Cell-based Assay

Andrei Leitão

Phenotypic screening in cancer drug discovery — past, present and future

John G. Moffat[1], Joachim Rudolph[2] and David Bailey[3]

A landmark study by Swinney and Anthony found that among the 183 small-molecule drugs across all therapeutic areas approved between 1999 and 2008, 58 (32%) were discovered using phenotype-based approaches. Importantly, 28 (56%) of the 50 small-molecule first-in-class new molecular entities (NMEs) identified in their study resulted from phenotypic screening approaches (phenotypic drug discovery; PDD), whereas 17 (34%) resulted from target-based approaches.

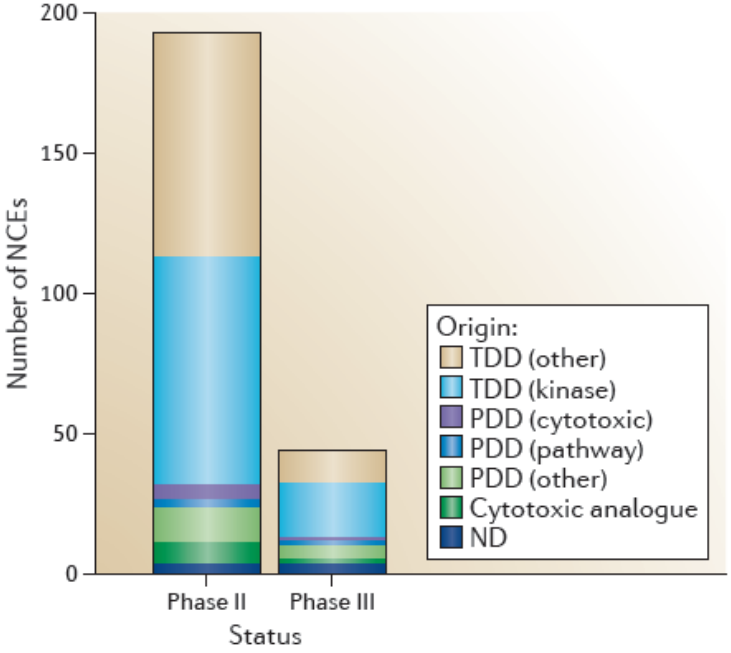

Figure 2 | **Origins of new chemical entities in clinical trials for cancer.** New chemical entities (NCEs) are classified by the assay strategy used to identify the starting chemical matter and/or the clinical candidate. 'ND' refers to cases where we were unable to determine the origin of the NCE from publicly available information. 'Cytotoxic analogue' refers to rationally designed analogues or prodrugs of a known cytotoxic drug. 'PDD analogue' refers to phenotypic drug discovery (PDD) assays that were used to select and optimize analogues of drugs or probes with known targets and/or mechanisms of action. 'PDD (cytotoxic)' refers to viability and cytotoxicity assays with cancer cell lines. 'PDD (pathway)' refers to assays for the activation or repression of molecular responses, including reporter gene expression or protein phosphorylation driven by a defined signalling pathway with multiple upstream steps and potential targets. 'TDD (kinase)' refers to the target-based drug discovery (TDD) of kinase inhibitors. The assignments were based on research using the Citeline Pharmaprojects database and citations therein in August 2013.

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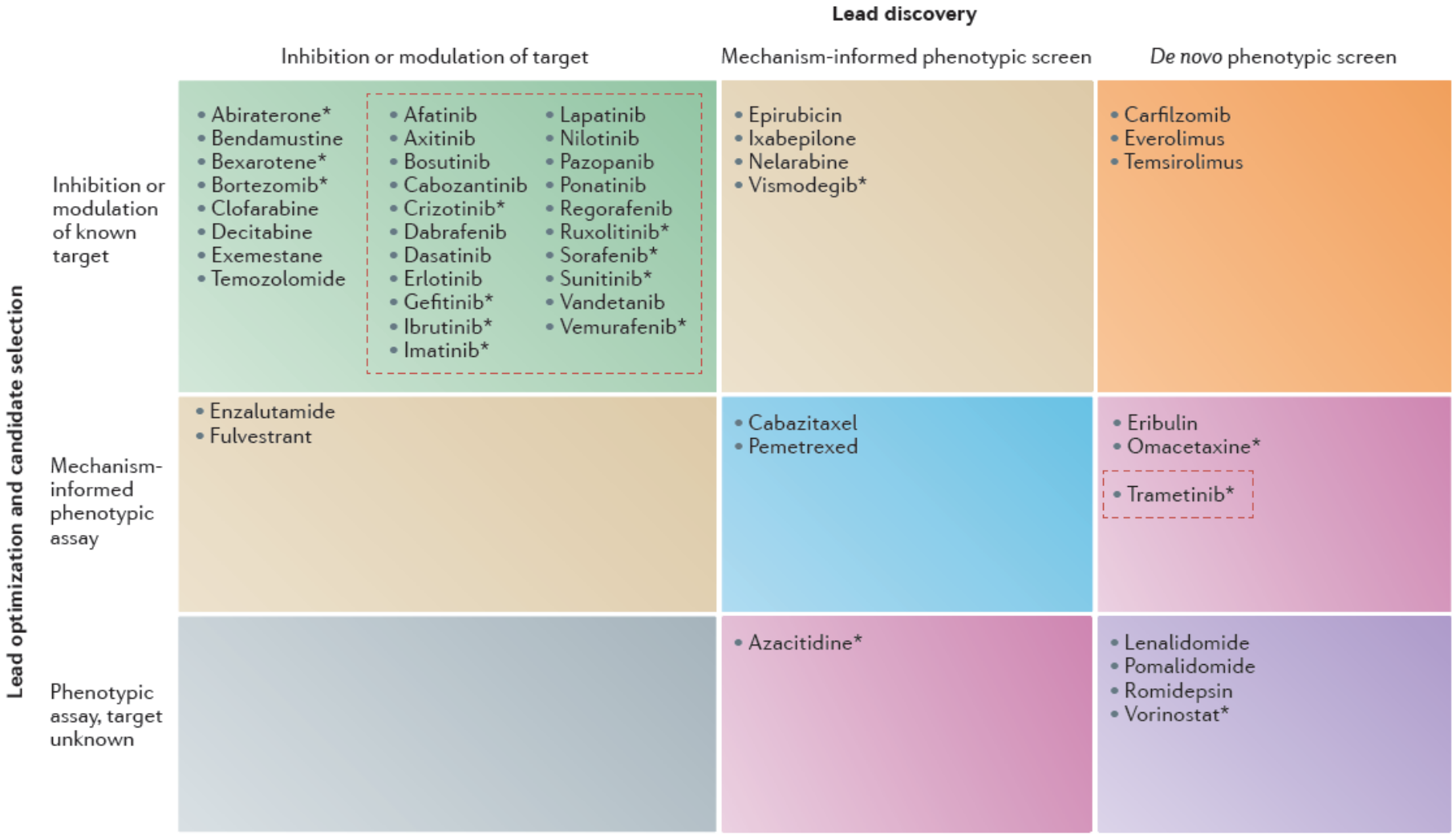
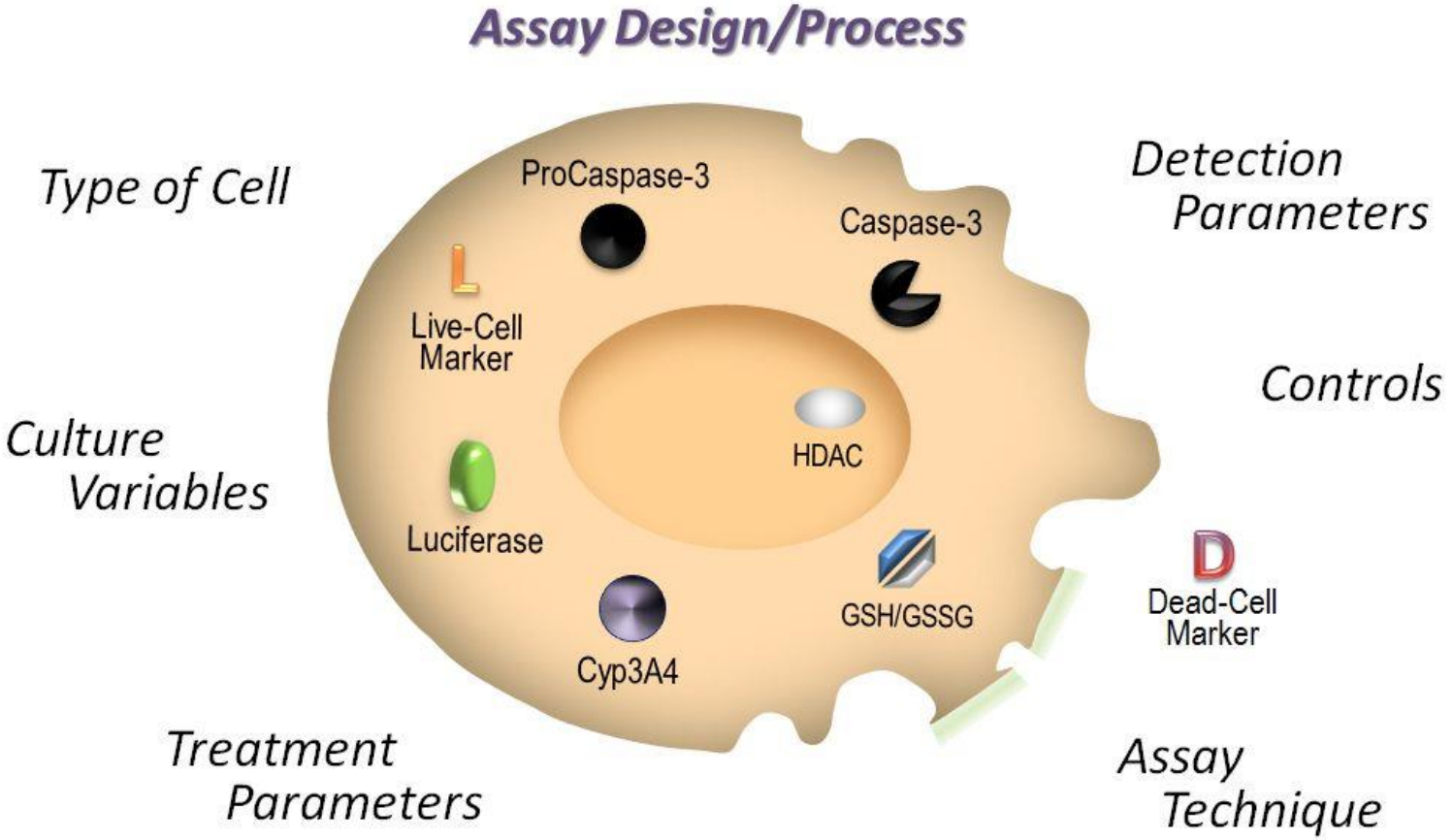


