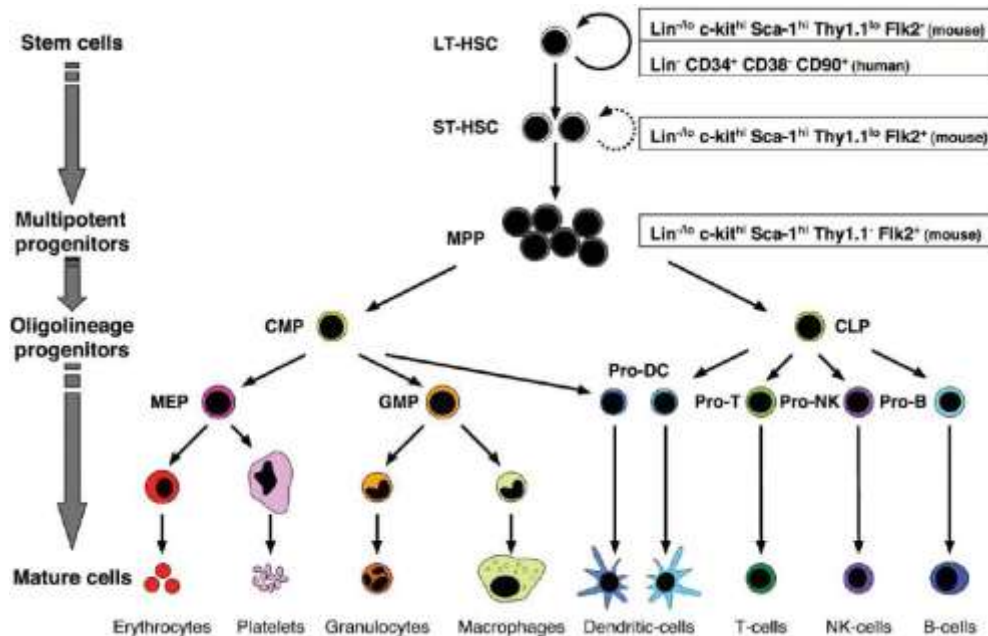
Experiment: Irradiated mouse, implantation of bone marrow, nodules on the spleen, each nodule contained all cells:

- Self renewable
- Clonal origin
- All cell lines

Transplantation of HSC: Helps an irradiated mouse to survive, transplantation in series possible

Identification of growth factors for HSC: Bone marrow cultivated on different stimuli, separated by chromatography and GF identified by colony assays, e.g. G-CSF, M-CSF

From bone marrow stem cell to hematopoietic progenitors:



Phenotypical separation of HSC: Flow through cytometry; separated by size of their shadow; markers; if the sorted cells were transplanted it needed much fewer cells for a sick animal to survive

Characteristics of blood-forming cells:

Frequency	< 1:10.000
Phenotype	not specific
RNA	none
Mitochondria	3
Protection	efflux channels

Transplantation of HSC in human: 1990 in Sweden (Nobel prize)

- Mobilisation of HSC to peripheral blood through G-CSF
- CD34⁺ selection
- Regeneration of hematopoietic system ✓ But leukemia cells will not be eliminated completely

1980-2010: Increase in transplantation activity, autologous cell transplantations on the rise

Indications for allogenic HSC transplantations:

- Acute high risk myelogenous leukemia (AML):
- Acute high risk lymphoblastic leukemia
- Chronic myelogenous leukemia
- (Non-) Hodgkin lymphoma
- SCID (severe combined immunodeficiencies)
- Hemoglobinopathies (sickle cell disease)
- Hurler's syndrome and other metabolic disorders
- Solid tumors
- Refractory anemia

Periphere Blutstammzellen <ul style="list-style-type: none"> • mobilisiert durch G-CSF • $3-5 \times 10^6$ CD34⁺/kg • kryokonserviert oder frisch • ggf. CD34⁺ selektioniert • rasche Regeneration 	Indikationen für eine SZT <ul style="list-style-type: none"> • Leukämien • Lymphome • Bestimmte solide Tumoren: <ul style="list-style-type: none"> - Neuroblastom, - Rhabdomyosarkom - Hirntumoren • Immundefekte • Anämien: <ul style="list-style-type: none"> - Aplastische Anämie - Sichelzellanämie - Thalassämie - Blackfan-Diamond Anämie • Speicherkrankheiten
Stammzellen aus Nabelschnurblut <ul style="list-style-type: none"> • $3-5 \times 10^7$ MNC/kg • kryokonserviert oder frisch 	

Summary on HSC:

