Tissue culture and practical Biotechnology

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Pharmaceutical research and production

- Research (models):

Tissue, cell culture, organism

- Production (models):

Microrganisms, cell culture

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Model organisms:

Organisms used as models:

→ for studying the biological processes

→ underlying states of health and disease, as well as

→ for studying the effects of particular compounds.

Among the model organisms most commonly studied are mice, yeast, worms, fruit flies, and zebrafish.

Because

- Known genes with cellular and physiological responses,
- Simple model organisms are expected to be highly useful in understanding evolution and development with a biomedical perspective.

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Tissue and Cell Culture

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Why is cell culture used for?

Areas where cell culture technology is currently playing a major role.

- Model systems

Studying basic cell biology, interactions between disease causing agents and cells, effects of drugs on cells, process and triggering of aging & nutritional studies

- Toxicity testing

Study the effects of new drugs

- Cancer research

Study the cancer biology and the function of various compounds

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- Virology

Used to study their infectious cycle.

- Gene therapy

Cells having a functional gene can be replaced with cells which are having the non-functional gene

- Genetic Engineering

Production of commercial proteins, large-scale production of viruses for use in vaccine production

- Tissue or Organ?

Animal cell culture can be used as replacement tissue or organs.

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Major development's in cell culture technology

- First development was the use of antibiotics which inhibits the growth of contaminants.
- Second was the use of trypsin to remove adherent cells to subculture further from the culture vessel (Flask, dish..)
- Third was the use of a chemically defined culture medium.

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Cell culture

In vitro cultivation of

cells at a defined temperature

- using an incubator & supplemented with a medium containing cell nutrients & growth factors

Different types of cell grown in culture

- fibroblasts, skeletal tissue, cardiac, epithelial tissue (liver, breast, skin, kidney) and many different types of tumor cells.

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Primary culture

- Cells when surgically or enzymatically removed from an organism and placed in a suitable culture environment will attach and grow are called primary culture
- Primary cells have a finite lifespan
- Primary culture contains a very heterogeneous population of cells
- Subculturing of primary cells leads to the generation of cell lines
- Cell lines have a limited life span, they passage several times before they become senescent
- Cells such as macrophages and neurons do not divide in vitro so can be used as primary cultures
- Lineage of cells originating from the primary culture is called a cell strain

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Permanent or established cell lines

- Most cell lines grow for a limited number of generations after which they ceases
- Cell lines which either occur spontaneously or induced virally or chemically transformed into continous cell lines
- Characteristics of continuous cell lines
  - Fast growth and have aneuploid chromosome number
  - Reduced serum and anchorage dependence and grow more in suspension conditions
  - Ability to grow up to higher cell density
  - different in phenotypes from donor tissue
  - but sometimes stop expressing tissue-specific genes

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Permanent or established cell lines

- Most cells are **anchorage dependent** and must be cultured while attached to a solid or semi-solid substrate (**adherent** or **monolayer culture**), while
- Others can be grown floating in the culture medium (**suspension culture**).

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Mammalian cells relevant for industrial processes can be characterized as follows

- **Primary cells** – cells taken from the tissue and further grown *in vitro*, without doubling.
- **Cell strains** – when these cells start to divide, one obtains a cell strain with finite lifetime (e.g., 30–40 generations) and the cells are unchanged.
- **Permanent or established cell lines** – cells that have gone through some transformations and can proliferate principally with an infinite life span. They can be effectively used for the expression of recombinant proteins.
- **Hybridoma cells** – cells obtained through the fusion of lymphocytes and tumor cells. They are particularly effective for the expression of monoclonal antibodies.

Cell Culture Technology -Ralf Pörtner, Uwe Jandt, and An-Ping Zeng
Industrial Biotechnology: Products and Processes, First Edition. Edited byChristophWittmann and JamesC. Liao.

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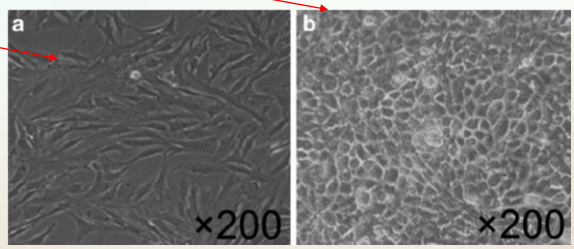
Types of cells

On the basis of morphology (shape & appearance) or on their functional characteristics. They are divided into three.

- Epithelial like-attached to a substrate and appears flattened and polygonal in shape
- Fibroblast like- cells attached to a substrate appear elongated and bipolar



- Lymphoblast like- cells do not attach remain in suspension with a spherical shape

ATCC → https://www.lgcstandards-atcc.org/?geo_country=it

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Biosafety in Microbiological and Biomedical Laboratories,

prepared by the Centers for Disease Control (CDC) and the National Institues of Health (NIH),

Biosafety Level 1 (BSL-1)

- BSL-1 is the basic level of protection common to most research and clinical laboratories and is appropriate for agents that are not known to cause disease in normal, healthy humans.

Biosafety Level 2 (BSL-2)

- BSL-2 is appropriate for moderate-risk agents known to cause human disease of varying severity by ingestion or through percutaneous or mucous membrane exposure. Most cell culture labs should be at least BSL-2, but the exact requirements depend upon the cell line used and the type of work conducted.

Biosafety in Microbiological and Biomedical Laboratories,
prepared by the Centers for Disease Control (CDC) and the National Institutes of Health (NIH),

Biosafety Level 3 (BSL-3)

- BSL-3 is appropriate for indigenous or exotic agents with a known potential for aerosol transmission, and for agents that may cause serious and potentially lethal infections.

Biosafety Level 4 (BSL-4)

- BSL-4 is appropriate for exotic agents that pose a high individual risk of life-threatening disease by infectious aerosols and for which no treatment is available. These agents are restricted to high-containment laboratories.

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Cell Culture Laboratory

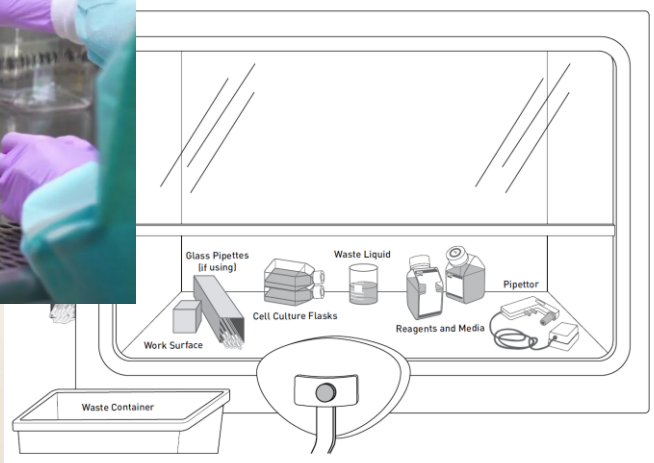

Figure 2.1 The basic layout of a cell culture hood for right-handed workers. Left-handed workers may switch the positions of the items laid out on the work surface.

