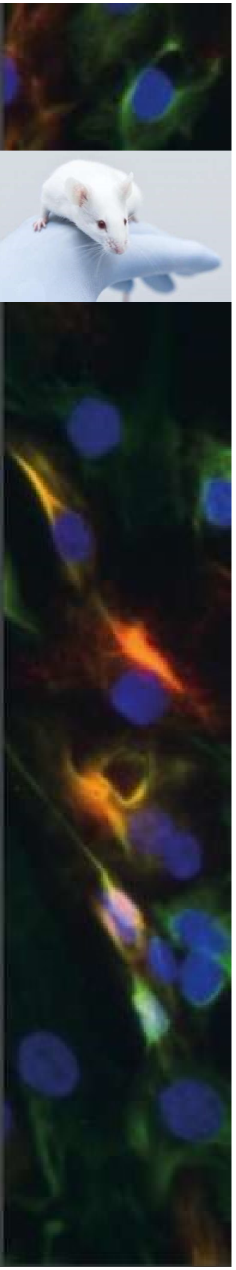


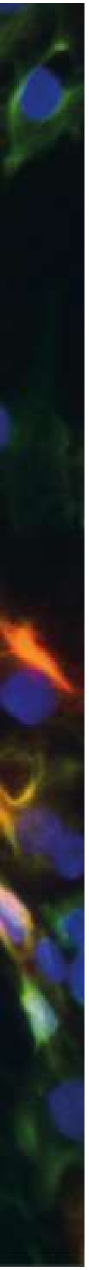
EUSAAT Virtual Seminar Series 2021 - June 10 – 5:00 PM CEST

3D cell cultures, bioprinted cancer rafts and their potential importance in experimental cancer research

Anna Sebestyén
Semmelweis University
1st Department of Pathology and Experimental Cancer Research
Tumour Biology, Tissue-, Cell Culture Laboratory

Tumour Biology – Tumour Metabolism Research Group

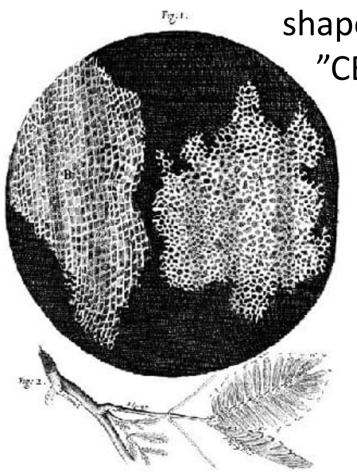
From the whole organism to the discovery of cells

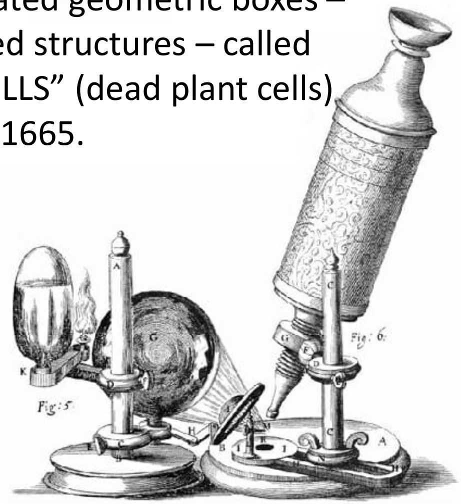


”First scientists”

Robert Hook

Repeated geometric boxes – shaped structures – called ”CELLS” (dead plant cells) 1665.





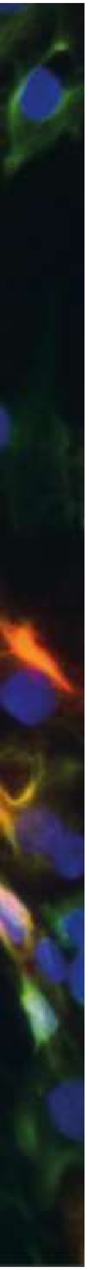
Anton van Leeuwenhoek – microscope
First obeserved living cells 1675.

Theodor Schwann – ”the animals are made of cells” - 1839
Rudolph Virchow - ”all living things are made of cells” - 1855

CELLS = building blocks of life
BASIC UNIT OF LIFE

1859. Virchow and Kölliker – Cellular Pathologie
Cell, as basic living unit.

Pathogene theory - **pathomorphology alterations of the cells cause several diseases**



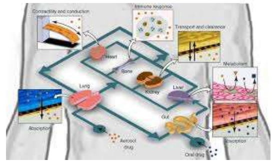
Milestones in the history of cell culturing

1885.
Wilhelm Roux
Chicken embrio
cells in vitro

1954. Nobel prize
J Enders, T Weller and F Robbins
polyo virus production in kidney
cell cultures (monkey)

L. Hayflick
Human fibroblast
in vitro max.
52 divisions

1975
1st mab producing
hybridoma cell line
Köhler and Milstein



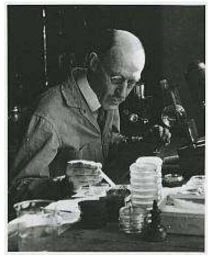
2012
Organ-
on-chip
Body-on
chip

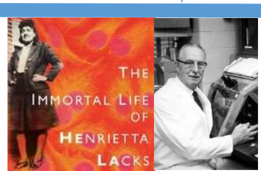
1998
Isolation of human
embrional stem cells
Thomson and Gerhart

After two world wars great advance in virology (vaccine production demand) resulted explosive development in cell culturing 40-50s

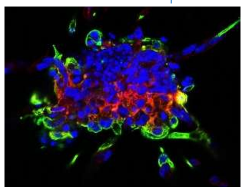
Development
of aseptic techn. (Carrel)

1907-10.
1st functional
in vitro
experiments
Ross Harrison
First publication





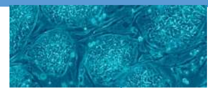
1951.
1st human
cancer cell line
HeLa (G. Gey)



1957.
1st primer
mammary organoids in vitro

1960-70
„modern"
wide spread of
Cell- and Tissue culturing

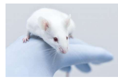
80's early studies
about the importance
of 3D culturing –
arteficial 3D matrixes



1992
Skinethic,
Episkin
Techniques
industrial spreading

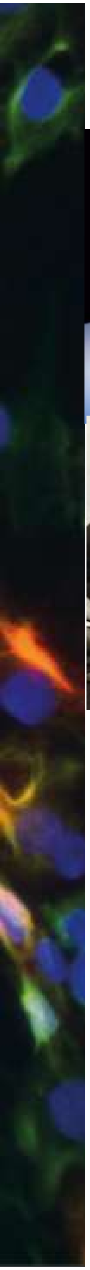
2004/5-2009
3D Bioprinting
(G. Forgács)

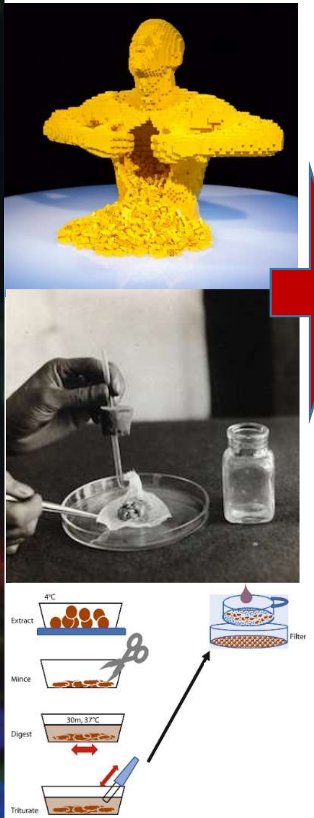
2012-2013
1st human
brain
organoids



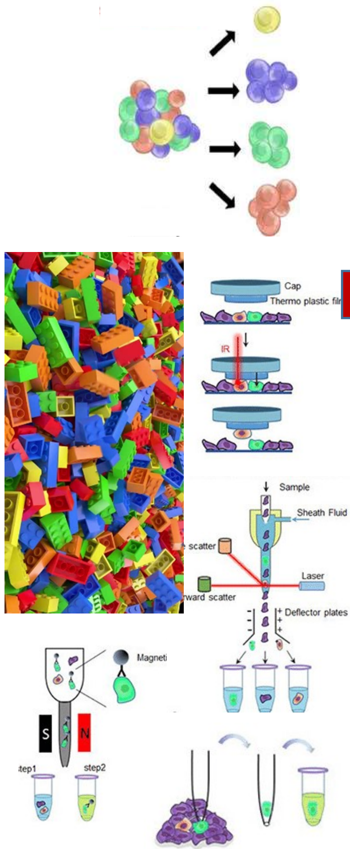
Developing in vitro cell culture-related models