Figure 1 | **Origins of new small-molecule cancer drugs approved by the FDA between 1999 and 2013.** Kinase inhibitors are highlighted within the dotted boxes. Information on the drugs to be analysed was obtained from the US Food and Drug Administration (FDA) website. *First-in-class drug.

Planning the assay



3D cell culture

Andrei Leitão

2D *versus* 3D models

Development, validation and pilot screening of an in vitro multi-cellular three-dimensional cancer spheroid assay for anti-cancer drug testing

Rati Lama [a], Lin Zhang [a], Janine M. Naim [a], Jennifer Williams [a], Aimin Zhou [a,b], Bin Su [a,b,*]

H292 non-small cell lung cancer cells

tubulin inhibitors

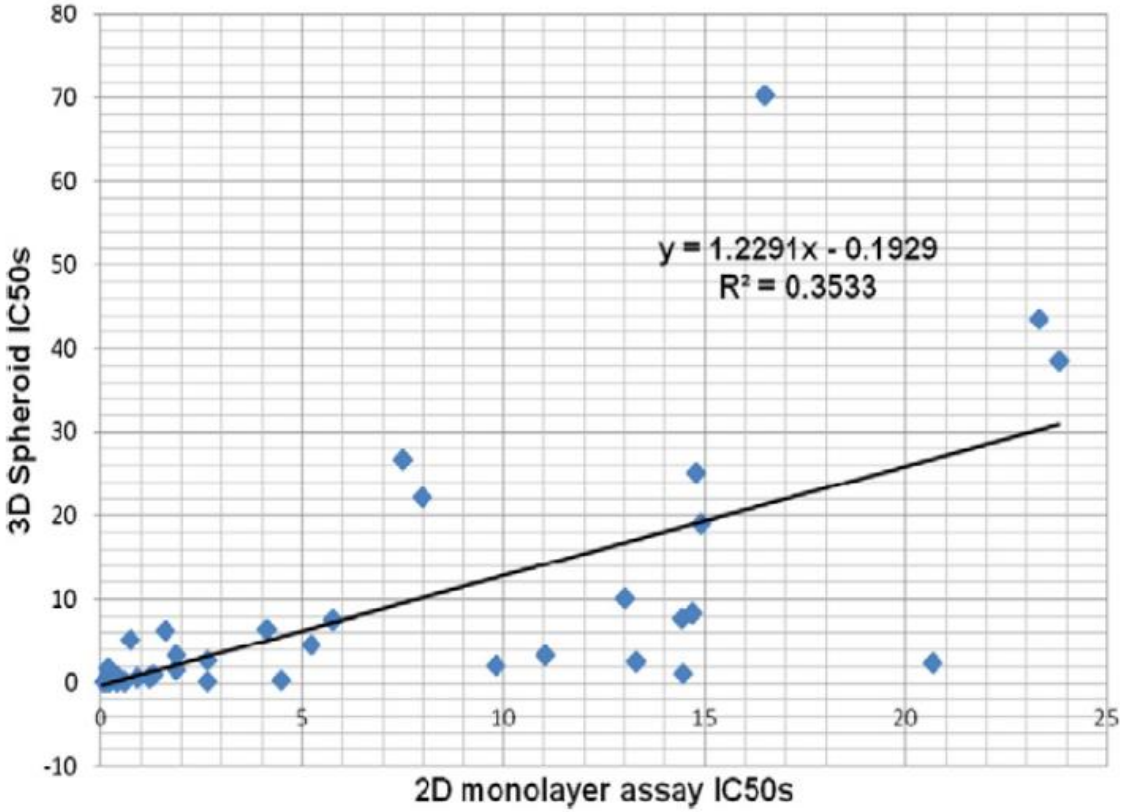


Figure 4. Correlation study of inhibitory effects of sulfonamide tubulin inhibitors on 2D monolayer cell proliferation and 3D spheroid growth.

Bioorganic & Medicinal Chemistry 21 (2013) 922–931

2D *versus* 3D models

Table 2. Comparison of drug testing results on 2D and 3D cultures.