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Basic equipments used in cell culture

- Laminar cabinet-Vertical are preferable
- Incubation facilities
  Temperature of 25-30 °C for insect
  37 °C for mammalian cells,
  CO_2 2-5% & 95% air at 99% relative humidity.
  To prevent cell death incubators are set to cut out at approx. 38.5 °C
- Refrigerators: Liquid media kept at 4 °C, enzymes (e.g. trypsin) & media components (e.g. glutamine & serum)
  at -20 °C
- Microscope: An inverted microscope with 4x to 100x magnification
- Tissue culture ware: Culture plastic ware treated by polystyrene

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Rules for working with cell culture

Never use contaminated material within a sterile area

Use the correct sequence when working with more than one cell line:

- Diploid cells (Primary cultures, lines for the production of vaccines etc.)
- Diploid cells (Laboratory lines)
- Continous, slow-growing line
- Continous, rapidly growing lines
- Lines which may be contaminated
- Virus-producing lines

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Basic aseptic conditions

- Swab all bottle tops & necks with 70% ethanol
- Avoiding placing caps & pipettes down on the bench;
- Practice holding bottle tops with the little finger
- Work either left to right or vice versa, so that all material goes to one side, once finished

Clean up spills immediately & always leave the workplace neat & tidy

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Culture media

- Choice of media depends on the type of cell being cultured
- Media is supplemented with antibiotics e.g. penicillin, streptomycin etc.
- Prepared media is filtered and incubated at 4° C

Common Media:

- Eagle's Minimal Essential Medium (MEM),
- Dulbecco's Enriched (modified) Eagle's Medium (DMEM),
- Ham's F12
- RPMI 1640…….

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Examples basal media in use:

E.g. 1: 1 mixture of IMDM (Iscove's Modification of Dulbecco's Medium) and Ham's F12

- *Salts* (CaCl2; CuSO4; Fe(NO3); FeSO4; KCl; MgSO4; MgCl2; NaCl; NaHCO3; NaH2PO4; and ZnSO4)
- *Vitamins* (biotin; D-Ca pantothenate; choline chloride; folic acid; i-inositol; nicotinamide; pyridoxal; riboflavin; thiamine; and vitamin B12)
- *Amino acids* (L-alanine; L-asparagine; L-arginine; L-aspartic acid; L-cysteine; L-glutamic acid; L-glutamine; L-glycine; L-histidine; L-isoleucine; L-lysine; L-methionine; L-phenylalanine; L-proline; L-serine; L-threonine; L-tryptophan; L-tyrosine; and L-valine)
- *Other compounds* (D-glucose; hypoxanthine (sodium salt); linoleic acid; lipoic acid; phenol red; putrescine; thymidine; and Na-pyruvate).

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Why sub-culturing?

- Once the available substrate surface is covered by cells (a confluent culture) growth slows & ceases.
- Cells to be kept in a healthy & growing state have to be sub-cultured or passaged (Split)
- It's the passage of cells when they reach 80-90% confluency in flasks/dishes/plates
- Enzyme such as trypsin, dispase, and collagenase in combination with EDTA breaks the cellular glue that attached the cells to the surface

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Culturing of cells

Cells are cultured as anchorage dependent or independent

- Cell lines derived from normal tissues are considered as anchorage-dependent grows only on a suitable substrate
  e.g. tissue cells
- Suspension cells are anchorage-independent e.g. blood cells
- Transformed cell lines either grows as monolayer or as suspension

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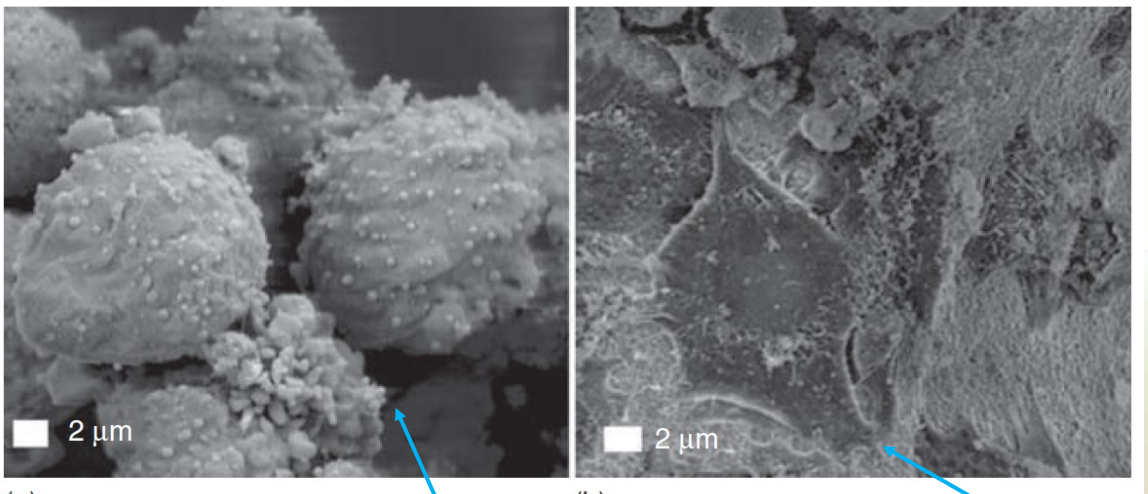

Figure 4.1 Examples for (a) cells for suspension growth (hybriodoma cells) and (b) adherent growing mammalian cells (HepG2 cells).

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Adherent Culture	Suspension Culture
Appropriate for most cell types, including primary cultures.	Appropriate for cells adapted to suspension culture and a few other cell lines that are nonadhesive (e.g., hematopoietic).
Requires periodic passaging, but allows easy visual inspection under inverted microscope.	Easier to passage, but requires daily cell counts and viability determination to follow growth patterns; culture can be diluted to stimulate growth.
Cells are dissociated enzymatically (e.g., TrypLE™ Express, trypsin) or mechanically.	Does not require enzymatic or mechanical dissocation.
Growth is limited by surface area, which may limit product yields.	Growth is limited by concentration of cells in the medium, which allows easy scale-up.
Requires tissue-culture treated vessel.	Can be maintained in culture vessels that are not tissue-culture treated, but requires agitation (i.e., shaking or stirring) for adequate gas exhange.
Used for cytology, harvesting products continuously, and many research applications.	Used for bulk production, batch harvesting, and many research applications.

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Adherent cells

- Cells which are anchorage-dependent
- Cells are washed with PBS (free of Ca & Mg) solution.
- Add enough trypsin/EDTA to cover the monolayer
- Incubate the plate at 37 °C for 1'-2'
- Tap the vessel from the sides to dislodge the cells
- Transfer the cell suspension to a 15 ml conical tube, centrifuge at 200-600g for 5' at RT and remove the growth medium by aspiration
- Add a complete medium to dissociate and dislodge the cells with the help of a pipette which are remained to be adherent
- Add complete medium depending on the subculture requirement either to 75 cm or 175 cm flask

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Suspension cells

- Easier to passage as no need to detach them
- As the suspension cells reach to confluency
- Aseptically remove 1/3rd of medium
- Transfer the cell suspension to a 15 ml conical tube, centrifuge at 200-600g for 5’ at RT and remove the growth medium by aspiration
- Replaced with the same amount of pre-warmed medium

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Cell Splitting Technique (Passaging)

https://www.youtube.com/watch?v=lXP9bS_sDgM

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Cell toxicity

- Cytotoxicity causes inhibition of cell growth
- Observed effect on the morphological alteration in the cell layer or cell shape
- Characteristics of abnormal morphology is the giant cells, multinucleated cells, a granular bumpy appearance, vacuoles in the cytoplasm or nucleus
- Cytotoxicity is determined by substituting materials such as medium, serum, supplements flasks etc.