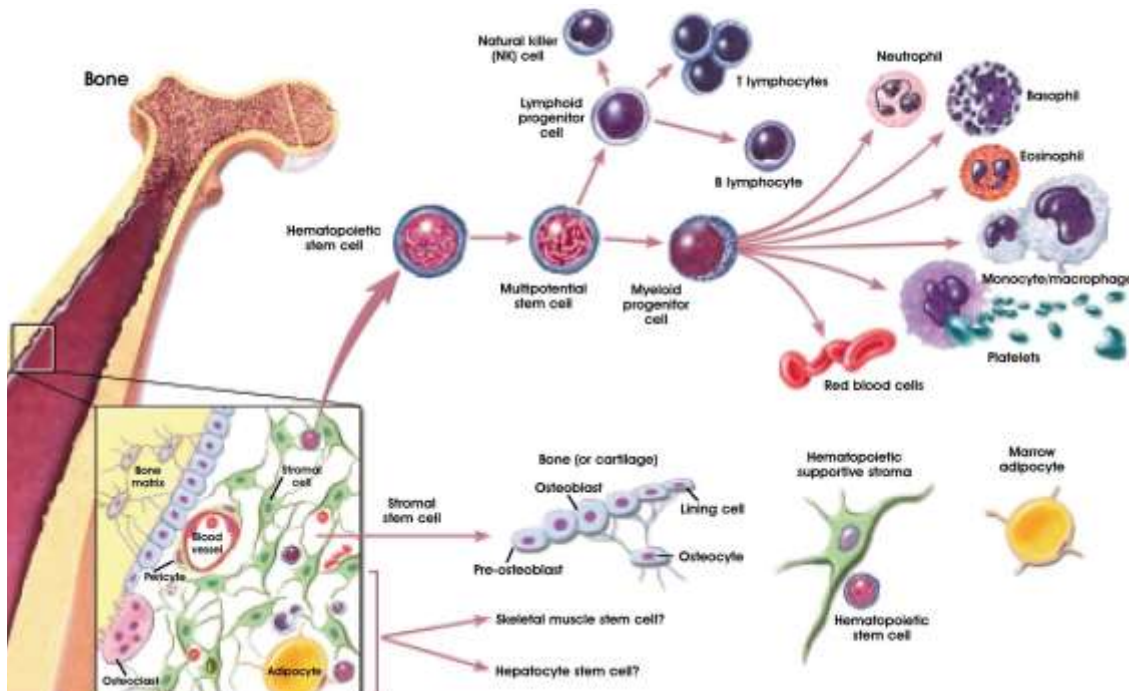
- Differentiation into all hematopoietic cell lines
- Self-renewal
- Continuous regeneration of tissues

Frequency	< 1:10.000
Phenotype	not specific
RNA	none
Mitochondria	3
Protection	efflux channels (MDR)

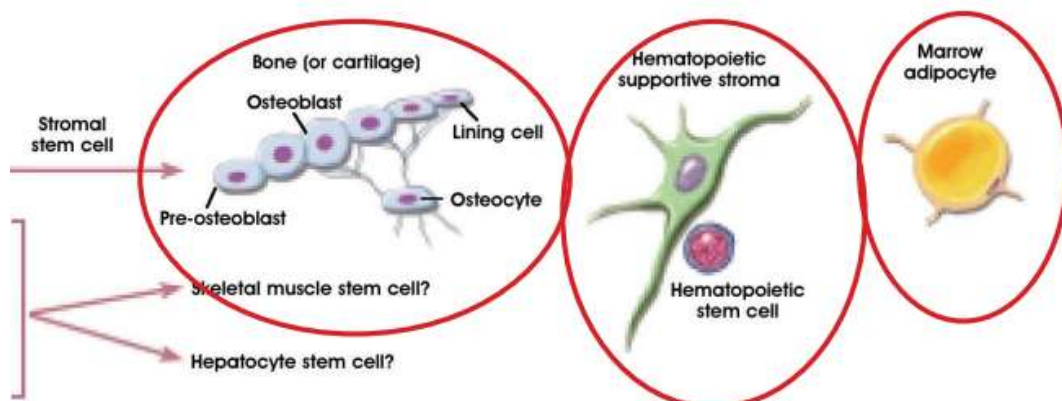
- In niches
- Activatable
- Mobilization
- Recircularization and Homing
- Autologous vs allogenic transplantation
- Therapy of hemic neoplasia
- Non-malign diseases

Part II: Microenvironment

- 1) Bone Marrow Anatomy
- 2) Stromal Cell Types
- 3) Contacts & Signals
- 4) Proliferation and Differentiation in the Microenvironment



|| Hematopoiesis takes place in the extravascular cavities between the sinuses of the marrow
The wall of the sinuses are comprised of adventitial reticular cells (ARC) + endothelial cells



Are stromal cells functionally important?

|| Stromal cells are the connective tissue cells of any organ that support the function of the parenchymal cells

1) Transplantation experiment

- 1970s in Moscow
- Transplantation of stromal cells (resembling fibroblasts) into animal model (rat liver)
- Formation of all lineages of HSC

|| Stromal cells are responsible for transferring the microenvironment of the hemopoietic tissues.