Cell Cultures	Drugs	2D	3D
HCT-116 wt HCT-116 wt/GFP	5-FU, oxaliplatin, irinotecan, melphalan	equally and highly sensitive to 5-FU, oxaliplatin, irinotecan and melphalan	resistant or almost totally resistant to 4 standard drugs
NHEK	gefitinib	antiviral activity in concentrations too high for in vivo applications	gefitinib at concentration 0.5 μM was sufficient to induce meaningful reduction of replication and spreading of virus
SW1353	DXR, CIS, CQ	cell viability in 2D cultures were lower than in 3D cell cultures	cell viability in spheroid cultures were higher than in 2D cell cultures
	SAL	similar results for monolayers and 3D cell cultures	

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3D models

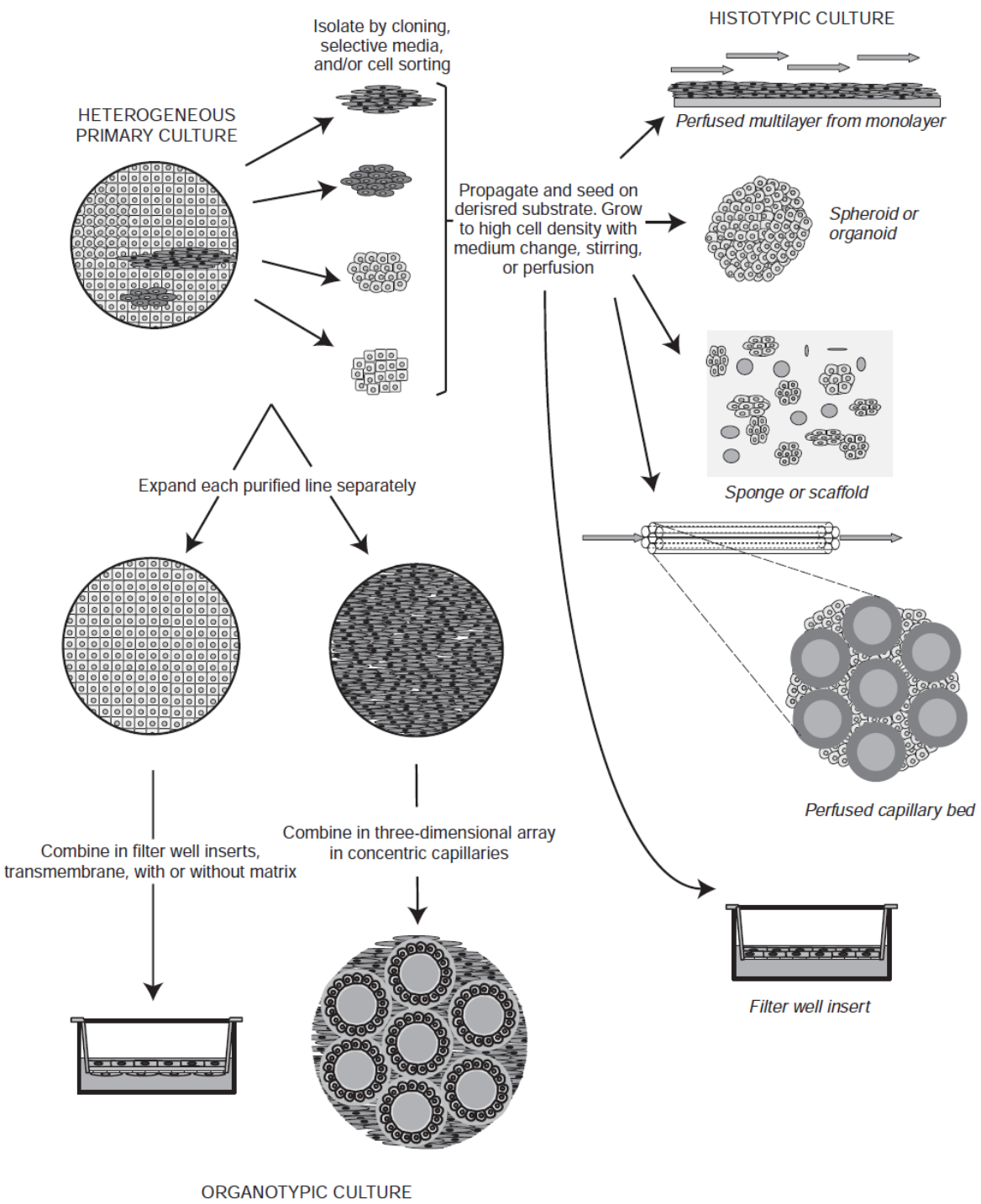
There are three main types of three-dimensional culture:

(1) *organ culture*, in which whole organs, or representative parts, are maintained as small fragments in culture and retain their intrinsic distribution, numerical and spatial, of participating cells;

(2) *histotypic culture*, in which propagated cells are grown alone to high density in a three-dimensional matrix or are allowed to form three-dimensional aggregates in suspension;

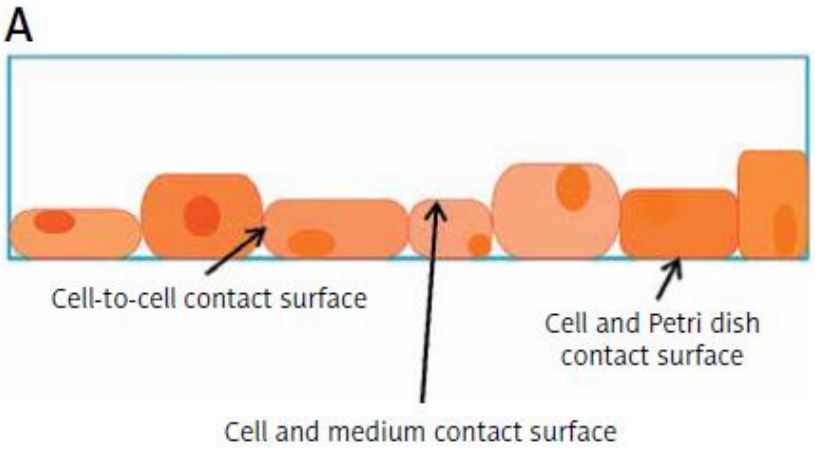
(3) *organotypic culture*, in which cells of different lineages are recombined in experimentally determined ratios and spatial relationships to recreate a component of the organ under study (Fig. 25.2)

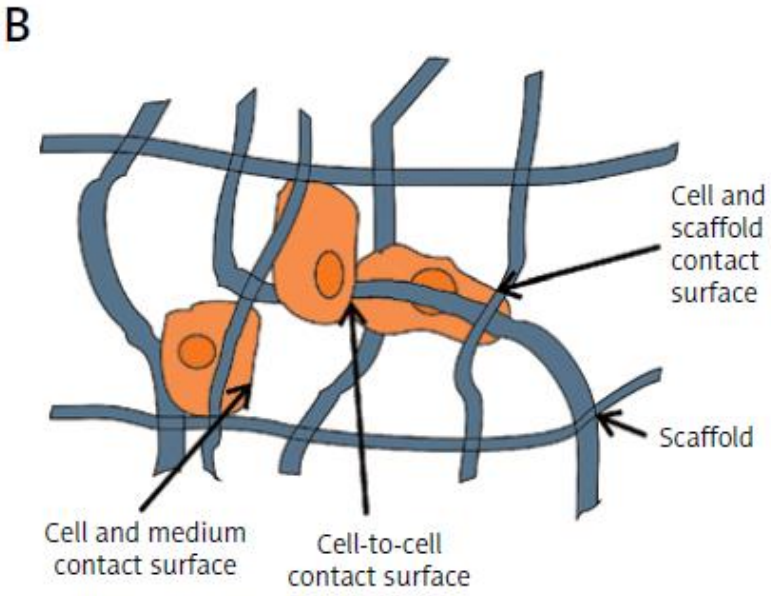
R. Ian Freshney, *Culture of Animal Cells: A Manual of Basic Technique and Specialized Applications, 6th ed.*, Chapter 25