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Working with cryopreserved cells

- Vial **from liquid nitrogen is placed into a 37 °C water bath**, agitate the vial continuously until the medium is thawed
- Centrifuge the vial for 10' at 1000 rpm at RT, wipe the top of the vial with 70% ethanol and discard the supernatant
- Resuspend the cell pellet in **1 ml of complete medium with 20% FBS and transfer to a properly labelled culture plate** containing the appropriate amount of medium
- **Check the cultures after 24 h to ensure that they are attached** to the plate
- Change medium as the colour changes, preferable use 20% FBS until the cells are established

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Freezing cells for storage

- Remove the growth medium, wash the cells with PBS and remove the PBS by aspiration
- Dislodge the cells by trypsin-EDTA
- Dilute the cells with a growth medium
- Transfer the cell suspension to a 15 ml conical tube, centrifuge at 200g for 5' at RT and remove the growth medium by aspiration
- **Resuspend the cells in 1-2 ml of freezing medium (e.g. 10 % DMSO in FBS/medium)**
- Transfer the cells to cryovials, and incubate the cryovials at -80 °C overnight
- Next day **transfer the cryovials to Liquid nitrogen**

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Contaminant's of cell culture

Cell culture contaminants of two types

- Chemical-difficult to detect caused by:
  - endotoxins, plasticizers, metal ions or traces of disinfectants that are invisible
- Biological-cause visible effects on the culture are:
  - mycoplasma, yeast, bacteria or fungi
  - also cross-contamination of cells from other cell lines

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Effects of Biological Contamination's

- They compete for nutrients with host cells
- Secreted acidic or alkaline by products interfere with the growth of the host cells
- Degraded arginine & purine inhibits the synthesis of histone and nucleic acid
- Maybe →produce H_2O_2 which is directly toxic to cells

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Detection of contaminants

In general indicators of contamination are:

- turbid culture media,
- change in growth rates,
- abnormally high pH,
- poor attachment,
- multi-nucleated cells,
- graining cellular appearance,
- vacuolization
- inclusion bodies and cell lysis

The best and oldest way to eliminate contamination is to
→ **discard the infected cell lines directly**

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Detection of contaminants

- **Yeast, bacteria & fungi** usually shows the visible effect on the culture (changes in medium turbidity or pH)
- **Mycoplasma** (bacteria) detected by direct DNA staining with intercalating fluorescent substances e.g. Hoechst 33258
- **Mycoplasma** also detected by enzyme immunoassay by specific antisera or monoclonal abs or by PCR amplification of mycoplasmal RNA

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Biological Contamination

E.Coli

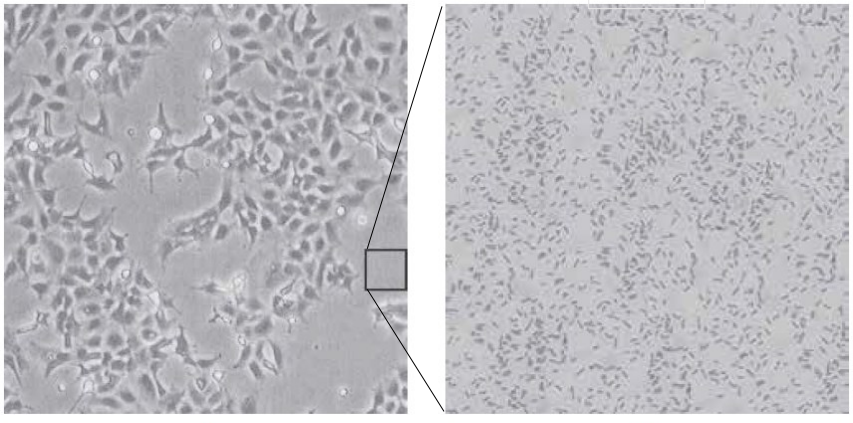

Figure 2.2 Simulated phase contrast images of adherent 293 cells contaminated with *E. coli*. The spaces between the adherent cells show tiny, shimmering granules under low power microscopy, but the individual bacteria are not easily distinguishable (panel A). Further magnification of the area enclosed by the black square resolves the individual *E. coli* cells, which are typically rod-shaped and are about 2 µm long and 0.5 µm in diameter. Each side of the black square in panel A is 100 µm.

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Biological Contamination

Yeast

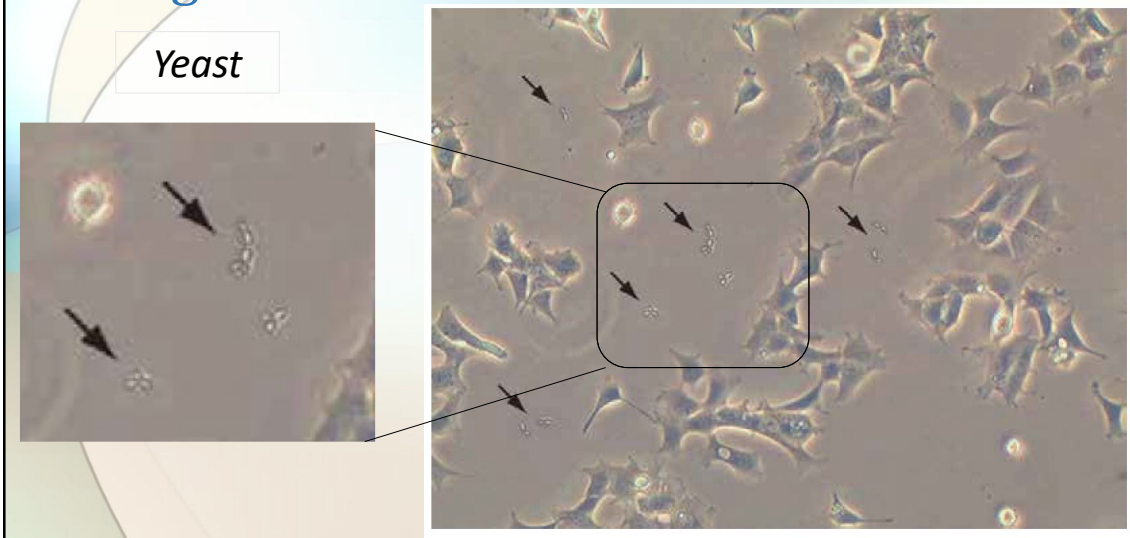

Figure 2.3 Simulated phase contrast images of 293 cells in adherent culture that is contaminated with yeast. The contaminating yeast cells appear as ovoid particles, budding off smaller particles as they replicate.