The main aim is to rebuild an arteficial complex in vitro model





Dissection



Selection

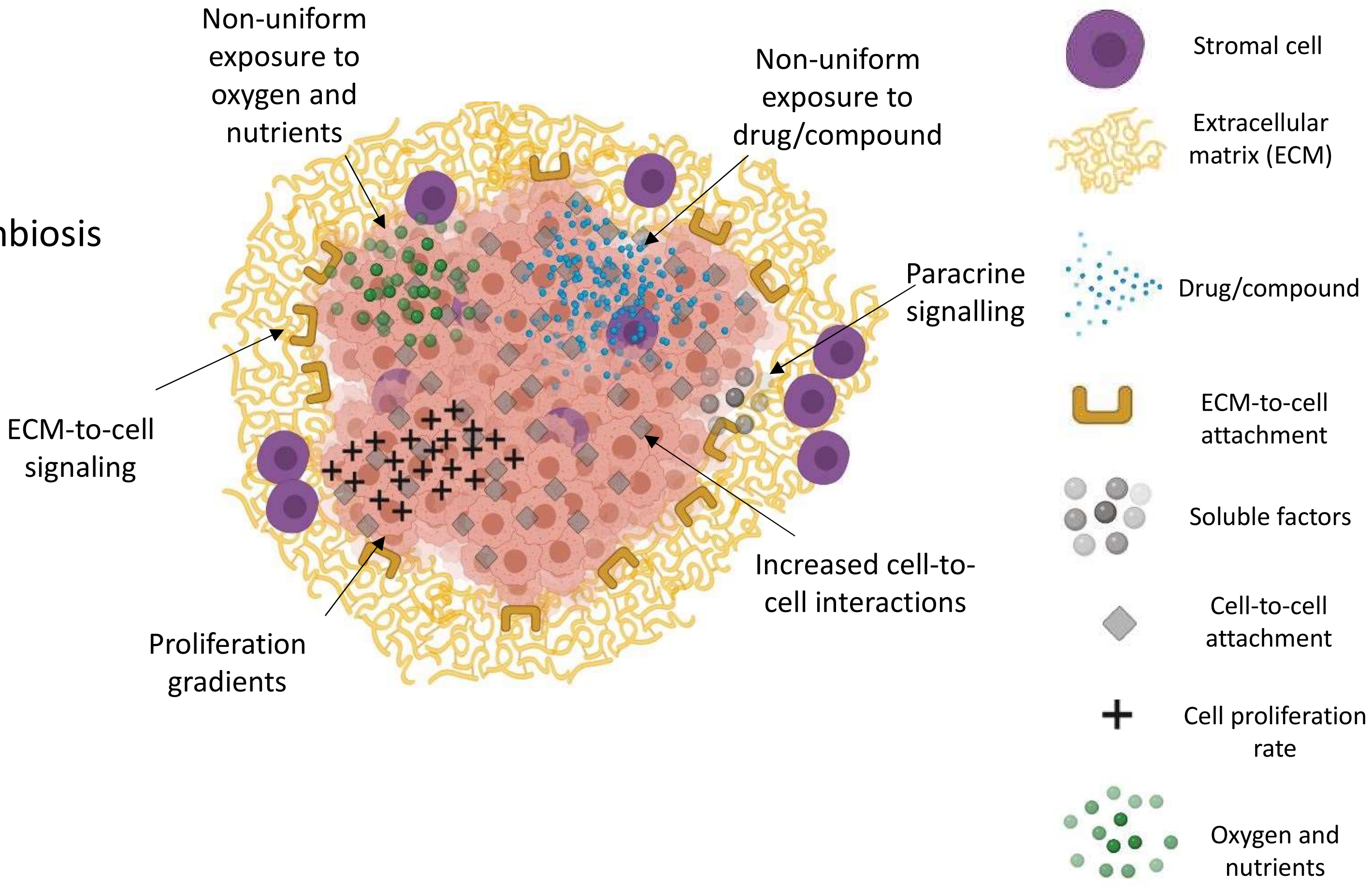
Cell cultures 3D cultures Co-cultures

3D cultures
Organoids
Organ-on-plate
Organ-on-chip

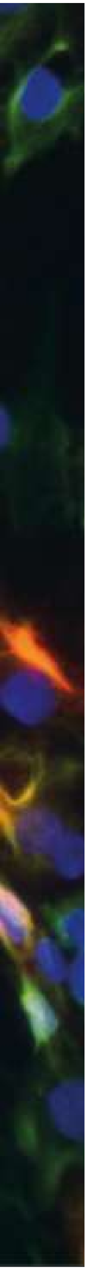
Body-on-chip

CELL line in in vitro cell culture is not TISSUE

Many different cell types are in symbiosis
3D structure , (non 2D)
Structured ECM (extracell. matrix)
Cell-cell connections are different
Cell polarity
Oxygen and nutrient diffusions are not equal
(2D cell cultures are not hypoxic)

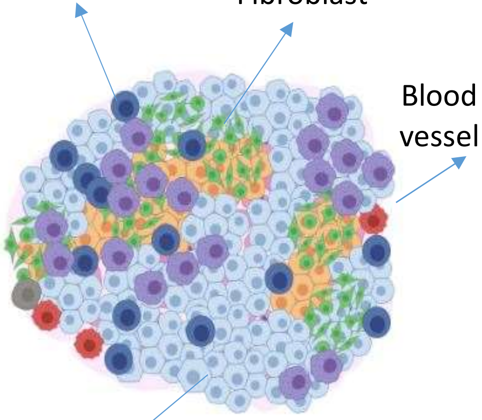


Lovitt C et al Advanced Cell Culture Techniques for Cancer Drug Discovery June 2014 Biology 3(2):345-367

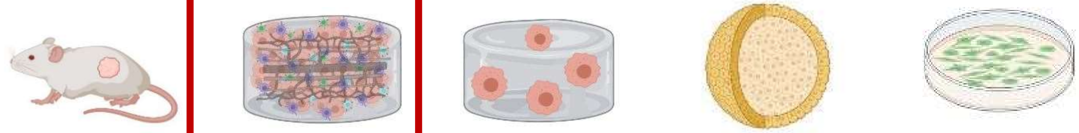


Different models to decrease differences between cell culture and in vivo systems

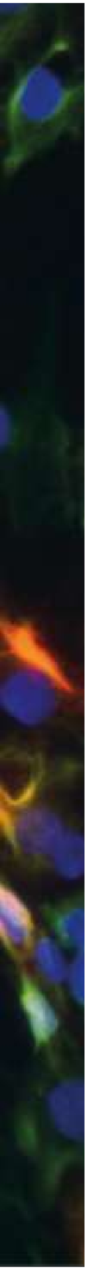
Tumour



Models

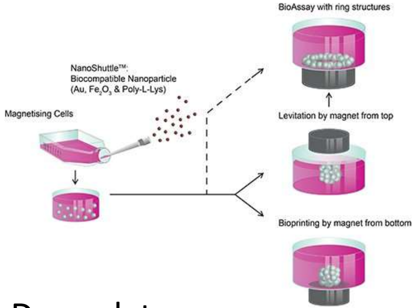


Xenograft	Tissue engineering	Gel embbending	Spheroids	Cell monolayer		
In vivo, 3D	In vitro, 3D	In vitro, 3D	In vitro, 3D	In vitro, 2D	Model type	Inherent features
+	++	-	-	+++	Reproducibility	
+++	++	++	++	- - -	Drug penetration	Tumour biomimetics
++	+++	+	+	- - -	ECM similarity	
++	+++	+	- -	- - -	Phenotype similarity	