Different types of cell culture

2D *versus* 3D models





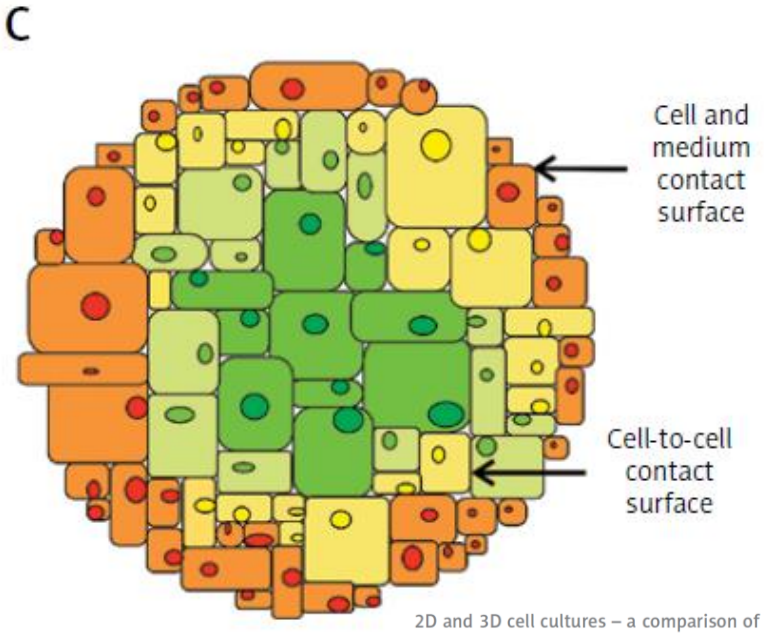


Figure 1. Types of cell culture methods commonly used in research studies. **A** – Cells flattened in a monolayer on the bottom of the culture vessel. They are in contact with the culture vessel, neighbouring cells, and the culture medium. **B** – Cells attached to a scaffold are in contact with the scaffolding, neighbouring cells, and the culture medium. **C** – A group of cells suspended in the culture medium or cultivated in gel-like substance; the cells are in contact with neighbouring cells and with the culture medium

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2D and 3D cell cultures – a comparison of different types of cancer cell cultures

Marta Kapałczyńska[1], Tomasz Kolenda[1,2], Weronika Przybyła[1], Maria Zajączkowska[1], Anna Teresiak[1], Violetta Filas[3], Matthew Ibbs[3], Renata Bliźniak[1], Łukasz Łuczewski[4], Katarzyna Lamperska[1]

2D *versus* 3D models

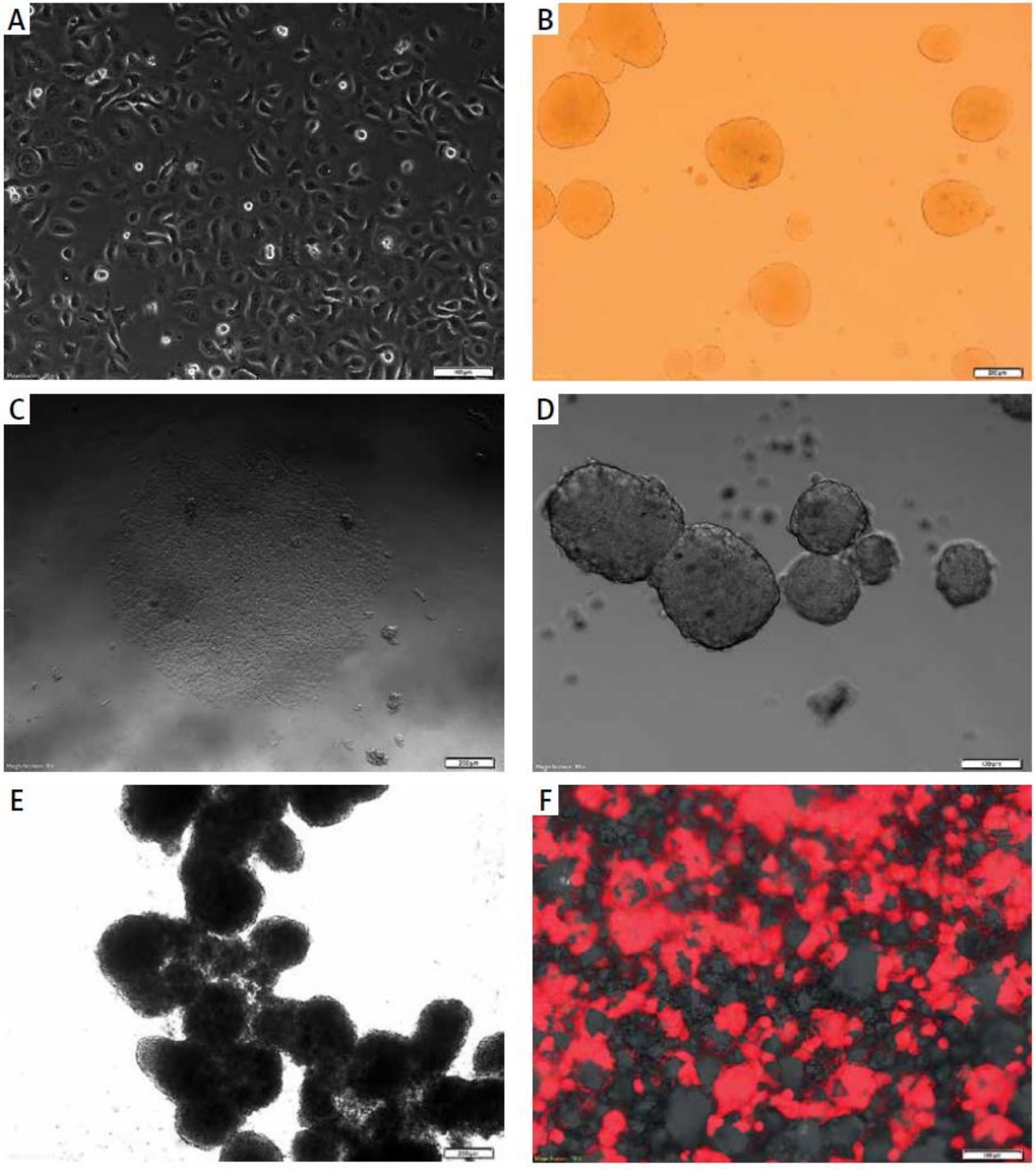

Figure 2. FaDu cell line cultured under various conditions. The FaDu cells were maintained in adherent conditions with standard medium (10% FBS) and next detached and placed as single cells in different (**A–F**) culture conditions in standard medium. **A** – flattened cells growing as a monolayer under 2D conditions (scale bar represents 100 µm); **B** – 3D structures in soft agar, single cells suspended in a gel are visible (scale bar represents 200 µm); **C** – adherent colonies formed between layers of soft agar (scale bar represents 200 µm); **D** – 3D structure formed on non-adherent plate (scale bar represents 100 µm); **E** – tissue-like structures formed by attached single spheres cultivated on ultra-low attachment plates (scale bar represents 200 µm); **F** – cells (red) cultured using 3D scaffold system with visible membrane pores (scale bar represents 100 µm)