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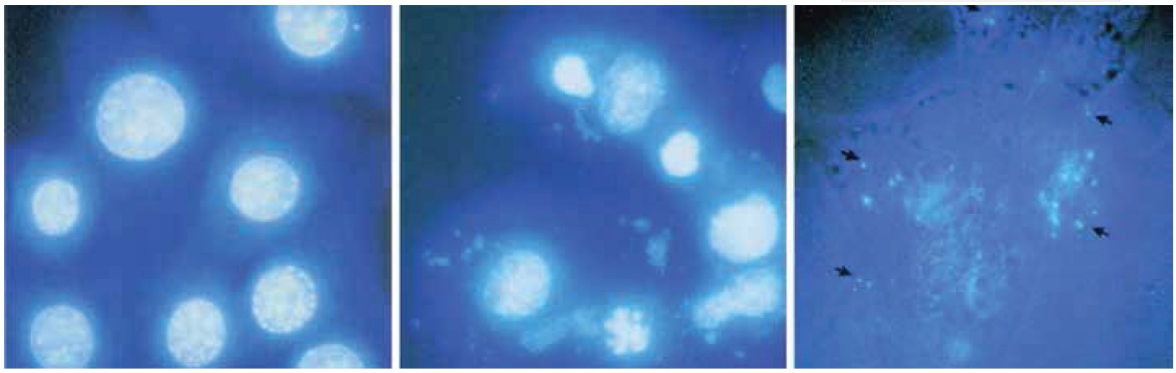
Biological Contamination

mycoplasma



A B C

Figure 2.4 Photomicrographs of mycoplasma-free cultured cells (panel A) and
→ cells **infected** with mycoplasma (panels **B and C**).

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Transfection of Host Cells and Recombinant Protein Production

- Introducing DNA into a mammalian cell is called transfection (but Transformation in *E. coli*).
- Methods to introduce DNA into a mammalian cell line. The plasmid DNA is complexed to
  - cationic lipids (such as Lipofectamine)
  - polymers (such as polyethyleneimines or PEI)
    - →The positively charged aggregates bind to the negatively charged cell membrane and are subsequently endocytosed
- Another way is through electroporation.
    - →During electroporation, an electric pulse is applied to the cells, which results in the formation of small pores in the plasma membrane.

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https://doi.org/10.1007/978-3-030-00710-2 -

Transfection methods

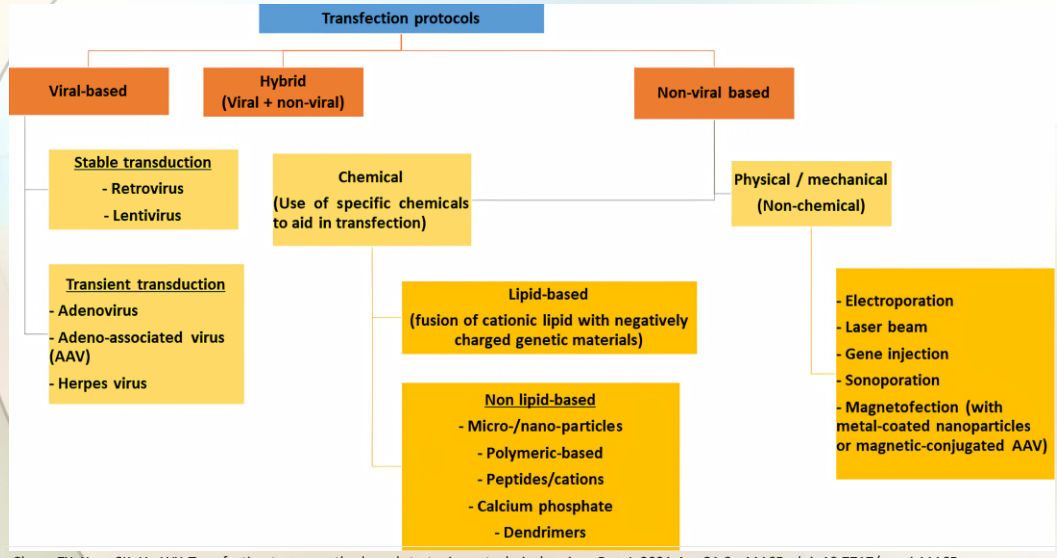
Examples:

- Calcium phosphate precipitation
- DEAE-dextran (dimethylaminoethyl-dextran)
- Lipid-mediated lipofection
- Electroporation
- Retroviral Infection
- Microinjection

Paras Yadav[1], Annu Yadav[1], P. Kumar[1], J.S. Arora[1], T.K.Datta[1], S. De[1], S.L. Goswami[1], Mukesh Yadav[2], Shalini Jain[3], Ravinder Nagpal[4] and Hariom Yadav[3]

[1]Department of Animal Biotechnology, [3]Animal Biochemistry Division and [4]Dairy Microbiology Division, National Dairy Research Institute, Karnal 132001 (Haryana), India; [2]SOS in Chemistry, Jiwaji University, Gwalior-474011, M.P., India

Different transfection protocols that can be divided into viral-based, non-viral based or combination of both (hybrid).



Chong ZX, Yeap SK, Ho WY. Transfection types, methods and strategies: a technical review. PeerJ. 2021 Apr 21;9:e11165. doi: 10.7717/peerj.11165.

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Transfection of Host Cells and Recombinant Protein Production

Transfection leads to

- **transient expression** of the introduced gene
  plasmids are rapidly diluted as a consequence of cell division or even degraded. However, it is
- **stably transfect cells** leading to long-expression periods.
  plasmid DNA has to integrate into the chromosomal DNA of the host cell.

- Stable transfection normally includes a selection gene into the expression vector (transfected cells have a selectable growth advantage) → the neomycin resistance gene (Neo^r) --> codes for a protein that neutralizes the toxic drug Geneticin, also known as G418.
  - → the selection process takes around 2 weeks, resulting in a tissue culture dish with several colonies.
  - →Each colony contains the descendants of 1 stably transfected cell.
  - →the cells from individual colonies have to be isolated and further expanded.
  - →quantification of the recombinant protein production to select cell culture with the highest yields.

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Production in Mammalian cells 1/3

Big challenge

→ **to scale up cell cultures** from lab scale (e.g., a 75 cm2 tissue culture bottle) to a large-scale production platform (like a bioreactor).

→ **Mammalian cells are relatively weak** and may easily become damaged by stirring or pumping liquid in or out a fermenter

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Production in Mammalian cells 2/3

Large-scale culturing of adherent (versus suspended) mammalian cells.

- The growth on the surface of small beads.
  Microcarriers *are* ***small beads****, either solid or macroporous, having a diameter of approximately* ***100–300 µm*** *with a density slightly higher than that of the growth medium*.
  - the surface of the beads will be completely covered (confluent) with cells,
  - it is necessary to detach the cells from the beads (usually the protease trypsin is used) and to redivide the cells over more (empty) beads
  - transfer to a bioreactor compatible with higher working volumes.