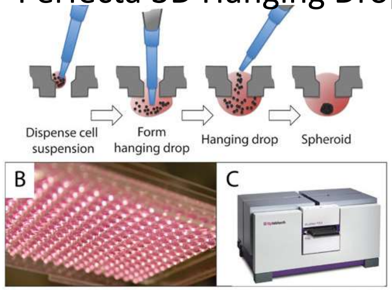


Scaffold free 3D culturing – co-culturing

Different hanging drop technologies

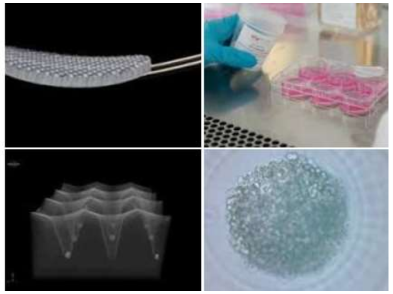


Perfecta 3D Hanging Drop plate



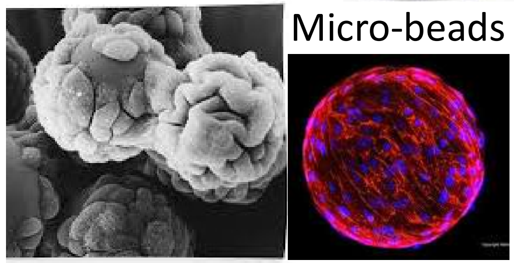
Rotation or spinner flasks

Low or no attachment technologies

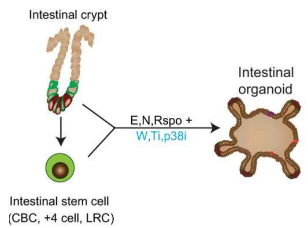


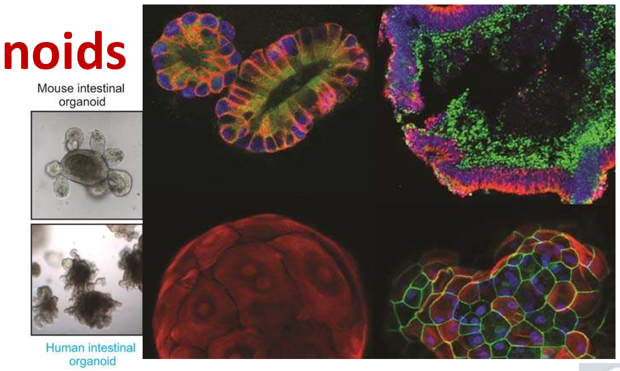
Scaffold-based culturing

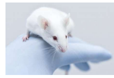
Micro-beads



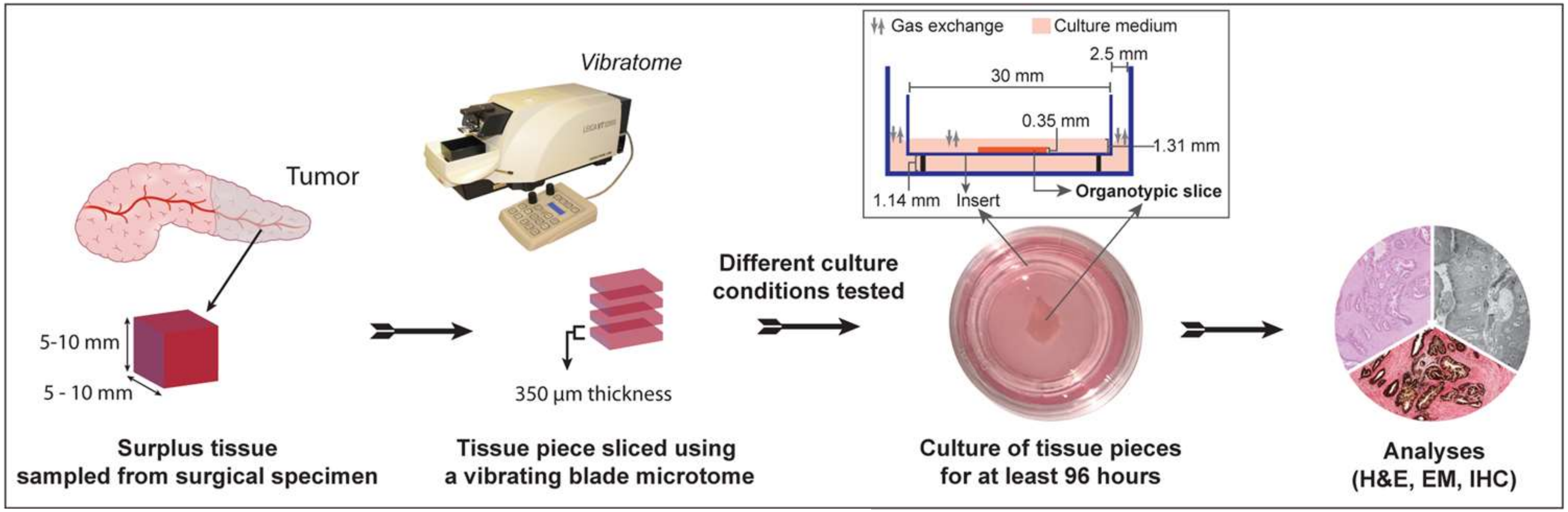
Organoids

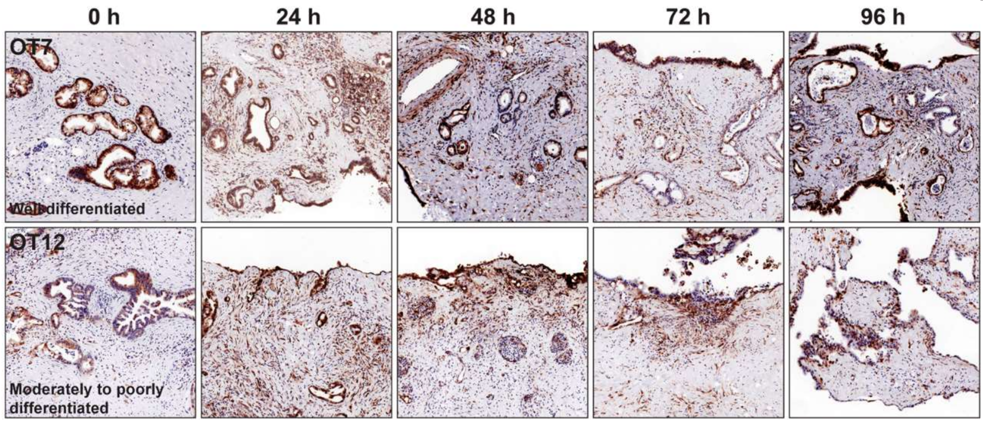


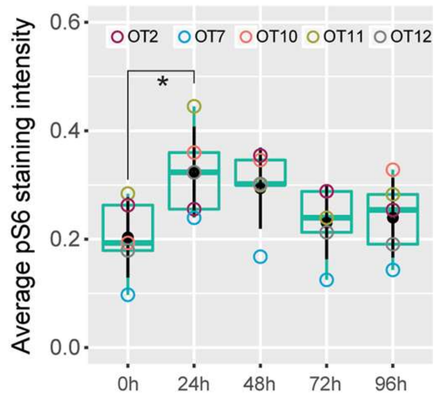




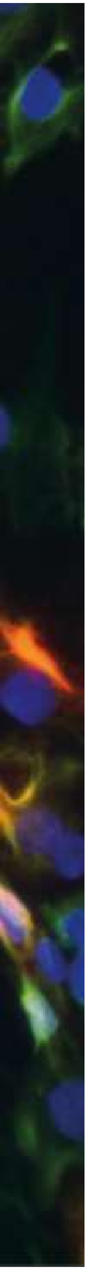
Tissue slice culturing, as an alternative





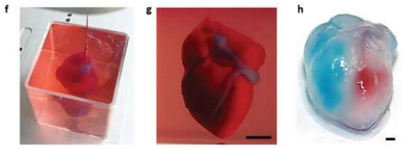


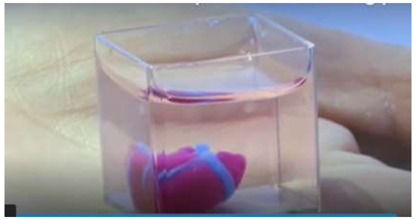
Misra S, Moro CF, Del Chiaro M, Pouso S, Sebestyén A, Löhr M, Björnstedt M, Verbeke CS. Ex vivo organotypic culture system of precision-cut slices of human pancreatic ductal adenocarcinoma. Sci Rep. 2019 Feb 14;9(1):2133.

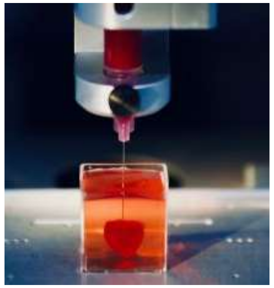


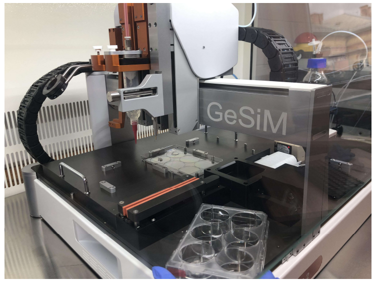
Possible real alternatives, bioprinted tissues, organs