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2D *versus* 3D models

Table I. Comparison of 2D and 3D cell culture methods

Type of culture	2D	3D	Ref.
Time of culture formation	Within minutes to a few hours	From a few hours to a few days	[11, 34, 57]
Culture quality	High performance, reproducibility, long-term culture, easy to interpret, simplicity of culture	Worse performance and reproducibility, difficult to interpret, cultures more difficult to carry out	[12]
In vivo imitation	Do not mimic the natural structure of the tissue or tumour mass	*In vivo* tissues and organs are in 3D form	[35]
Cells interactions	Deprived of cell-cell and cell-extracellular environment interactions, no *in vivo*-like microenvironment and no "niches"	Proper interactions of cell-cell and cell-extracellular environment, environmental "niches" are created	[13, 28, 29, 36, 37]
Characteristics of cells	Changed morphology and way of divisions; loss of diverse phenotype and polarity	Preserved morphology and way of divisions, diverse phenotype and polarity	[1, 14–17, 20, 38]
Access to essential compounds	Unlimited access to oxygen, nutrients, metabolites and signalling molecules (in contrast to *in vivo*)	Variable access to oxygen, nutrients, metabolites and signalling molecules (same as *in vivo*)	[10, 46]
Molecular mechanisms	Changes in gene expression, mRNA splicing, topology and biochemistry of cells	Expression of genes, splicing, topology and biochemistry of cells as *in vivo*	[23–26, 42–45]
Cost of maintaining a culture	Cheap, commercially available tests and the media	More expensive, more time-consuming, fewer commercially available tests	[8, 48, 58, 75]

3D models

Table II. Characteristics of different 3D cell culture methods

Type of 3D system	Description of cell culture	Advantages	Disadvantages	Ref.
Suspension cultures on non-adherent plates	• Single cells are seeded on non-adherent plates with medium • 3D structures can be observed after 3 days of culture	• Simplicity, easiness and speed of conducting culture • Bacterial plates or non-adherent culture plates can be used but only for some cell lines • Cells can be easily extracted from the medium and used for further experiments	• Some cell lines need expensive plates coated with specific materials, for example polystyrene or covalently bound hydrogel, because of strong adhesion abilities of cells • Formation of aggregates of cells as a result of cells' movement in medium	[8, 48, 58, 59]
Cultures in concentrated medium or in gel-like substances	• Single cells grow in medium containing substances with gelling properties: i) dissolved low-melting agarose with cell medium is poured on plate and incubated until solidifying to obtain the first, lower layer; the top layer consisting of agarose and the medium with single cells is added; ii) the cells are flooded in Matrigel (multiprotein hydrogel) • 3D structures can be observed after 7 days of culture	• Soft agar allows to study both the growth of a single cell regardless of attachment and the phenomenon of escape from anoikis • Cells cultured in Matrigel can be easily recovered for further analysis • Cells in Matrigel have three-dimensional interactions with the local environment and form tissue-like structures • Used to study the aggressiveness of the cells and their potential for metastasis	• Difficulty in obtaining spheres for certain lines, inconvenient and time-consuming preparation of the two layers of agar and requirement of long-term cultures • Low repeatability of the results • The difficulty of extracting cells from the agar and immunofluorescence staining of spheres, • Materials constituting the Matrigel contain endogenous bioactive ingredients that influence the structure formation	[7, 48, 58, 59, 75–81]
Cultures on scaffold	• The cells can migrate among fibres and attach to the scaffold, made of biodegradable material such as silk, collagen, laminin, alginate, and fill the space among fibres, grow and divide	• System is compatible with commercially available functional tests, as well as with DNA/RNA and protein isolation kits • Easy to prepare for immunohistochemical analysis	• Cells attached to the scaffolds flatten and spread like the cells cultured under adherent conditions • Scale of scaffolds and topography of cell distribution may cause various behaviour of the cell • Materials used to construct the scaffold may affect the adhesion, growth and cell behaviour • Cell observation and cell extraction for some analyses are restricted	[7, 8, 37, 82–90]

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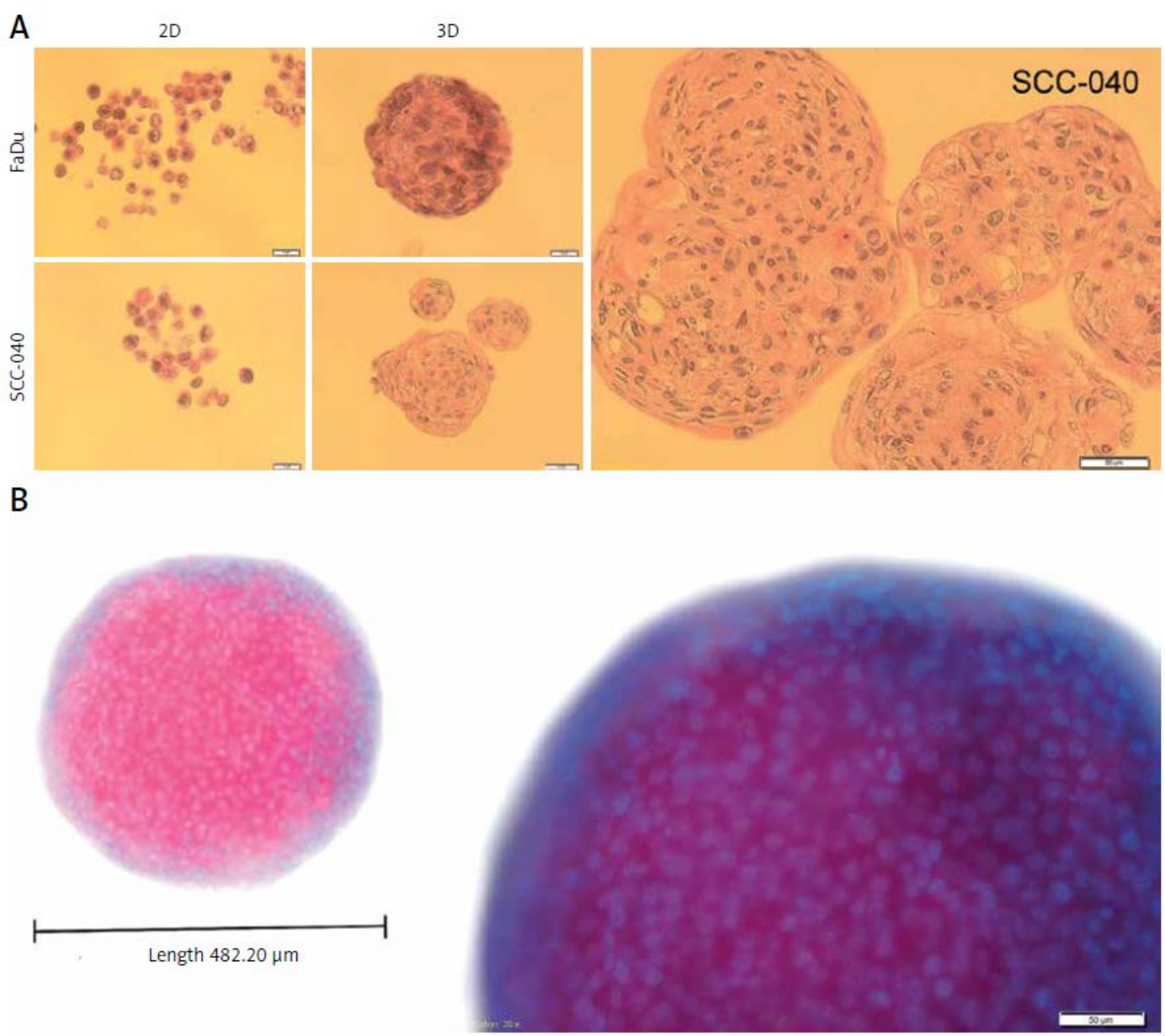
Spheroids