Important trypsinization process is well timed:

→if too short, many cells are still on the beads,

→if too long, the cells will lose their integrity and will not survive

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Cell Culture Technology -Ralf Pörtner, Uwe Jandt, and An-Ping Zeng
Industrial Biotechnology: Products and Processes, First Edition. Edited byChristophWittmann and JamesC. Liao.

Production in Mammalian cells 3/3

Some companies scaled up by **culturing and expanding** in **roller bottles.**

These bottles revolve slowly (between 5 and 60 revolutions/h), which bathes the cells that are attached to the inner surface with medium

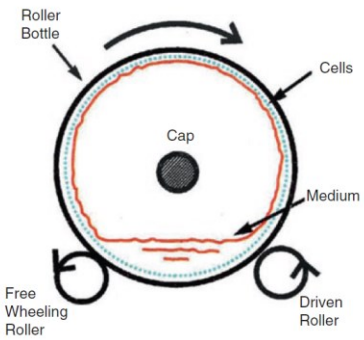

Figure 1.12 ■ Cell culturing in roller bottles

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https://doi.org/10.1007/978-3-030-00710-2 -

Recent Advances in Medium Development

- **Optimization of Growth Media** for recombinant protein expression
  - Industrial cell culture processes
    - **Media must be consistent in large-scale** manufacturing and preparation.
    - Dry powder and liquid **media formulations should be optimized for attributes affecting manufacturability**, such as component solubility, media filtration, sterilization, storage stability, media preparation scalability, and raw material consistency with a minimal lot-to-lot variation.
- **Modern cell culture manufacturing** processes utilize chemically defined, serum-free and animal-component free culture media.
  - This addresses issues around potential contamination with adventitious microbial or viral agents arising from serum or animal components, as well as consistency and reproducibility of undefined media components

Ritacco FV, Wu Y, Khetan A. Cell culture media for recombinant protein expression in Chinese hamster ovary (CHO) cells: History, key components, and optimization strategies. Biotechnol Prog. 2018 Nov;34(6):1407-1426. doi: 10.1002/btpr.2706.

Recent Advances in Medium Development

- Mammalian cell culture **media complex**,
  - often including **greater than 50 components**.
  - this complexity makes it challenging to ensure **scalable and reproducible media** preparation,
  - Typically, **a medium comprised of 1–2 premixed powders is ideal** for minimizing the complexity of media preparation.
  - **Additional components may be added individually** or in the form of supplements, depending on the attributes of the component in terms of solubility, stability, and sterilization requirements.

Ritacco FV, Wu Y, Khetan A. Cell culture media for recombinant protein expression in Chinese hamster ovary (CHO) cells: History, key components, and optimization strategies. Biotechnol Prog. 2018 Nov;34(6):1407-1426. doi: 10.1002/btpr.2706.

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Recent Advances in Medium Development

- Mammalian cell culture **media complex**,
  - **Virus control** (in medium preparation) is another significant manufacturability concern.

→ **Inactivation methods** employ ultraviolet (UV) irradiation, gamma irradiation, heat, extremes in pH, or solvent/detergent exposure.

→ For chemically defined media, ultrafiltration and high temperature-short time (HTST) treatments have been used to reduce the risk of viral introduction.

Ritacco FV, Wu Y, Khetan A. Cell culture media for recombinant protein expression in Chinese hamster ovary (CHO) cells: History, key components, and optimization strategies. Biotechnol Prog. 2018 Nov;34(6):1407-1426. doi: 10.1002/btpr.2706.

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Recent Advances in Medium Development

The optimization of a medium formulation can be very challenging
(greater than 50 components)

- **one-factor-at-a-time (OFAT)** approach to media optimization is very labour and resource-intensive for such complex formulas.

- **statistical design of experiments (DoE)** has been demonstrated to streamline and improve this effort, by evaluating multiple components and their interactions at once, **reducing the necessary size and number of experiments**, and allowing observation of complex interactions between medium components.

Ritacco FV, Wu Y, Khetan A. Cell culture media for recombinant protein expression in Chinese hamster ovary (CHO) cells: History, key components, and optimization strategies. Biotechnol Prog. 2018 Nov;34(6):1407-1426. doi: 10.1002/btpr.2706.

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Recent Advances in Medium Development

- New technologies can support automated and aseptic sampling of bioreactors,
  - **real-time analytics and diagnosis** via Process Analytical Technology (PAT) approaches (capacitance and Raman spectroscopy) allow the **development of feedback loop adjustments to medium composition.**
- High-throughput cell culture equipment and methodologies to decipher and control product quality, (product quality for **biosimilars**, sustain high cell densities long periods to enable continuous processing).

- → **The need for the development of highly customized media**, optimized for specific cell lines and processes to maximize cellular productivity.

Ritacco FV, Wu Y, Khetan A. Cell culture media for recombinant protein expression in Chinese hamster ovary (CHO) cells: History, key components, and optimization strategies. Biotechnol Prog. 2018 Nov;34(6):1407-1426. doi: 10.1002/btpr.2706.

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Recent Advances in Medium Development

- **Advances in "multiomics" technologies**

single cell: genomic, epigenomic, transcriptomic, proteomic, metabolomic etc.

→ enable an integrated and complete picture of metabolism and its control (**adjustments to medium composition)**.

Ritacco FV, Wu Y, Khetan A. Cell culture media for recombinant protein expression in Chinese hamster ovary (CHO) cells: History, key components, and optimization strategies. Biotechnol Prog. 2018 Nov;34(6):1407-1426. doi: 10.1002/btpr.2706.

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Cell culture systems used for the production of biopharmaceuticals

- **The available cell culture systems:**
  - non-agitated (multiwall plates, dishes and flasks, culture bags)
  - agitated
- **Cell culture bioreactors considered:**
  - gentle agitation without cell damage
  - well-controlled environment
  - respect to pH, temperature,
  - dissolved oxygen, and dissolved CO_2 concentration;
  - low levels of toxic metabolites (ammonia, lactate);
  - high cell and product concentrations;
  - optimized medium utilization;
  - surface for adherent cells;
  - scalability.

Cell Culture Technology -Ralf Pörtner, Uwe Jandt, and An-Ping Zeng
Industrial Biotechnology: Products and Processes, First Edition. Edited byChristophWittmann and JamesC. Liao.

