Pharmaceutical Nanotechnology

General introduction

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Contents

• General introduction to pharmaceutical nanotechnology

• Examples:
  • Tissue engineering
  • (i.e. nanotechnology for cell culture and transplantation)
  • Drug Delivery
  • (nanotechnology for delivering drugs to the target cells in the body)
**Basic requirements in drug treatment**

- Drug treatment should be effective and safe to develop effective and safe medicines. One should investigate:
  - Specific effect (drugs acts primarily in target --> off-target effects should be minimised)
  - Optimal pharmacokinetics (drug should reach the target in active form)
  - Safety (drug or its metabolites should not have unacceptable adverse effects)

**Drug Discovery & Development**

Cost of drug development is > 800 MEUR/drug

Problem 1: 90% of the clinical trials fail.
Problem 2: Many drugs fail even after market approval.

Informative tools are needed to augment the selection of successful compounds to the clinical phases and to improve the drug properties.
Why NanoBioScience?

- Nanotechnology is a promising set of methods and materials for generation of new functional materials and devices at nanoscale (less than 1000 nm).
- In biology, nanoscale multifunctional materials with precise functions are common.
- Nanobioscience may bring bioinspired materials to nanotechnology → more precise and controlled functionalities.

Why pharmaceutical nanotechnology?

Nanotechnology provides new tools that facilitate the development of effective and safe drug treatments.
Nano-1

**Lab-on-chip technology**

- miniaturised systems for the analyses
- goal: generate data with small quantity of compounds
- nanotechnology small scale controlled manipulation of the surfaces
- different read-outs
- various technologies
  - immobilisation of target compounds on lipid
  - microfluidics for sample handling
  - binding of DNA on the surface (DNA arrays)
  - immobilisation of DNA transfer nanoparticles
  - cell growth on the chip (cell array)

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**Systems Biology and Nanotechnology**

- **Background**
  - Human genome project generated basis for understanding the cell as machinery.
  - Human genome is 23 000 genes; sequences are known, but the functions of the encoded proteins are still unclear.
  - Genomics, proteomics and metabolomics analyse the changes in the cells at the universal level.
Nanotechnology:

for example immobilisation of DNA probes to a chip
--> attachment of fluorescent RNA strands from the sample are detected on the chip
--> universal expression data
--> describes cell response to the test compound
**Imaging tools**

- Imaging tools are needed in the cell studies and in vivo studies.

- Provides non-invasive information about the test compound disposition in the cell, animal or human.

- Nanotechnology:
  - Fluorescent quantum dots for cell and animal imaging
  - Multifunctional nanoparticles for imaging and diagnosis (e.g. localisation in tumour)

- Cell culture for tissue engineering and improved cell models

- Improved drug delivery systems
Pharmaceutical Nanotechnology: Drug Delivery

Contents

1. Background
2. Controlled Drug Release
3. Decreased Drug Clearance
4. Drug Targeting
5. Gene Delivery
6. Cell Therapy
in successful drug therapy
the drug must gain access
in active form
to its site of action
at adequate concentration and
for sufficient time period
Informative tools are needed to augment the selection of successful compounds to the clinical phases and to improve the drug properties.

Drug delivery reasons lead to compound failures in the clinical phase.

**problematic cases**
- Biotechnological drugs (proteins, genes)
- Difficult target sites: intracellular, brain, tumour, back of the eye
- Drugs with serious side-effects (cytostatics)
- Poorly soluble drugs
- Improved delivery technologies are needed
**Delivery of Drugs to Different Sites**

Drugs should escape from blood stream to access the target cells. Distribution is controlled by the blood flow and the barriers between the blood and tissues.

**2. Controlled Drug Release**

- oral prolonged action tablets
- implants
- slowly dissolving injectables
- microcapsules
- newer developments
Concentration Profiles; Modified with Controlled Drug Release. Rate Controlling Factor

Absorption does not control elimination

Advantages: long duration of action, less concentration changes, patient compliance

Chemical and Physical Hydrogels

Absorption controls elimination

Chemical Hydrogel

Physical Hydrogel
**Gel Formation by Cross-Linking**

- Bi-functional monomers and polymers
- Multifunctional crosslinkers

**pH-Dependent Hydrogels**

(a) Acidic solution
- Anionic hydrogel

(b) Basic solution
- Cationic hydrogel

Drug Discovery Today
**External Control of Hydrogel and Drug Release**

Example: Ocular Drug Delivery

- **TEAR FLUID**
- **CORNEA**
- **ANTERIOR CHAMBER**
- **POSTERIOR CHAMBER**
- **IRIS**
- **LENS**
- **VITREOUS BODY**
- **SCLERA**
- **CILIARY BODY**
**Surgery and Drug Release**

- Surgical implants with drug release (eye, bone, cardiac)
- Tissue engineering products
  - Polymer scaffolds
  - Immobilisation of growth factors
  - Controlled release of the growth factors is crucial