FaDu: Hypopharyngeal squamous cell carcinoma

SCC-040: Tongue squamous cell carcinoma

Figure 3. Structural architecture of 3D spheroids. The SCC-040 and FaDu cells were maintained in adherent condition with standard medium (10% FBS) and next detached and placed as single cells on non-adherent plates in standard medium. The created spheroids were taken to make the formalin-fixed paraffin-embedded tissue sections (FFPET) and H&E staining as well as DAPI staining. **A** – cross section through the cells growing in 2D and 3D cultures of SCC-040 and FaDu cell lines, H&E staining (scale bars represent 20 µm and 50 µm, respectively); **B** – 3D structure stained with DAPI; blue – nuclei, pink – cells (scale bar represents 50 µm)

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Spheroids: core & nutritional facts

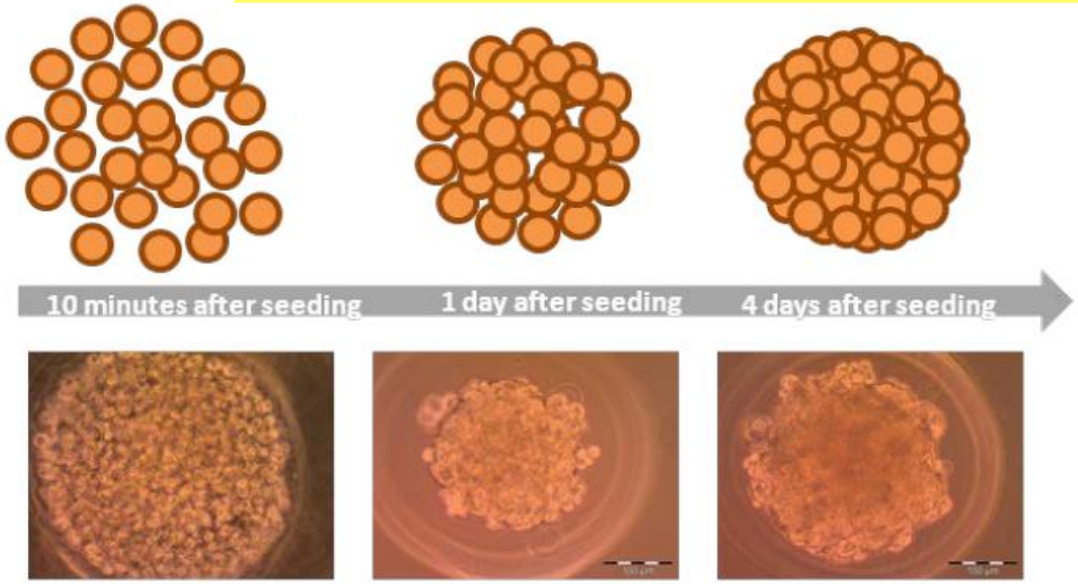
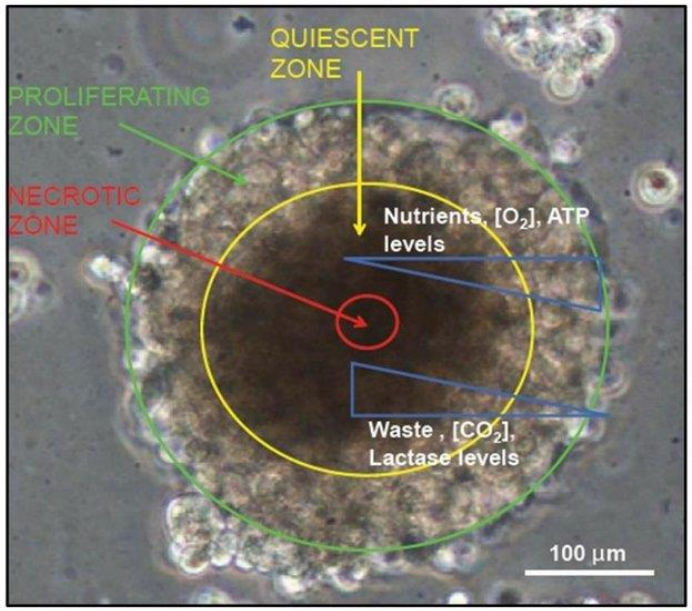
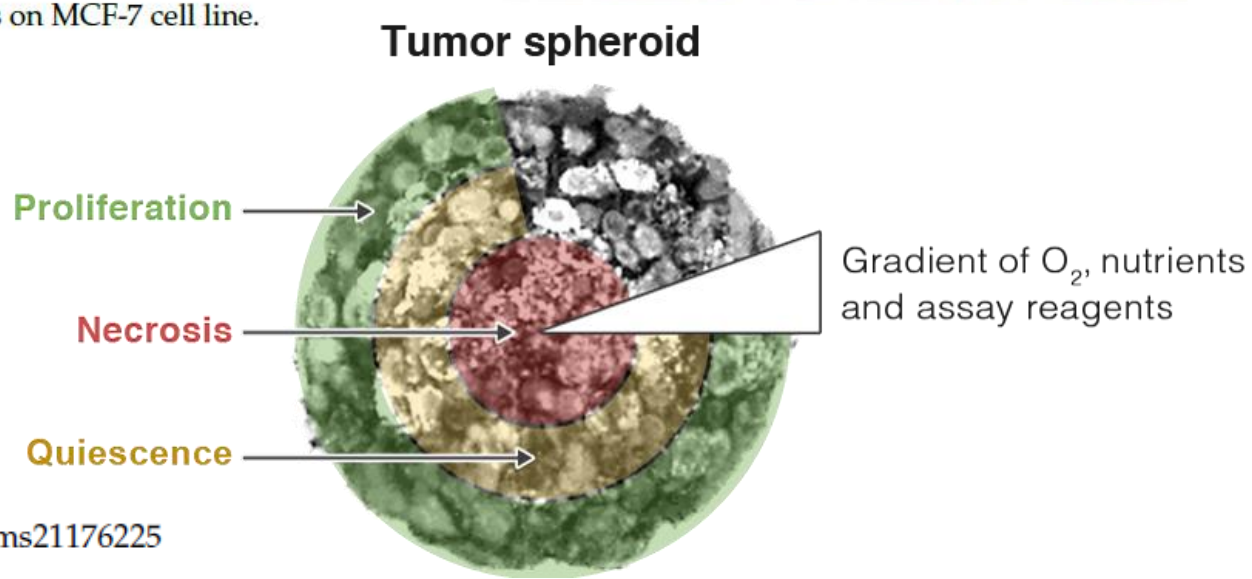


Figure 1. Formation of spheroids on MCF-7 cell line.