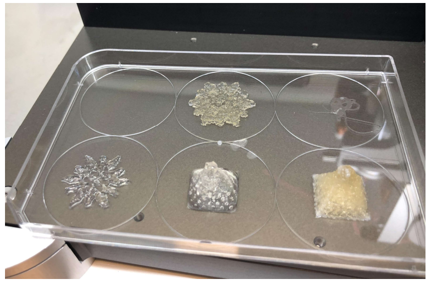
SCIENCE FICTION ?! → REALITY

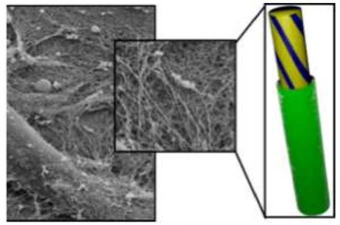


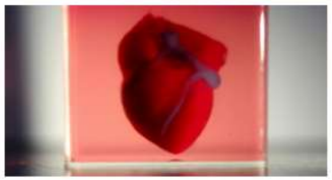


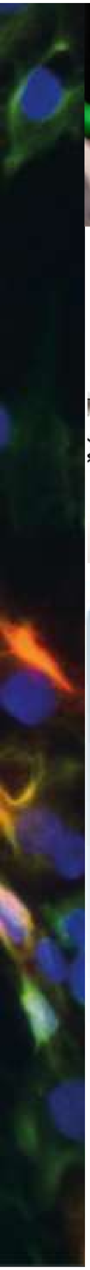


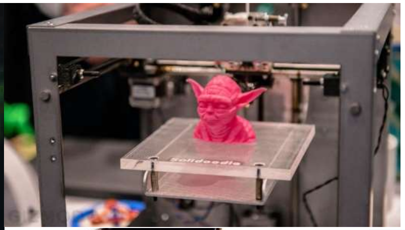




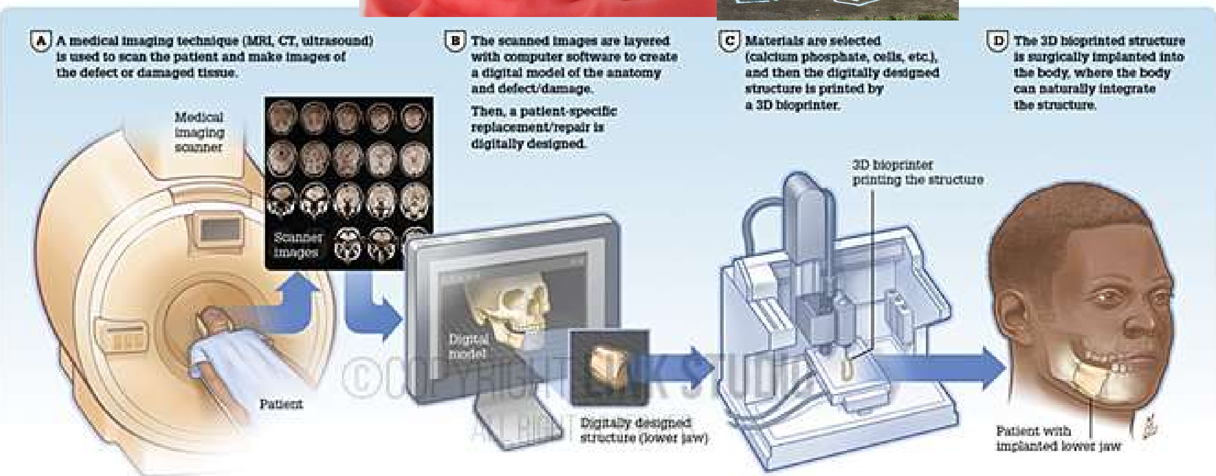




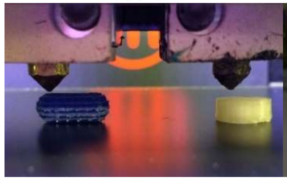


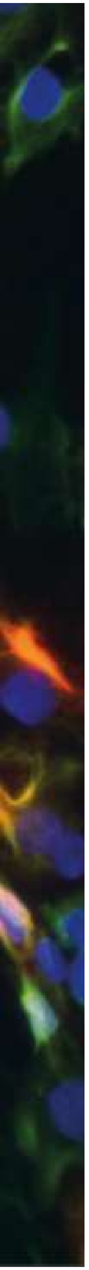


3D Bioprinting areas in medicine
3D Bioprinting in biotechnology and in tissue engineering



- precision personalised prostetics
  (bones, prostetic dentistry, teeth, braces…),
- medical aid equipment
  (arch-supports, exoskeletons, special prostetics)
- biosimilar material development
- CT derived 3D biomedical models for special surgery, and trachea or orthopediatric replacement
- Personalised pills





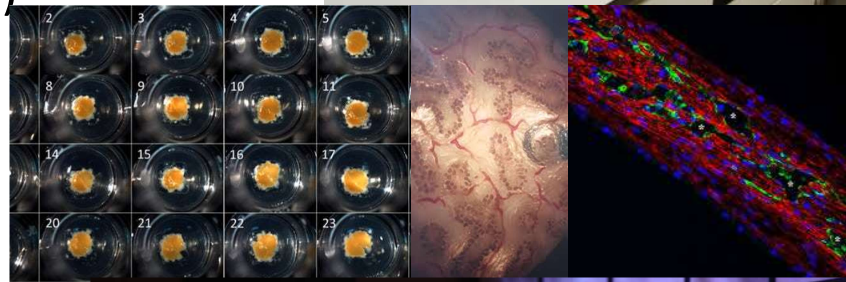
3D Printing – 3D Bioprinting with living cells

2009 organovo

Gábor Forgách (Missouri University – ORGANOVO 1st Bioprinter)

Hydrogel-based scaffold and living cells

Injekt printhead uses ECM mimetic gel and cell suspensions as BIOINK and builds more layers or circles, spheroids… (3D)

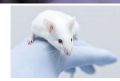


printheads for cell containing scaffold gels with nutrients and other materials depending on cell needs

Calibration by laser and special softwares

e.g. capillary for bypass and liver tissue samples

In the future complete organ printing, skin, kidney….



Sterile Bioprinters – tissue engineering using stem cells – ”replacing anything from own stem cells” in the FUTURE

Reality is

Is it science fiction or not???

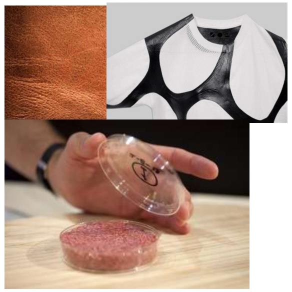
animal experiments replacement

2-3 cellular components and capillaries

L’Oreal

Bioprinted human skin development for testing cosmetics

Modern Meadow

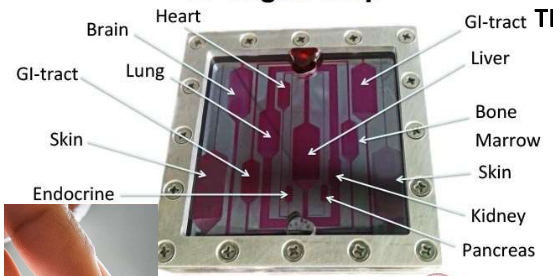


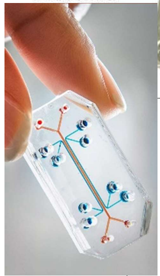
Bioprinted leather for BMW and textil-fashion industry….

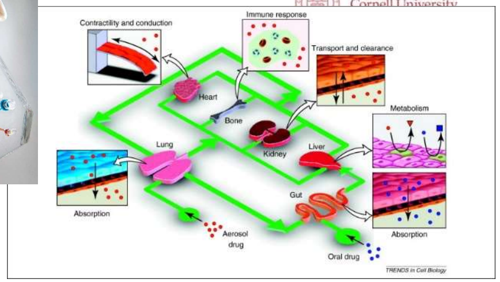
Bioprinted meat, hamburger.

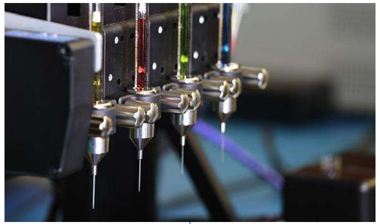
Body-on-chip technology in pharmaceutical development

10-Organ Chip









In the US 100 000 people are on the organ waiting lists,
20 people dies daily because of transplantation delay

WORDWIDE CAPACITY and ORGAN DEFICIT

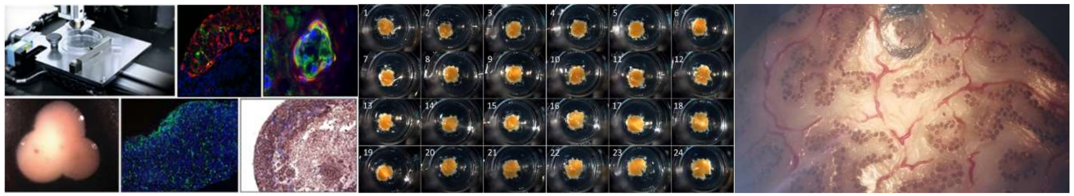
TISSUE ENGINEERING IS AN OPTION

Organovo

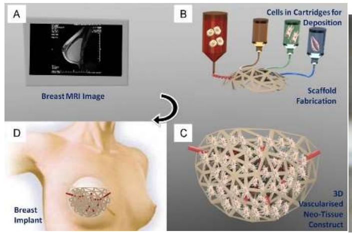
Bioprinted organs

Liver, kidney, skin, capillaries

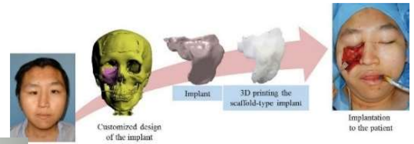
- Arteficial bioprinted liver or kidney in capsules, nanocapsules eliciting hemodialysis
- other

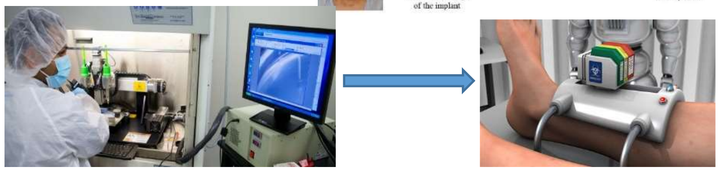


” Living implants”