3. **Decreased Drug Clearance**

- Prolongation of half-life for injectable proteins
- Conjugation with PEG
- Avoid elimination by liver
- Stealth effect
**4. Drug Targeting: Background**

Drug is accessible to tissues with high blood flow and leaky blood vessels.
Targeting needs: eye, brain, tumours
Targeting at Cellular Level

Drug targeting systems
- nanoparticles
- micelles
- complexes
- dendrimers
- liposomes
- other vesicles
- polymeric prodrugs
5. Gene delivery

- Transfer of Therapeutic Gene
- delivery system
- Expression of Transgene Product
- promoter in plasmid
Delivery of Plasmid DNA and Other Gene Medicines

- large molecular weight
- charges of phosphates
- enzymatic lability
- per oral administration does not work
- inadequate distribution in the body
- poor intracellular delivery
- problems of viral vectors
DNA Complexation

cationic polymer or liposome

Controlled packing structure-property relationships?

OLIGO/LIPID

POLYMER-DNA
Steps of Non-Viral Gene Transfer

DNA + kationinen polymeeri

solun sitoutuminen

kohdesolu

hoidollinen proteiini

+kuma

* Soluunotto (FACS)

* confokalimikroskopia

* geenikompleksikko

* gelelektroforeesi

* kokojakauma (DLS)

* TEM

* betagalaktosidaasin

ilmentyminen (ONPG)

kompleksit

koossa

DNA vapautunut

tumaan

solun pinnan

GAGit

* betagalaktosidaasin

ilmentyminen (ONPG)

hoidollinen proteiini

6. Cell Therapy

cells are modified to act as bioreactors that produce the drug in the body
6. Cell therapy: CELL MICROENCAPSULATION

Nano-1 Solut, ARPE-19
Na-alginaatti 1,2%
päällystys 0,1% PLL 20 kDa
0,125% Na-alginaatti
DNA geenikytkin
ravinteet ja happi
aineenvaihdunta-tuotteet
immuunivaste
kapseleiden päällystys ja pesu
terapeuttinen proteiini
farmakokineettinen malli

in vivo -kokeet
in vitro -kokeet
farmakokineettinen malli

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Cell microcapsules
Release of SEAP as a function of microencapsulated cells

In vivo 68 mM Ca\(^{2+}\) + 20 mM Ba\(^{2+}\) + PLL for 8 days in rats abdominal cavity. Size and shape are the same compared to in vitro situation.

Capsule transplantation studies, Wistar rats, abdominal cavity

In vivo 20 mM Ba\(^{2+}\) + PLL capsules become covered with massive growth of biological material (probably macrophage cells).
Conclusions:

Successful nanomedicine must overcome several steps

- Drug Targeting with Nanosystems

- Controlling of the drug targeting and its release

- Activation of the system upon request
Liver Cell Cultures in Drug Discovery

- Liver is the major site of drug elimination
  - metabolism
  - biliary excretion
- Liver is the major site of drug induced toxicity
- Pharmacokinetic and toxicity problems are important reasons for failures of drug candidates in the clinical phases.
- Can we predict the kinetics / toxicity of drug candidates early?
Liver in Pharmacokinetics

- **metabolism**
  - phase I: CYP450
  - phase II: UGT and other conjugating enzymes
- **transporters**
  - efflux transporters, organic anion transporters...several others
  - transporters pump drug from the blood into hepatocytes, and from hepatocytes into the blood and bile
  - interplay between the enzymes and transporters
- **prediction of kinetics and toxicity**: hepatocytes should have correct morphology, expression profile and correct location of the enzymes and transporters to mimic the in vivo situation

Current test models

- isolated liver microsomes
  - metabolic reactions are studied; no transporters
- hepatocyte cell lines
  - expression profiles not correct; easy to grow
- primary cells
  - not good for routine use; not polarised; no 3-d
- sandwich culture system
  - polarization, elementary bile secretion system; can be followed using fluorescence imaging
Current methods

- stem cells
  - in principle allows proper differentiation to hepatocytes
  - not working yet
- animal models
  - enzyme expression different than in humans
  - not predictive
- hepatocytes or stem cells in 3-d culture
  - biomaterials support used to guide the cell growth to obtain right orientation of the cells and vectorial transport
  - how to measure secretion into the bile

Microenvironmental interactions *in vitro* and *in vivo*
Extra Cellular Matrix – Should we try to mimic it?