Janina Kuen (Thesis) Influence of 3D tumor cell/fibroblast co-culture on monocyte differentiation and tumor progression in pancreatic cancer. Faculty of Biology, University Würzburg



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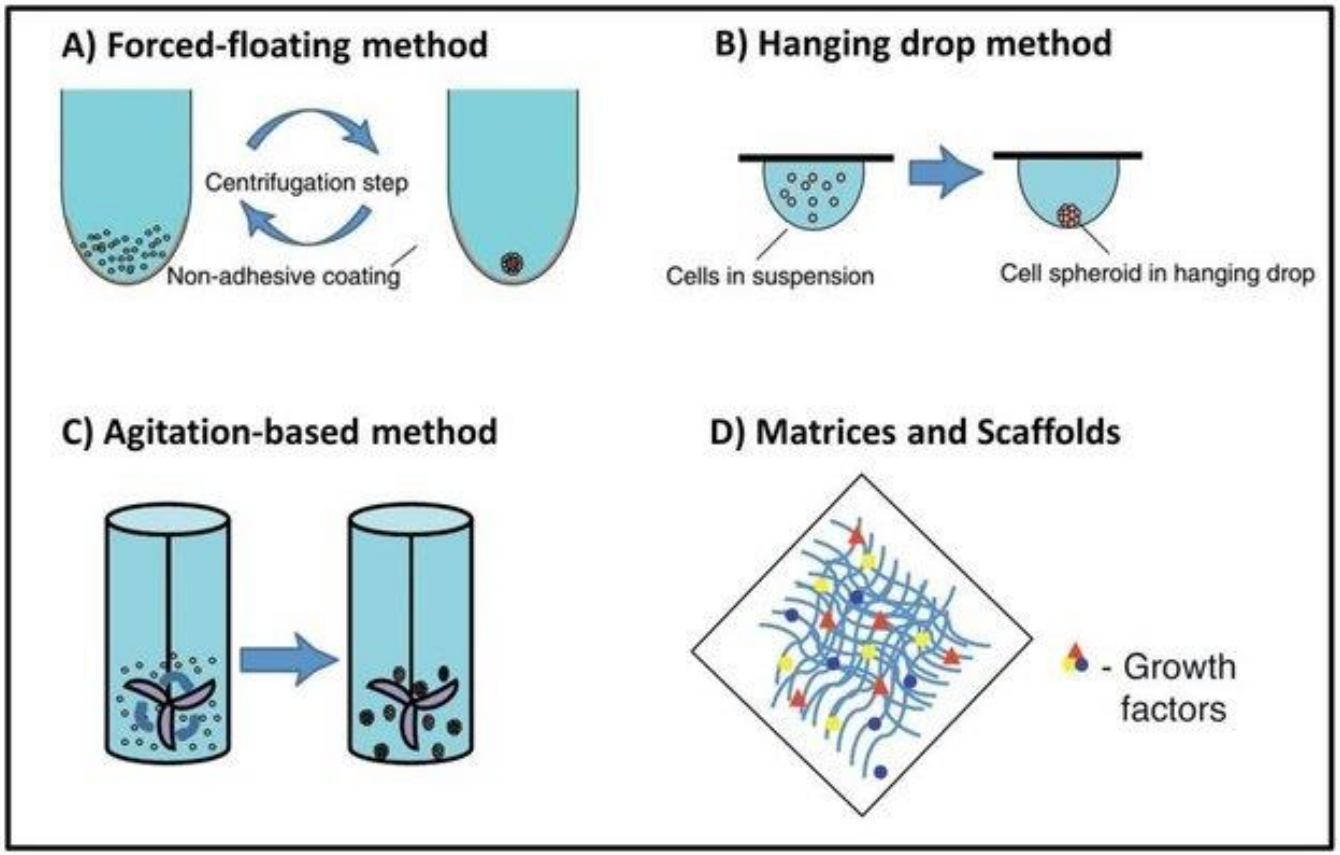
https://www.promega.com.br/resources/guides/cell-biology/3d-cell-culture-guide/

Common methods to produce spheroids



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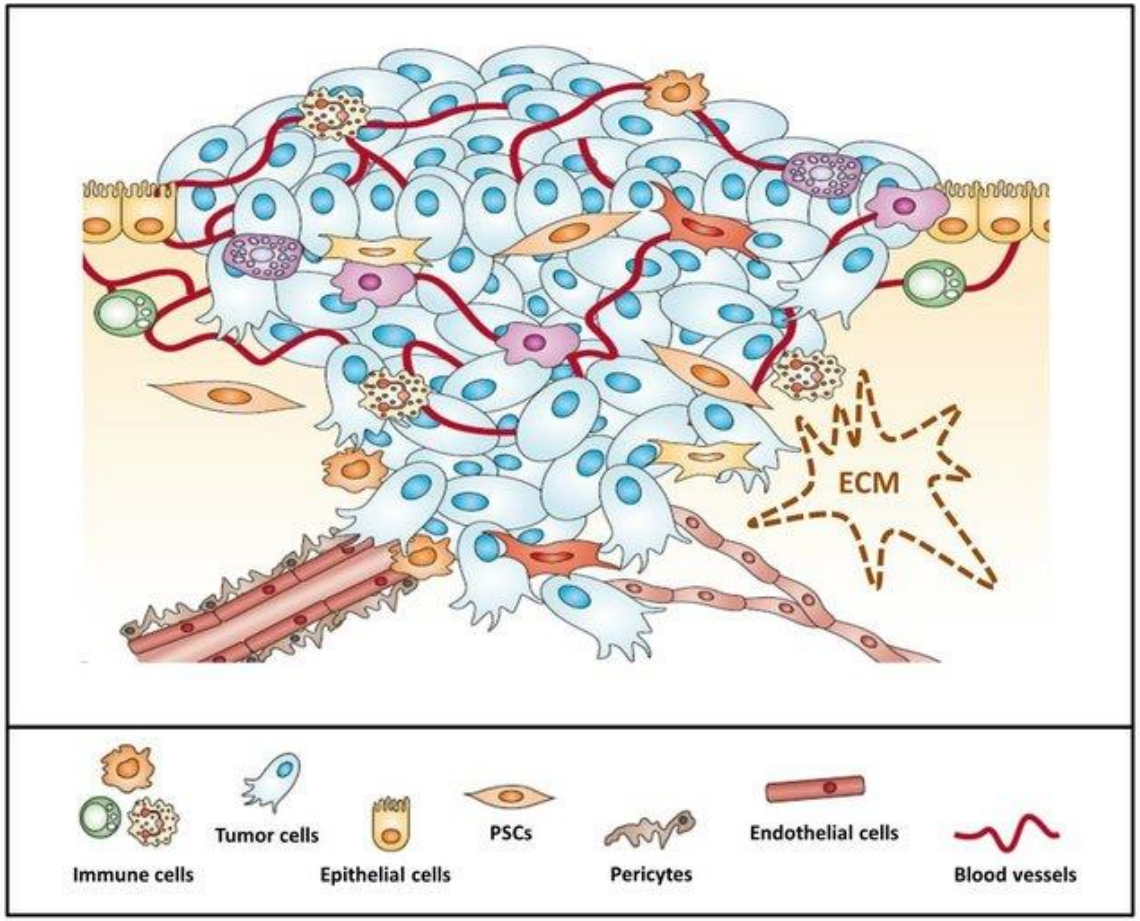
Primary tumor environment



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Co-culture of cells: 2D versus 3D