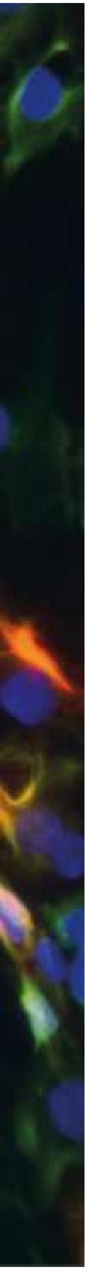


Bone,
Trachea and face reconstituions



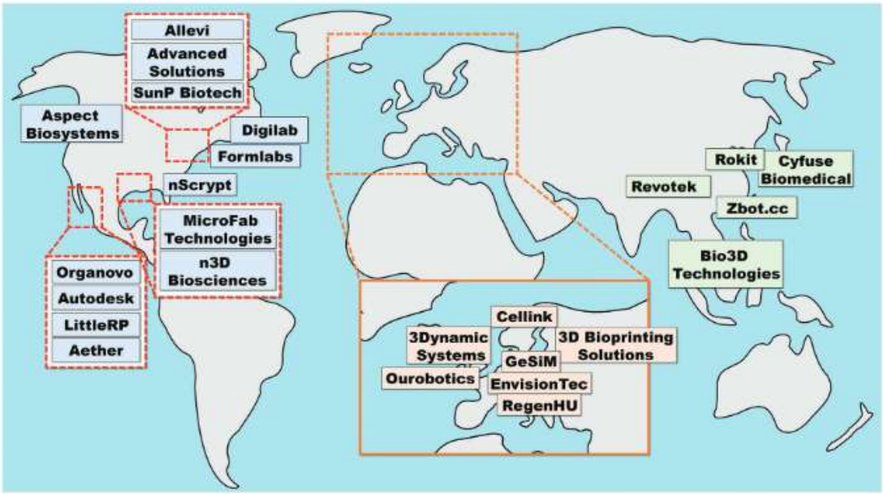


Direct skin replacement in the operating room



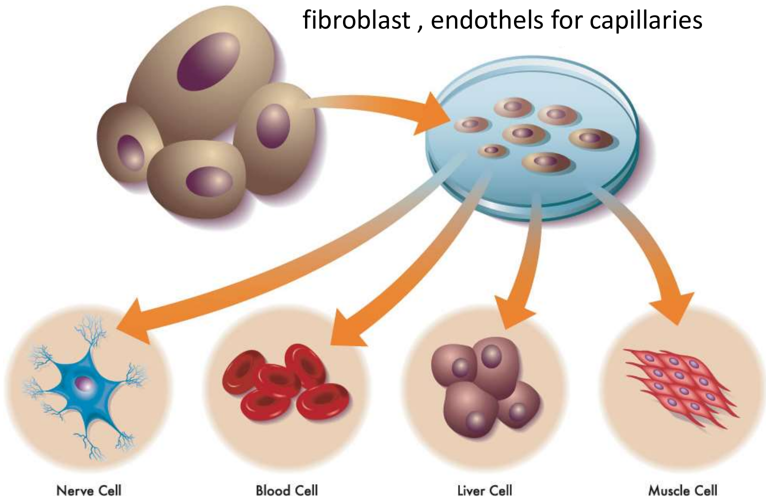
Minimum requirements for bioprinting

Bioprinter, software, special printer heads



Tissue cell culture laboratory (stem cell lab)

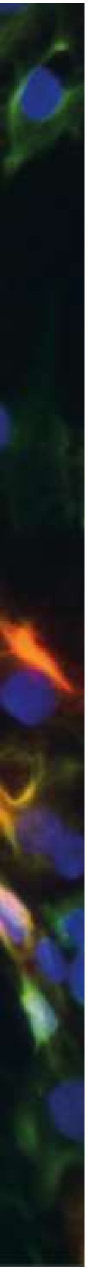
Stem cells,
tumour cells,
fibroblast , endothels for capillaries



BIOINK

adding cytokines, growth factors and
induce, inhibit proliferation
tailoring the differentiation programs

PRODUCTION OF HUGE AMOUNT OF CELLS (Bioreactors)
ADDITION SPECIAL MATRIX



3D Bioprinting has been started in our University, as well

tissue cell culture lab

we bought GESIM Bioprinter one year ago

WHY our research group is interested in 3D Bioprinting:

- new biological models and tests
- 3D Bioprinting completes microfluidica in body-on-chip technology

Cancer research institute
Tumour Biology
Tissue and Cell Culture
Laboratories

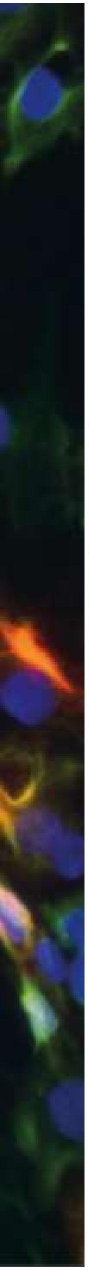
- cancer target research
- anti-cancer drug tests

AS NEW RESEARCH TECHNOLOGY DEVELOPMENT

Our main interest is metabolic disregulation in cancer

- known metabolic differences between 2D, 3D and in vivo systems
- in vitro tumour metabolism and therapy resistance studies need 3D culture systems

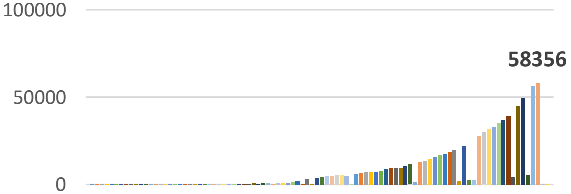
TUMOUR METABOLISM RESEARCH GROUP

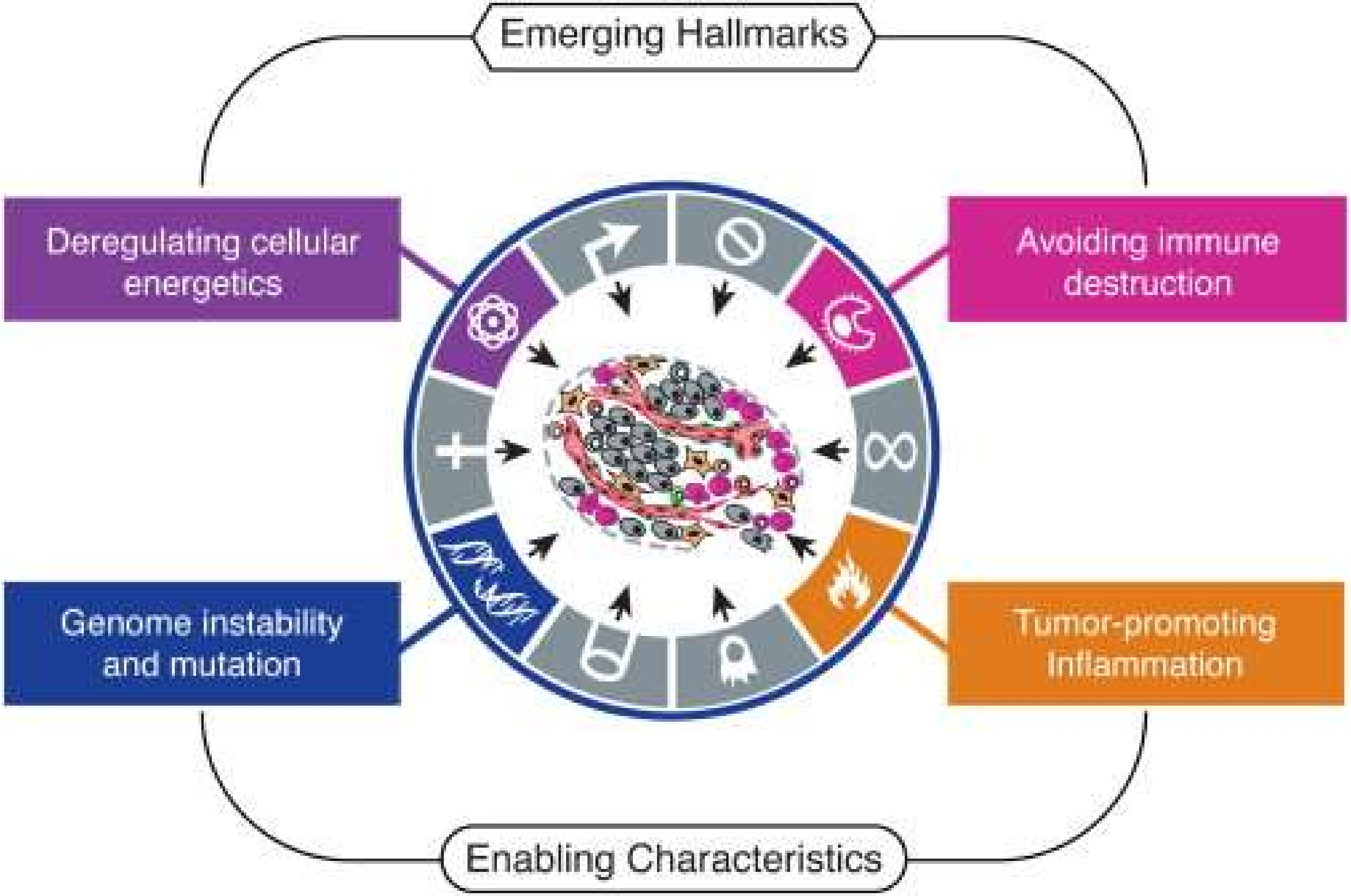


Hallmarks of cancer cells (2002)