2) Culture experiment

- 1975 in Manchester
- Huge impact of culture conditions (temperature, serum, ...)

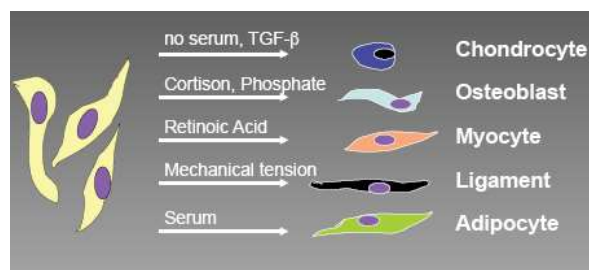
Stromal cell types

1 Adventitial Reticular Cell (ARC) = "Fibroblast"

In vitro forms colonies (CFU-F = MSC), fat cells, bone cells

In vivo: Transplantable microenvironment

"Marrow Stromal Cells" = "Mesenchymal Stem Cells" from Bone Marrow



→ Adipocytes

- No lipolysis
- Insulin-independent
- Glucocorticoid-dependent
- Are regulated ~ hematopoiesis:
 - \uparrow Hyperregeneration
 - \downarrow Hypertransfusion
- Differentiate from ARC
- Produce colony-stimulating activity

2 Macrophage (perisinial & central)

- 1) Enucleation (erythrocytes)
- 2) Development (iron uptake, granulopoiesis)

3 Endothelial cell

Contacts & Signals

|| Hematopoietic cells induce proliferation and differentiation stimuli in their microenvironment

|| Hematopoietic colony stimulating factors regulate hematopoiesis via stromal cells

|| Hematopoietic cells are stimulated by extracellular matrix substances in their microenvironment
(→ adhesion, survival, differentiation)

|| ECM components regulate differentiation (eg. Fibronectin regulates hematopoiesis)

Matrix component	Cell type	Reference
Laminin	kidney epithelia neuron hepatocytes	Klein et al., 1988 Sanes, 1989 Caron 1990
Thrombospondin	neuron	Neugebauer et al., 1991 O' Shea et al., 1991
Fibronectin	erythroblast	Patel and Lodish, 1987
Collagens	mammary epithelia colonic epithelial	Hall et al., 1982 Pignatelli and Bodmer, 1988
Vitronectin	neuron	Neugebauer et al., 1991
Tenascin	neuron	Chiquet, 1989

Growth factors

CSFs (mobilization for transplantation!)

SCF, FLT3L

IL-1, IL-6, LIF, VEGF

Microenvironment Messages To Take Home		
Bone Marrow	Sinuses; Islands; Stem Cell Niche	
Stromal Cells	ARC Culture: Fibroblasts MΦ, EC → Adipocytes; Osteoblasts	
Extracellular Matrix	Fn, Lam, Col etc.	Integrin → Adhesion → Development
Growth Factors	CSFs, SCF, Ang1 Notch / jagged Pathway	→ Survival → Proliferation → Decision
Mobilization	Integrins, Chemokines,	> G-CSF → Adhesion/Migration

Part III: Interdisciplinary Development of Cellular Therapies, → Ausdruck

Donor-lymphocytes, MSC, cellular therapeutics for cardiac regeneration increasing since 1990s

Zelltherapiezentrum: Cord blood, bone marrow, MSC / NK (selection and expansion)

Zelltherapeutika: HSC, MSC, immune cells from monocytes

HSC:

CD34⁺ selected → expansion → + cytokines → dendritic cells / progenitor cells for hematopoietic regeneration

Tumor therapy:

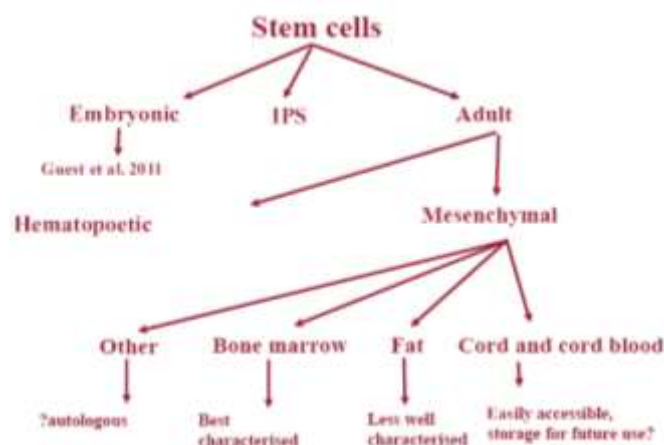
Monocytes + gm-csf/il-4 → immature DC → TLR cocktail (RNA transfection) → mature DC → human

Translation into the clinic: Manufacturing permission, approval of clinical trial, ethical vote

GUEST LECTURER: Ribitsch, Veterinary Regenerative Medicine



CELL THERAPIES



Problems: Cell source
Application
Safety
Functionality

1 Cell choice: undifferentiated / progenitor cells

Embryonic SC, iPS cells, umbilical cord SC, MSC

Different stem cell techniques:

- Non-stem cell products
- Direct bone marrow
- Bone marrow nucleated cell fraction
- Bone marrow + culture
- Fat - no culture
- Fat - culture

Applications: Tendon regeneration, bone regeneration, degenerative joint disease

TENDON REGENERATION (Tenonitis etc.)

Exercise-accelerated aging, increased tendon load → Cumulative fatigue damage

Little or no possibility of repairing microdamages in tendon → Eventually rupture of the tendon

Superficial digital flexor tendon (Achillessehne)

Clinical consequences → Altered biomechanics, reduced performance, high frequency of re-injury

Scar tissues is hard and not functional → Doesn't do the job

- The tendon itself acts as a scaffold, with the vascularized granulation tissue inside
- As a cell source MSC are introduced
- Growth factor source is the BM supernatant
- Constant movement on the tendon

1. Draw bone marrow
2. Grow cells in 2-3 weeks
3. Estimate the size of injury (5 mio cells / cm³ are needed)
4. Apply SC under ultrasound
5. Rehabilitation

- It takes about 1 year until the horse can be used in sports
- The healing is not faster with stem cells, but better
- Trials have shown that tendon tissue only was created
- Core lesions were filled quickly without inflammation
- 1500 clinical cases treated
 - 5 cases: Inflammation after intrasynovial administration
 - 1 case: Mineralisation observed, but far off the injection site

- Histologically the outcomes showed good linear organisation and no abnormal tissue
 - ✓ Reduced stiffness
 - ✓ Reduced cellularity (scar!)
 - ✓ Improved linear organisation
 - ✓ Less reinjury

MSC therapy routine for horses (if money available)

BONE REGENERATION

Experimental applications for sheep and horses

Clinical applications on smaller animals (less weight)

Applications in the dog

- Nonunion
- Malunion
- Loss of bone
- Radio curvus
- OCD

Essential components of bone tissue engineering: Cells and scaffolds (biomaterials)

→ Interaction of these two components is critical for the success of the tissue construct

1. Cell isolation from native tissue
2. Seeding of cells onto the matrix
3. Implantation of tissue construct back in vivo

BMSC directly seeded on scaffolds of different composition may generate osteoinductive grafts:

- ✓ High standardization
- ✓ High safety
- ✓ Reduced costs & time

Different biomaterials have been proposed:

Osteoconduction: Property to induce bone formation on the scaffold surface

Osteoinduction: Property to induce cell differentiation and thus formation of new bone

Osteointegration: Physical and chemical property to induce bone formation without fibrous tissue

Scaffold requirements: Biocompatible, flexible to moulding, right mechanical properties, promote development of bone tissue, reduce inflammation, biodegradable

Scaffold characteristics: Porosity, morphology of the granules, composition and molar proportion between calcium and phosphorous

|| Amelioration of the performance possible through osteoprogenitor cells and bone morphogenic protein (bmp)

Use of bone powder:

Bone cysts (Hohlraum, schwächt den Knochen)

Cells + powder injected into the cyst

Large bone defects: Use of bone plate and screws or other systems of fixation of the scaffold are essential

DEGENERATIVE JOINT DISEASE

- Most common reason for early retirement from athletic use
- Pathogenesis: Abnormal joint - normal forces; normal joint - excessive forces
 - Cartilage trauma
 - Intraarticular trauma
 - Ligament or meniscal tears
 - Synovitis, etc.
- Little knowledge so far, no advanced therapy so far
- Problematic: Animals cannot be immobilised, cartilage damage usually noticed very late