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Cell culture systems used for the production of biopharmaceuticals

Cell culture processes can be operated similarly to other biotechnological **processes modes**:

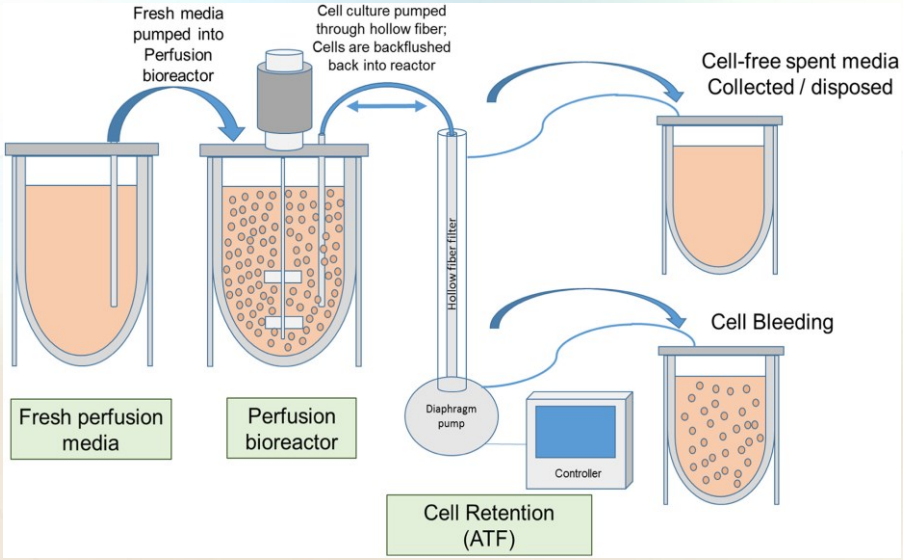
- Discontinuous (batch, repeated-batch, or fed-batch)
- Continuous (chemostat, perfusion)

Cell culture systems used for the production of biopharmaceuticals

Continuous system

- Chemostat: is a valuable tool for research (e.g., kinetic studies), but is **rarely considered for the production-scale** operation.
- Perfusion: the ability to **grow cells** to a very **high density**, the ease of **handling media exchanges** for fresh feed and **product harvest**, product stability associated with short residence time in the culture (an advantage for the production of labile proteins), the **easy removal of metabolites** and other **inhibitors**, and the prospect of **easy scale-up**.

Cell culture media for recombinant protein expression in Chinese hamster ovary (CHO) cells: History, key components, and optimization strategies



Biotechnology Progress, Volume: 34, Issue: 6, Pages: 1407-1426, First published: 05 October 2018, DOI: (10.1002/btpr.2706)

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	Step	Techniques and purpose
Upstream	Seed-train	Step-wise increase in scale to provide inoculum
	⇩	
	Cultivation	Bioreactor on production scale, process strategy (*batch*, *fed-batch*, perfusion), chemically defined medium
	⇩	
Downstream	Harvesting and clarification	Centrifugation/filtration for removal of cells and debris →clarified culture prior to chromatography
	⇩	
	Capture	Protein A chromatography → highly purified product
	⇩	
	Virus inactivation	Low pH → inactivation of endogenous or adventitious viruses
	⇩	
	Polishing	Ionexchange chromatography → removal of product/process related impurities nanofiltration → removal of endogenous or adventitious viruses, hydrophobic interaction chromatography, ultra-/diafiltration → bulk formulation
	⇩	
	Bulk product	

Figure 4.4 Scheme of steps involved in biopharmaceutical production.

Cell Culture Technology -Ralf Pörtner, Uwe Jandt, and An-Ping Zeng
Industrial Biotechnology: Products and Processes, First Edition. Edited byChristophWittmann and JamesC. Liao.

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Permanent cell lines	Characteristics
Chinese hamster ovary (CHO)	Adherent cells, can be adapted to suspension, used for production of recombinant proteins (e.g., HBstg, tPA, factor VIII; Therapeutic antibodies ….)
COS (monkey kidney) HEK-293 (human embryonic kidney)	Used for transient protein expression
Baby hamster kidney (BHK)	Adherent cells, can be adapted to suspension, used for production of foot and mouth disease vaccines, rabies vaccine, recombinant proteins (e.g., factor VIII)
MDCK (canine kidney)	Adherent cell line with good growth characteristics, animal vaccines
MRC-5 (human embryonic lung cells)	"Normal" cells with a finite life span, vaccine production
NAMALWA (human lymphatic tissue)	Used for production of alpha-interferon
NS0 and SP2/0 (mouse myeloma from B-lymphocytes)	Used for antibody production
PERC.6 (human embryonic retina cells)	Immortalized cell line, well characterized, produce high levels of recombinant proteins and viruses
Vero (long-tailed monkey kidney)	Established cell line, but with some characteristics of the normal diploid cells
WI-38 (human embryonic lung cells)	"Normal" cells with a finite life span, vaccine production

Cell Culture
Industrial Biotechnology: Products and Processes, First Edition. Edited byChristophWittmann and JamesC. Liao.

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3D In Vitro Tumor Models: What Cancer Hallmarks Are Accessible for Drug Discovery?

DOI: (10.1002/adhm.201701174)

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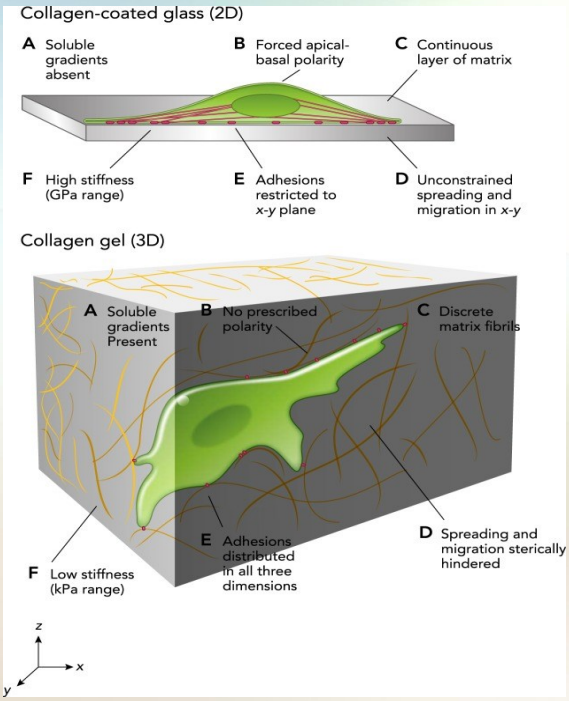
Cells in 2D and 3D

Cells in 2D and 3D microenvironments interact differently with their surroundings due to differences in the cues, mechanical and chemical, that they experience

Reprinted from Ref. 2, with permission from Journal of Cell Science.

Physiology (Bethesda). 2017;32(4):266–277. doi:10.1152/physiol.00036.2016



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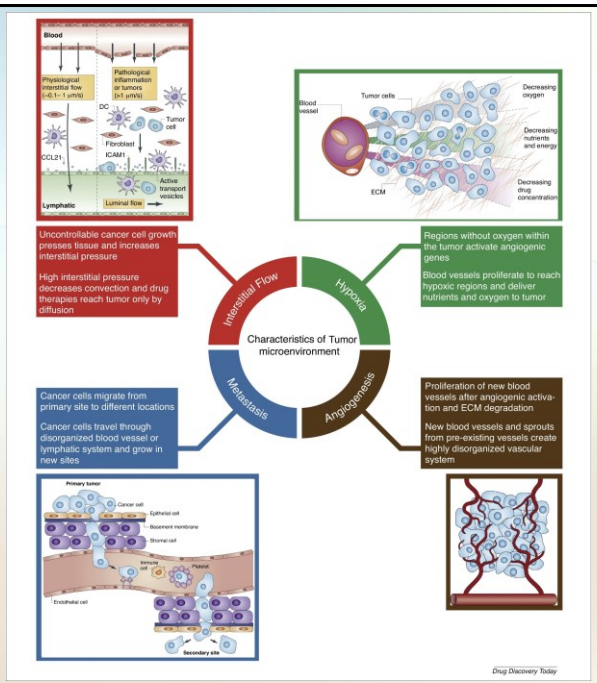
Characteristics of the tumour microenvironment

Abnormal interstitial flow decreases the efficacy of drug therapies.