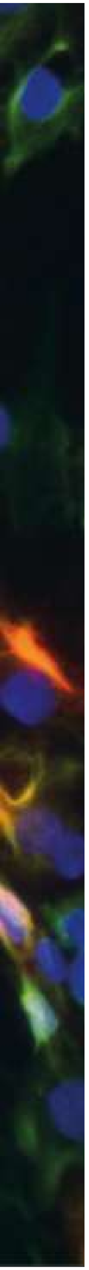
Deregulating cellular energetics as a ”new” hallmark of cancer cells (2011)

J Gen Physiol. 1927 Mar 7; 8(6): 519–530.

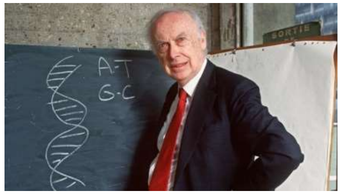




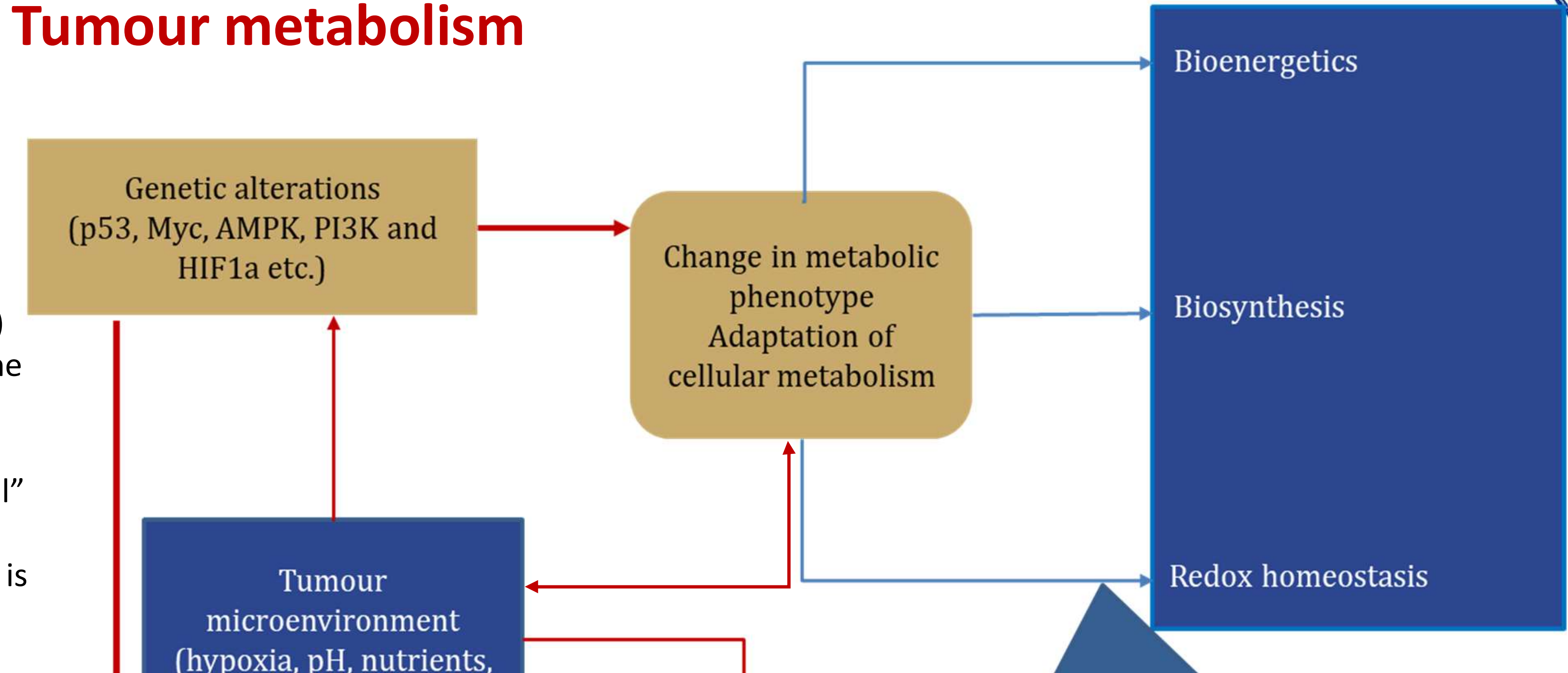
Hanahan and Weinberg 2011.

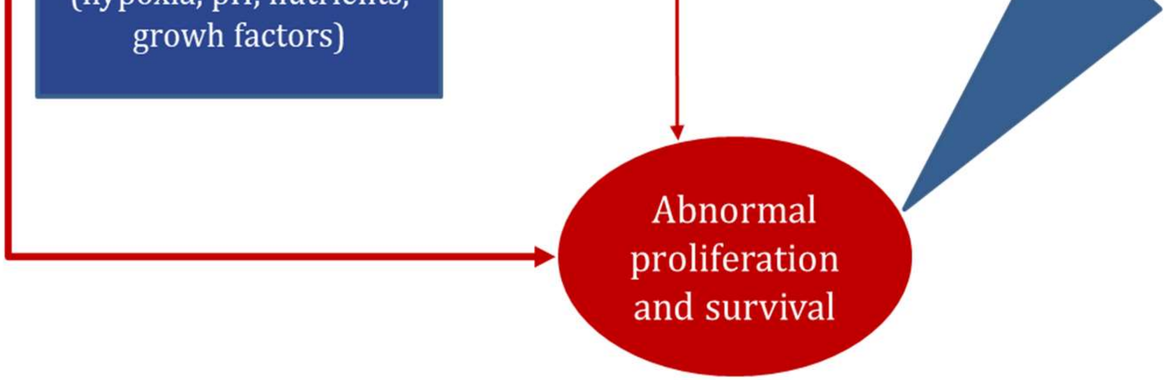


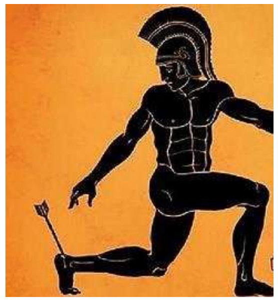
Tumour metabolism

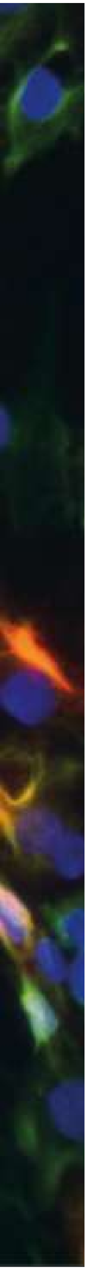


(1988. – 30 years ago) Watson – "Locating the genes that cause cancer has been "remarkably unhelpful" — the belief that sequencing your DNA is going to extend your life a cruel illusion. If he were going to cancer research today, he would study biochemistry than molecular biology"