Nano-1 3D cell culture

- HepG2 cell line is cultured in peptide-based hydrogel matrix = Puramatrix that promotes cell attachment and migration
- Consists of standard amino acids 1% and water 99%
- RGD = Arginine-alanine-aspartic acid
- Pore size of the hydrogel 5-200 µm
**Puramatrix**

Nano-1

AcN-RADARADARADARADA-CONH2 (RAD16-I)
- arginine-alanine-aspartic acid-
- in the presence of salts, self-assembles into a network of nanofibers of about 10 nm diameter
- Pore size of the hydrogel 5-200 µm

Capillary-like network formation by endothelial cells in Puramatrix,
Enhanced expression of angiogenic growth factor VEGF
(Narmonova et al. 2005)

Induced differentiation of rat hepatocyte progenitor cell line (Lig-8) in Puramatrix,
Increased albumin-synthesis, expression of CYP1A1, CYP1A2 and CYP2E1
(Semino et al. 2003)

Puramatrix was functionalized with peptides either from collagen IV or from laminin-1,
Some advantageous effects on endothelial cultures was observed (Genove et al. 2005)
Why polymers?

- We can engineer their properties
  - Chemical
  - Degradation
  - Biological
  - Mechanical
  - Electrical
  - Engineer structures

Examples of various types of polymers

- Natural
  - Proteins / peptides
  - Collagen
  - Elastin
  - Polysaccharides / agarose / HA
  - ...

- Synthetic
  - PEG
  - PLGA
  - HEMA
  - MMA
  - ...

- Degradable
  - PGA
  - Most natural polymers
  - ...

- Non-degradable
  - MMA / HEMA/PEG
  - PLA
  - ....
Tissue Eng. Example 1: Degradable scaffolds

Cells
Osteoblasts
Chondrocytes
Hepatocytes
Enterocytes
Urothelial Cells

Biodegradable Polymer Scaffold

In vitro Tissue Culture

In Vivo Implantation

New
Bone
Cartilage
Liver
Intestine
Ureter

Other examples

- Nerve Regeneration
- Wound Healing
- Artificial Skin, Lung, Liver, Pancreas
Control of Degradation Rate

* PH 7.70°C, buffer solution
**Electrospinning Instrumentation**

- Electrospun mat of fibers

**Polymer solution**

**Needle**

**High voltage power supply**

**Collector**

Without cells

1 d

3 d

7 d

PVP in 95% EtOH, M_w 100,000 g/mol
Nanofibers for tissue engineering applications

Ref. Huang et al. 2005

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<td>Endothelial cells (ECs) and SMCs</td>
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</tr>
</tbody>
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Advanced matrices

Figure. Schematic drawing of an artificial mimic of an extracellular matrix. The fibrillar skeleton is composed of electrospun biodegradable polymer fibers. The matrix is modified by incorporating cell adhesion ligands on the surface of nanofibers to improve the cell attachment. Hydrophilicity is increased with grafted polymer chains and the cell culturing is controlled with a controlled release of growth factors.
Cartilage Tissue Engineering

BEFORE cell seeding

AFTER 2 weeks in culture

Robert Langer, MIT
Soft lithography for microfabrication

Controlling Cell Microenvironment
- Cell & protein patterning
  - PEG
  - Polysaccharides
- Patterned co-cultures
- Layer-by-layer
- Microfluidics
  - High-throughput
  - Material gradients

Fundamental biology, improved tools and assays
Regenerative medicine, drug delivery, diagnostics
Cell and protein patterning with PEG

- Clonal analysis of cells and their progeny
- Controlling cell shape
- Track cells and number of cells on an island

Can we immobilize cells to particular regions of a dish and control the size of cell aggregates?

- PEG dimethacrylate (PEG-DMA) was used for the crosslinking
Patterning with heat crosslinkable PEG

Microstructures (MS)
Polymeric self-assembled monolayer (PM)

Control of EB Size, homogeneity and shape

PEGMA Microstructures
Seeded cells
Cells washed and grown
Controlling Cell Microenvironment

- Cell & protein patterning
  - PEG
  - Polysaccharides
- Patterned co-cultures
- Layer-by-layer
- Microfluidics
  - High-throughput
  - Material gradients
- Fundamental biology, improved tools and assays
- Regenerative medicine, drug delivery, diagnostics

Micro/nanofluidics

- Cover slip
- Inlets
- Outlet
- Controlling cell microenvironment
  - High-throughput / diagnostic
  - Tissue engineering
Protein arrays inside microchannels

Cell arrays inside microchannels

Fig. 1  (A) Schematic of the microfluidic device consisting of two PDMS layers: a top fluidic channel and a bottom microgrooved surface. (B) The PDMS layers are aligned and bonded. (C) Cells are docked within the microgrooves in a microfluidic device.

Lab Chip. 2008, 8, 747–754
Cells were captured within microstructures of various shapes inside microfluidic channels.

Conclusions

- Techniques are developed to control cellular microenvironment in vitro
- Challenge is to scale up this into 3D
Generation of 3D cell culture systems

Conclusions

Nanobioscience is a multidisciplinary field

Principles and materials from biology are utilised to generate new functional materials and devices with new properties