Hypoxia caused by absence of oxygen results in the activation of angiogenic genes.

Angiogenesis resulting from the activation of angiogenic genes causes the proliferation of blood vessels and a disorganized blood network.

Tumor metastasis results from the migration of cancer cells from the primary site to different



Drug Discov Today. 2017 Nov;22(11):1654-1670. doi: 10.1016/j.drudis.2017.06.010.

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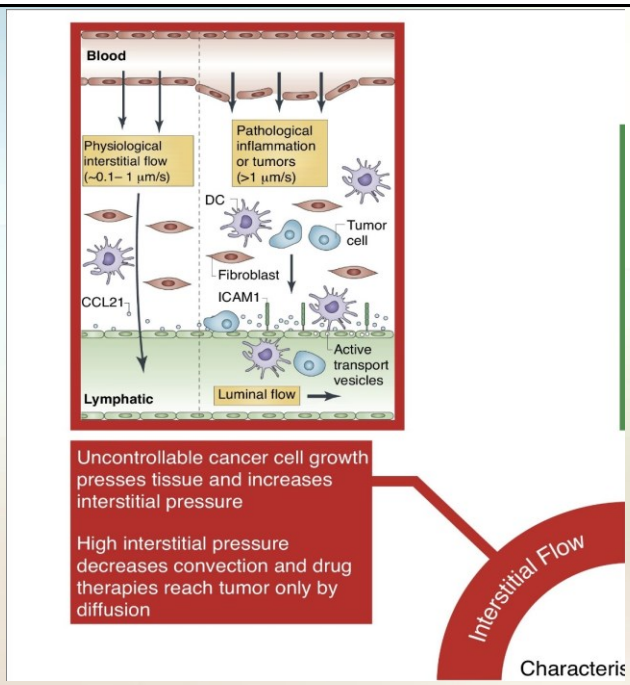

Drug Discov Today. 2017 Nov;22(11):1654-1670. doi: 10.1016/j.drudis.2017.06.010.

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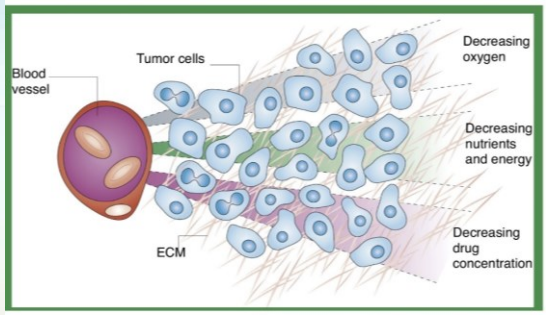

Regions without oxygen within the tumor activate angiogenic genes

Blood vessels proliferate to reach hypoxic regions and deliver nutrients and oxygen to tumor

Hypoxia

stics of Tumor

Drug Discov Today. 2017 Nov;22(11):1654-1670. doi: 10.1016/j.drudis.2017.06.010.

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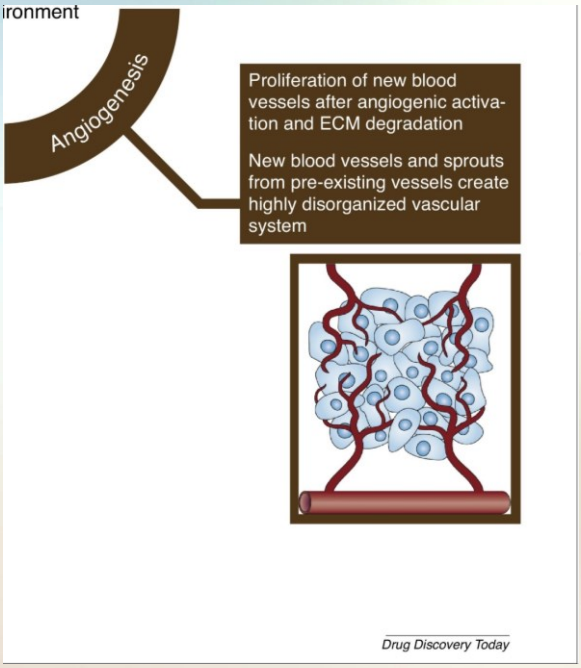

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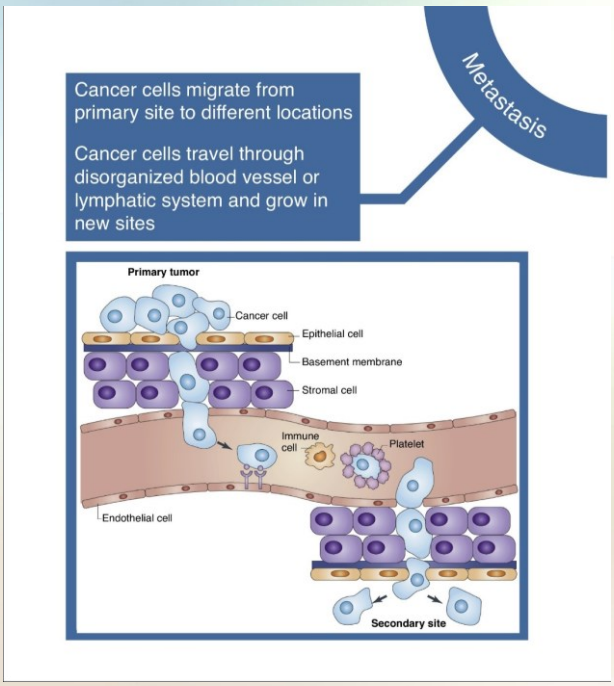

Drug Discov Today. 2017 Nov;22(11):1654-1670. doi: 10.1016/j.drudis.2017.06.010.