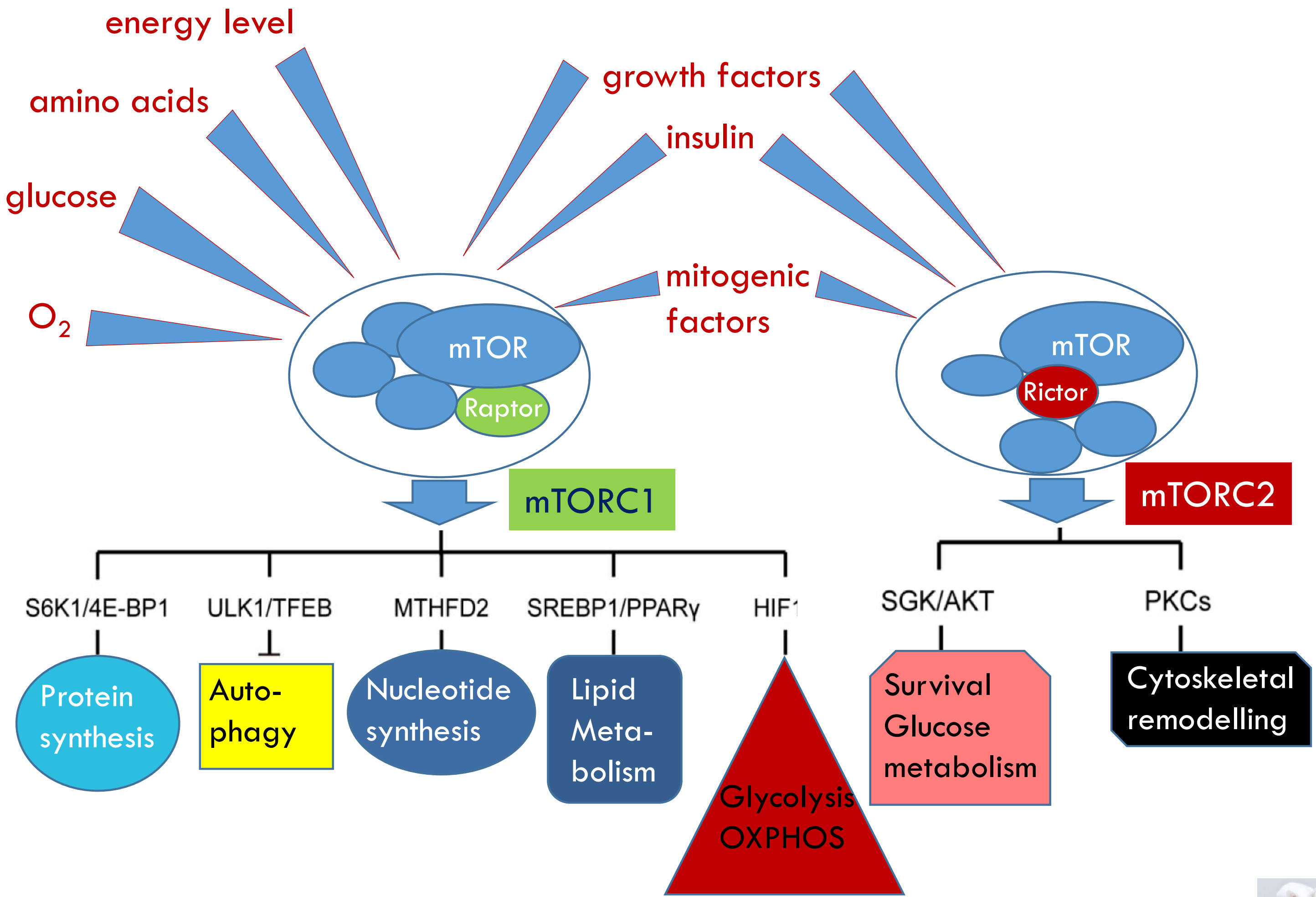


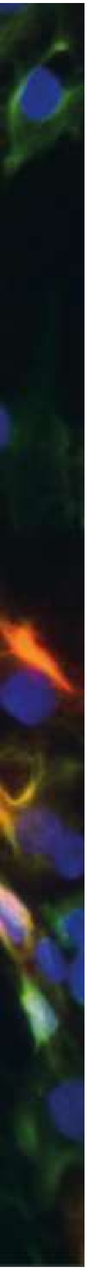


mTOR kinase is a hyperactivated ”crossroad” in many cancers

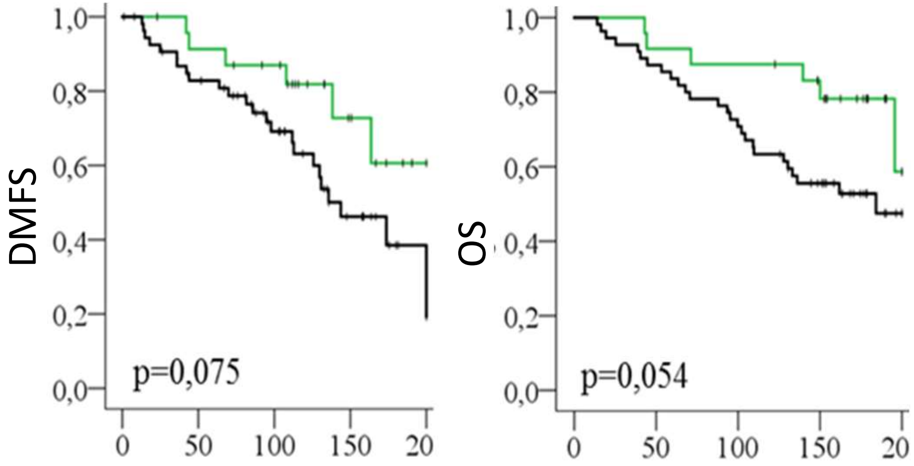
mTOR as a METABOLIC CROSSROAD in cellular signalling network

Metabolic inputs
and regulatory effects
related to mTOR complexes:



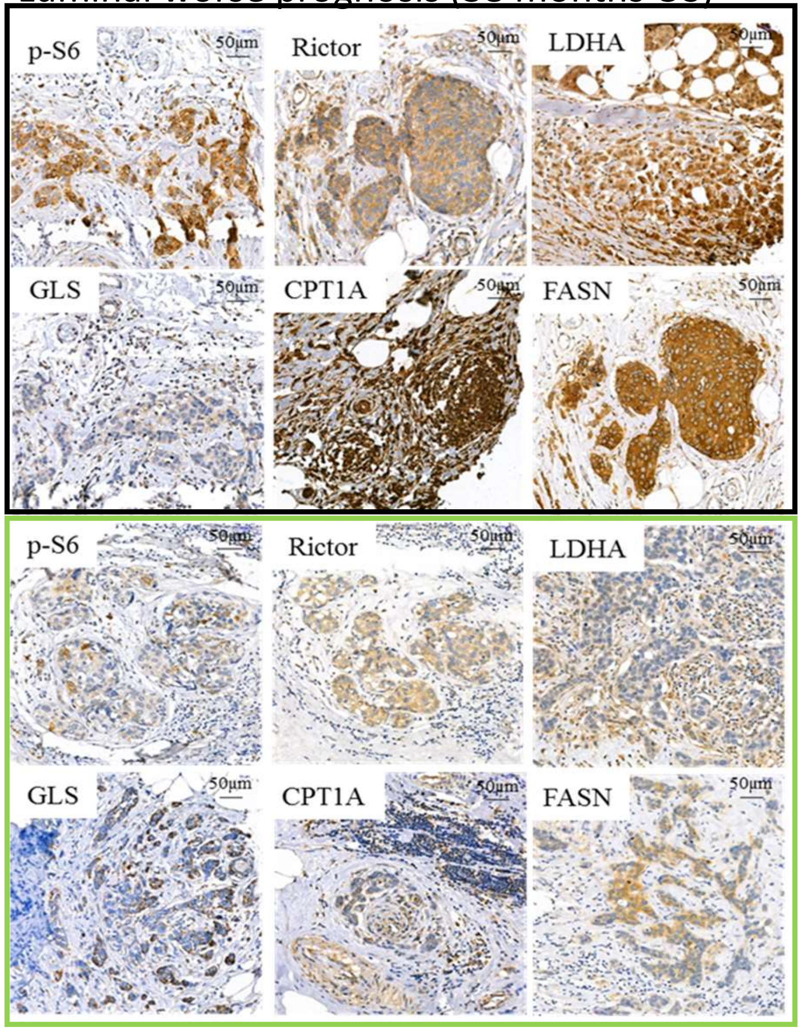


Metabolic plasticity correlates to worse prognosis in breast cancers

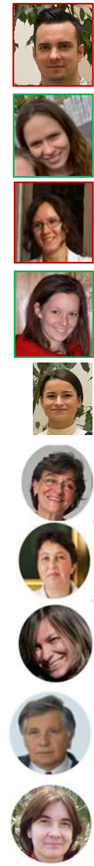


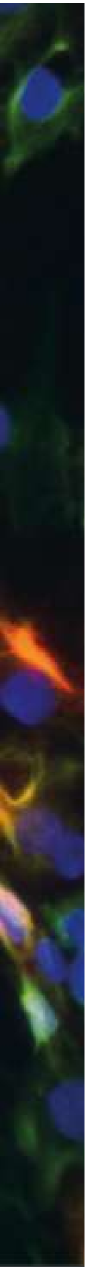
Metabolic plasticity +
(High mTOR actvity scores
and high score evaluation
in minimum two other metabolic enzymes)
No high metabolic plasticity

Luminal worse prognosis (38 months OS)