Synovial joint: Joint capsule, articular cartilage, joint cavity, synovial fluid

Therapy methods:

- Autologous chondrocyte transplantation
- Matrix associated chondrocyte transplantation
- Autologous membrane induced chondrogenesis: Microdamage to the bone → cells come out → covered with periosteal flap

PROBLEMATIC: When SC are introduced as a suspension, they will simply disappear → Covering them with a periosteal flap, SC also go inside the capsule to calm the inflammation → Qualitatively high synovia & fluid can be reached

Results in clinical studies: Reduction of pain killers was possible, in most of the cases no regeneration but inflammation could be reduced

CELLFREE THERAPY

ACS (autologous conditioned serum)

- Patient's own serum (cell-free) that is conditioned (surface increase & temperature)
- Cells are forced to give off growth factors (e.g. IL-1 receptor antagonist IL-1Ra → anti-inflammatory and chondroprotective)
- ACS is used in e.g. degenerative joint disease (osteoarthritis) → shorter inflammatory phase

Procedure: Draw blood from the animal, incubate at 37°C, reinject only the GF rich plasma

Mode of action: Cytokines released during an inflammatory response → destruction of hyaline cartilage and matrix → IL-1Ra has anti-inflammatory and chondroprotective properties!

PRP (platelet rich plasma)

- High concentration of thrombocytes, leucocytes and other plasma proteins (e.g. fibrinogen, GW: TGF- β , VEGF, IGF)
- GF induced cytokine and cell recruitment
- Vasoinvasion
- ECM formation

Application: Tendinopathies, suspensory ligament desmitis, demopathies, meniscus tears, bone cysts, cartilage defects: Intralesional & intraarticular injection

Preparation: Double centrifugation of citrated blood (coagulation blocked), no closed system → only superficial application recommended!

FUTURE APPLICATIONS AND CHALLENGES

The techniques are still rather crude

Understanding why things work, the exact mode of action (SC or factors?)

Legislative hurdles → Arzneimittelgesetz

Extending the use of regenerative medicine

Issues to address on current techniques

Does it need to be a stem cell?

- Allogenic / xenogenic cell lines
- ESC (migrate and survive in higher numbers)
- iPS
- Other cells
- Understanding the exact mechanism of action behind stem cell therapy (cell surface markers, trophic effect, anti-inflammatory effect, ...)
- Evidence of efficacy (controlled experiments, randomised clinical trials, follow-ups, ...)
- Delivery (dose, vehicles, routes, repeated treatments, ...)
- Better definition of products (markers, link with the outcome → interpretation, ...)

TRANSLATION INTO HUMANE MEDICINE

Tendinopathies

- Similarities to humans
 - Achilles tendinopathy
 - Intarsynovial lesions
 - Rotator cuff
- Problematic and common diseases
- Similar biomechanical properties

Degenerative Joint disease: Same pathophysiology in humans and horses (and dogs)

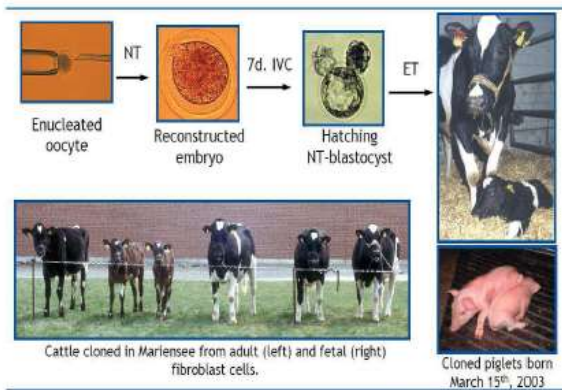
CLONING

→ Dolly

Current status of somatic cloning in mammals

Species	Viable offspr. (%)	No. cloned anim. (est.)
Cattle	15-20	~4000
Sheep	8	<500
Goat	3	<600
Pig	<1 (-5%)	<1500
Cat	<1	~10
Mouse	<2	>350
Rabbit	<2	<10
Mule	<1	3
Horse	<1	~30
Rat	1	~10
Dog	1	~200
Ferret	1	2
Wolf	<1	1
Buffalo	1-2	3-5
Camel	2	2
Monkey	1	1

Somatic cloning of cattle and swine



Potential problems with somatic cell nuclear transfer

Viable offspring (e.g. 15-20 % in cattle, only 8% in sheep)

- Time and labour intensive
- Inefficient
- Offspring not completely identical
- Telomere shortening
- Loss of genetic variability
- „Large offspring syndrome“ (large babies)