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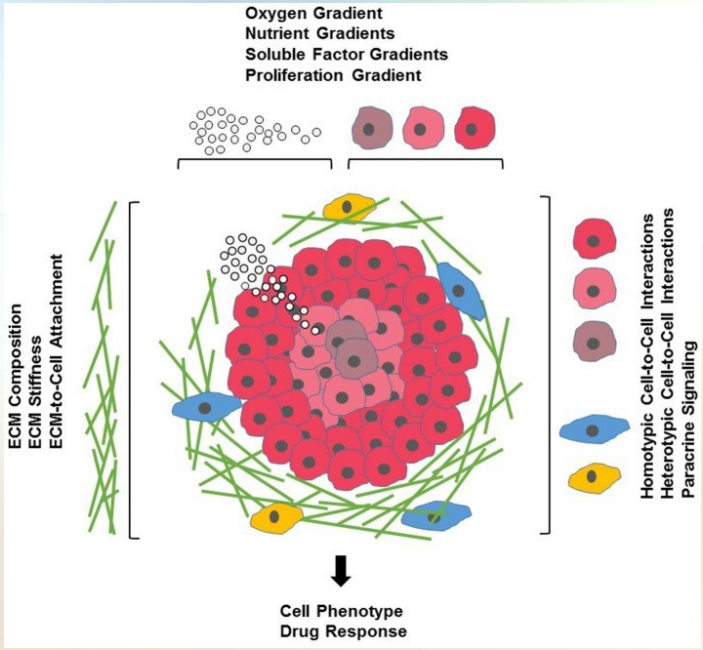
Cells and their microenvironment



Sigrid A. Langhans - Front Pharmacol. 2018; 9: 6 - doi: 10.3389/fphar.2018.00006

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Summary of 2D and 3D in vitro cell culture models

TABLE 1

Summary of 2D and 3D *in vitro* cell culture models

Study	2D	3D	Refs
Cytotoxic response of trastuzumab in breast cancer (SKBR-3) and ovarian cancer (SKOV-3) in 2D and 3D *in vitro* models	Trastuzumab only slightly reduced cell proliferation in the monolayers (~10% inhibition)	48% proliferation inhibition seen for SKBR-3 and ~35% inhibition for SKOV-3	[44]
Study of tumorigenic capability of breast cancer cells (MCF-7) cultured in 2D and 3D models	2D-derived tumor implanted into mice and, after 5 weeks, resulted in a tumor weighing 0.17 ± 0.27 g	3D-derived tumor implanted in mice created a tumor weighing 0.7 ± 0.26 g after same 5 weeks	[57]
Impact of ECM on therapeutic effect of doxorubicin on human cervical (HeLa) cells cultured in 2D and 3D collagen matrices	After 24 h of exposure to doxorubicin, toxic effect of drug in 2D cell culture generated IC_{50} = 1.2 ± 0.3 μM	Effect of doxorubicin in 3D cell models was lower than in 2D cultures, with IC_{50} = 3.6 ± 1.33 μM	[58]
Effect of matrix stiffness on breast cancer cells (MCF-7)	Cells cultured in a petri dish showed a flat shape	3D-cultured cells maintained a circular shape, forming 300-μM spheroid clusters in less stiff alginate matrices	[59]

Drug Discov Today. 2017 Nov;22(11):1654-1670. doi: 10.1016/j.drudis.2017.06.010.

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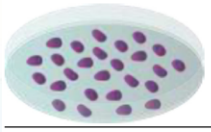
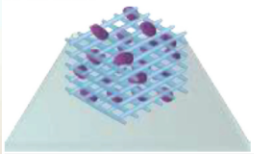
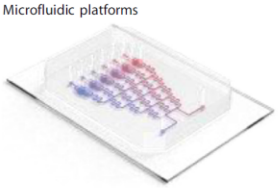
Advantages and disadvantages of in vitro cell culture models

TABLE 2
Advantages and disadvantages of *in vitro* cell culture models

Culture model	Advantages	Disadvantages
2D cell culture 	Methodology well established Simplicity to work with cell monolayer	Static conditions Uniform concentration of nutrients and drugs Lack of 3D environment Large reagent volumes
3D cell culture 	Cell–cell and cell–ECM interactions Sensitivity to cytotoxic agents similar to *in vivo*	Failure to produce dynamic environment Lack of fluid flow perfusion
Microfluidic platforms 	Higher control of environment Diffusion of nutrients and drugs Cost-effective Combination of CGG, biosensors, and mechanical stimuli High-throughput assays	Nonstandardized protocols PDMS can adsorb molecules Perfusion of more than one growth medium can be challenging

Drug Discov Today. 2017 Nov;22(11):1654-1670. doi: 10.1016/j.drudis.2017.06.010.

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The Current Landscape of 3D In Vitro Tumor Models: What Cancer Hallmarks Are Accessible for Drug Discovery?

Validate biological relevance

- Demonstrate clinical relevance of data for a specific disease.
- Demonstrate can identify (ideally) a novel target that validates in vivo

Creation of industrialization pipeline

- Establish standards in performance and reproducibility and define required benchmarking assays.
- Design with scaling and manufacturing in mind.
- Demonstrate feasibility to implement scalable manufacturing processes and to establish model distribution mechanisms.
- Establish scalable data analysis pipelines

Customize/define application space

- Integrate primary patient cell materials
  - Define medium formulation
  - Assess cell proportions and phenotype over time to help assess usability time.
  - Define how complex is enough.
- Define domains of validity for specific assays
- Create criteria to define/select appropriate disease biology to model *in vitro*.

Quantify value proposition

- Demonstrate types of novel biology accessible that lead to clinically relevant target identification.
- Quantify impact of model use on overall drug development process (can you find targets faster and cheaper?)
- Increase communication between academia and industry to ensure models are being designed to add value to drug development pipeline.

The Current Landscape of 3D In Vitro Tumor Models: What Cancer Hallmarks Are Accessible for Drug Discovery?, Volume: 7, Issue: 8, First published: 19 January 2018, DOI: (10.1002/adhm.201701174)

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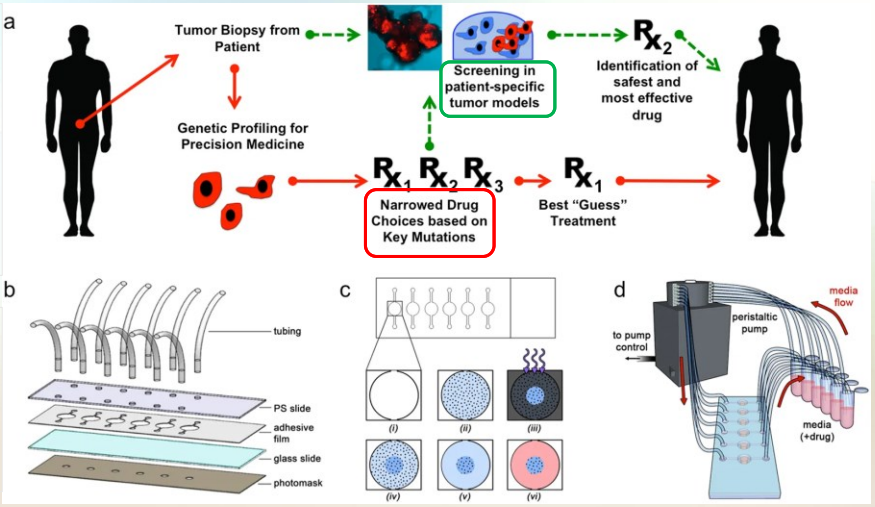
In vitro patient-derived 3D mesothelioma tumor organoids facilitate patient-centric therapeutic screening

Red arrows: The current state of the art precision medicine pipeline, in which treatments are identified for patients based on their tumor genetic profiles. In practice, even after identification of key mutations, oncologists are often left with several potential drug options, resulting in a best guess of the optimal treatment.

Green arrows: Implementation of organoids created with patient cells can supplement genetic screens of biopsied tumor cells, ultimately predicting the optimal therapies for patients.



Scientific Reports **volume 8**, Article number: 2886 (2018)