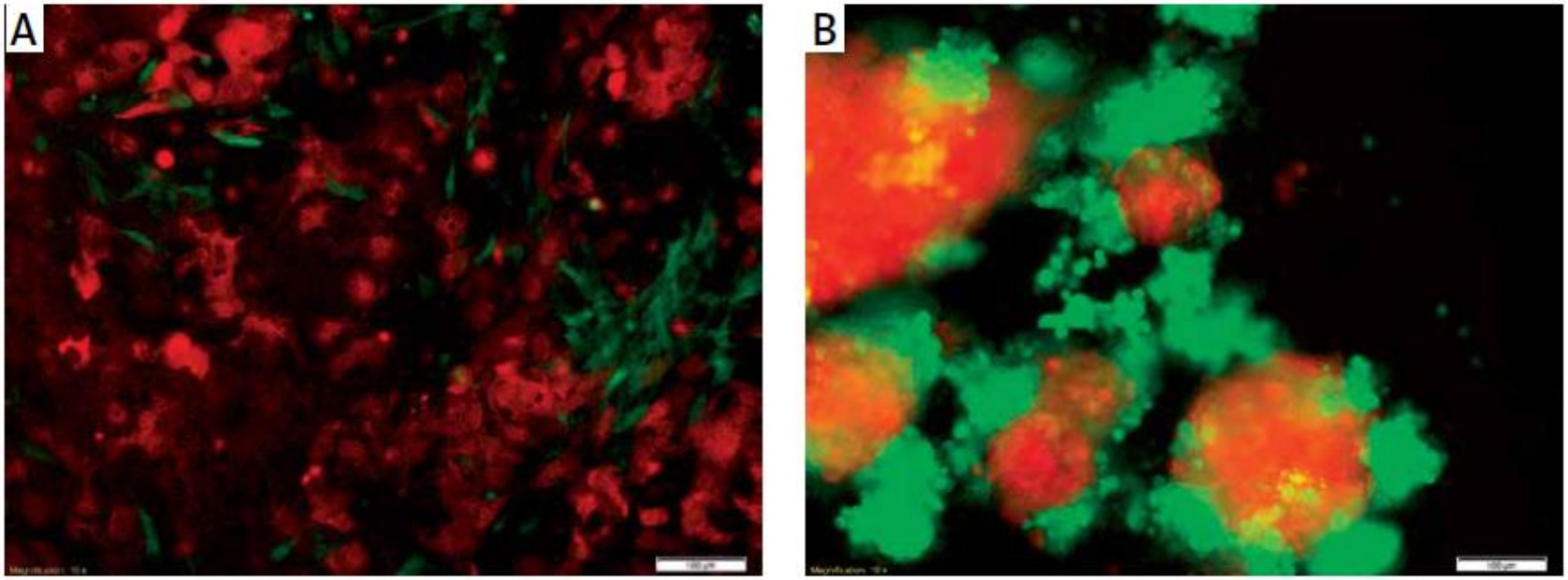

Figure 4. Co-culture of epithelial SCC-25 (red) and fibroblast MSU-1.1 (green) cell lines (scale bar represents 100 µm). **A** – cells cultured under 2D conditions are flattened and attached to the plate surface. The epithelial SCC-25 cells (red) have typical rhombus-like shape and MSU-1.1 cells (green) are spindle-like and surround SCC-25 cells; **B** – SCC-25 (red) and MSU-1.1 (green) cells cultured under 3D conditions changed their own morphology due to the lack of attachment. Cells lose their typical shape and aggregate, creating more (SCC-25) and less (MSU-1.1) compact structures

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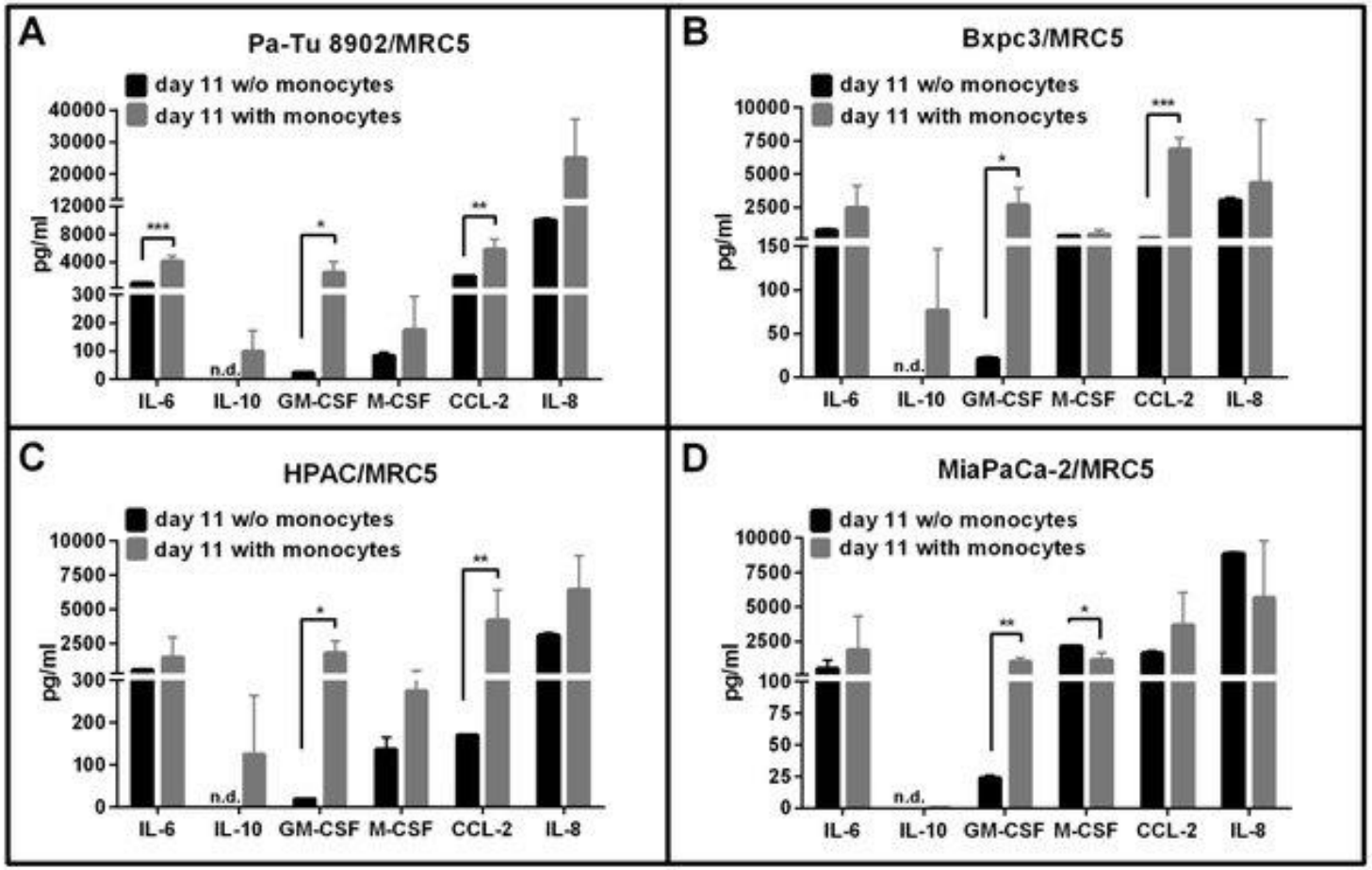
3D Co-culture of cells: induction of constituent production



3D tumor cell/fibroblast co-culture with monocytes induces differential secretion of cytokines, chemokines and growth factors.

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