TNBC good prognosis (189 months) *Petővári et al. Cancers 2020.*

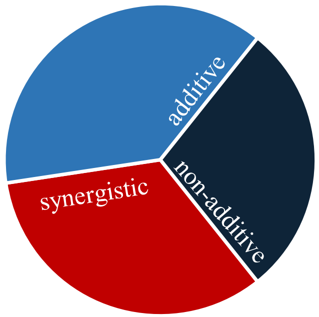




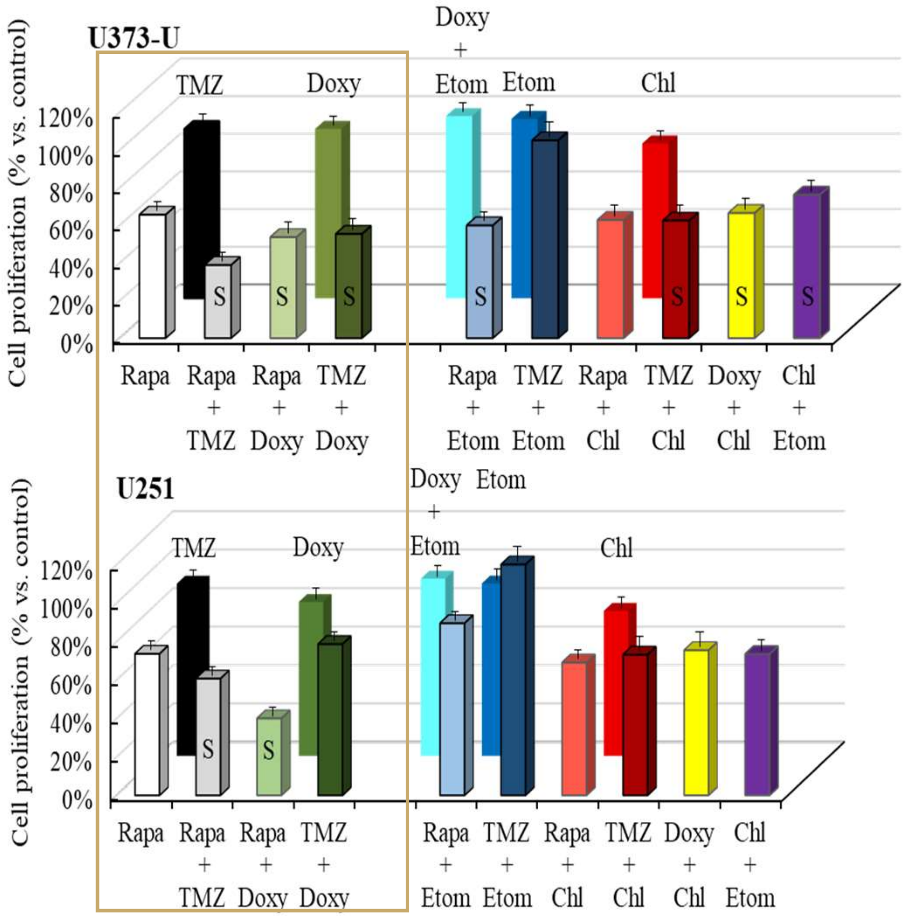
Importance of metabolic alterations in cancers and cancer model systems

In our metabolic inhibitor studies we tested many different drugs:

- mTOR inhibitor (mTOR is a master regulator of cellular metabolism)
- Disease specific chemotherapeutics (TMZ – glioma cells)
- Doxycycline (tetracycline antibiotics)
- Etomoxir (lipid synthesis inhibitor)
- Chloroquine (autophagy inhibitor)



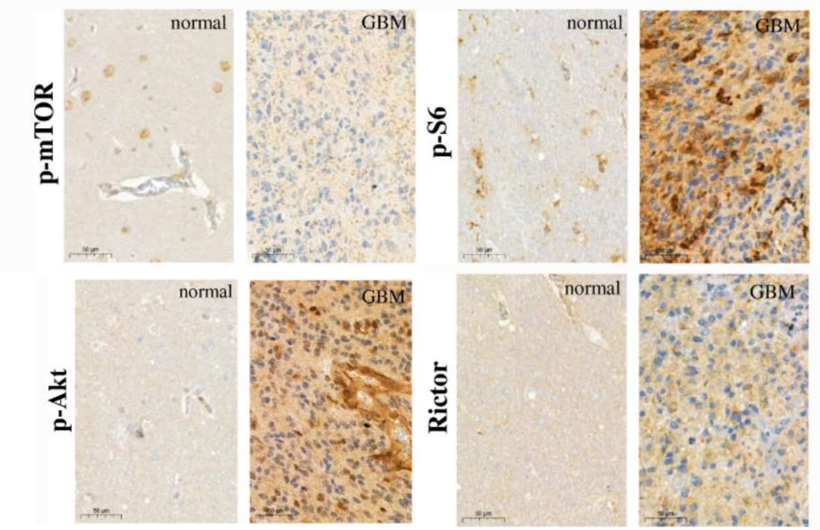
25 human cc. cell lines were tested with rapamycin and doxycycline combination: In 2/3 of these lines, the Rapa+Doxy combination significantly (<50%) reduced the cell proliferation *in vitro* and these effects were additive or synergistic.



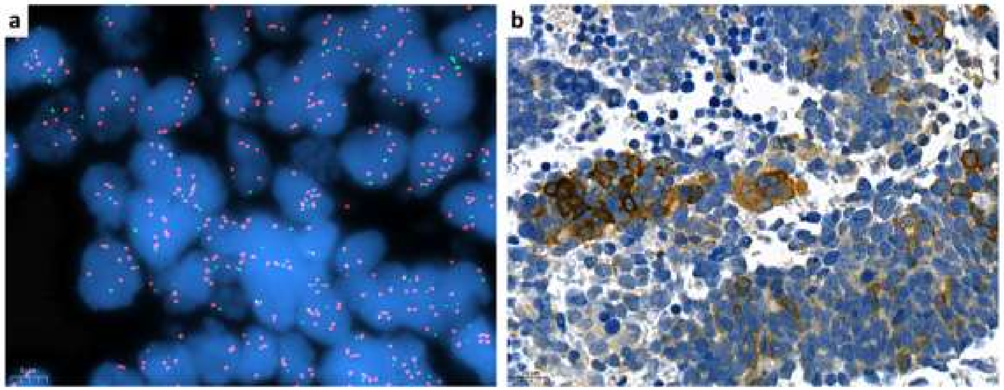
(Hujber et al. J Clin Exp Cancer Res 2018, Petővári et al. Cancer Cell Int 2018)

Characterisation of mTOR hyperactivity
(breast cancers, rhabdomyosarcomas, lung tumours, gliomas – metabolic characterisations simultaneously
colon cancers, medulloblastomas, GISTs, lymphomas and leukemias)

Cancers 2020, Humanpathology 2019,2018, 2017, Pathol Oncol Res 2020, 2019, J Neurolpathol Exp Neurol 2018, J Clin Pathol 2017,
J Exp Clin Canc Res 2018, 2017; Modern Pathol 2012; BMC Cancer 2013, PlosOne 2013, Diagn Mol Pathol 2011…

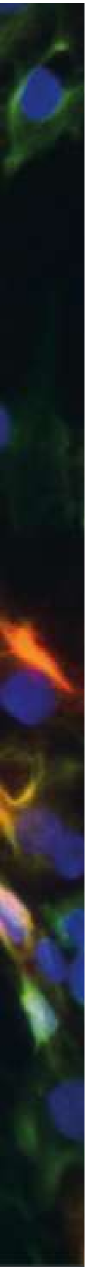


mTORC2 hyperactivity in human glioma biopssies



Rictor amplification and its correlation with the detected Rictor ovreexpression and high mTORC2 activity in SCLC biopsies, recently started VISTUSERTIB (C1-C2 inhibitor) therapy

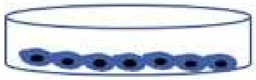
Despite encouraging preclinical data, mTOR inhibitor mono- and combined therapies have shown poor anti-tumour results and individual side-effects in patients (promising in some advanced carcinomas, pancrease tumours, sarcomas)



What could be the reasons:

- cytostatic effects (surviving cell populations)
- mTORC1 and 2 have complex functions in cell signalling network and in metabolism
- metabolic heterogeneity in situ in tumour tissues and complex metabolic regulation
- metabolic symbiosis in adaptation mechanisms
- we need better patient selection and therapy markers

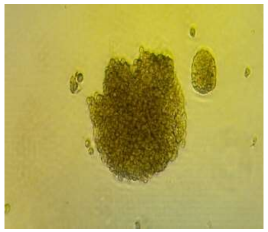
2D preclinical models and xenograft animal models (SCID or other mice)

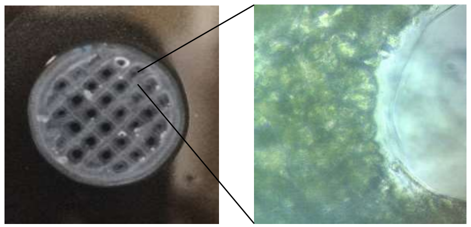


No real structure and
single type of cells

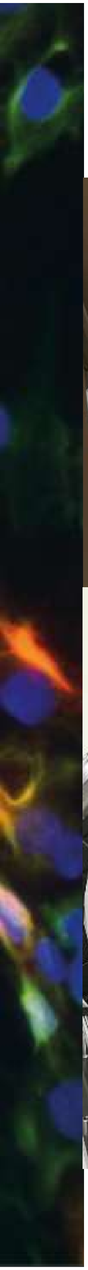
No human
ECM and microenvironment

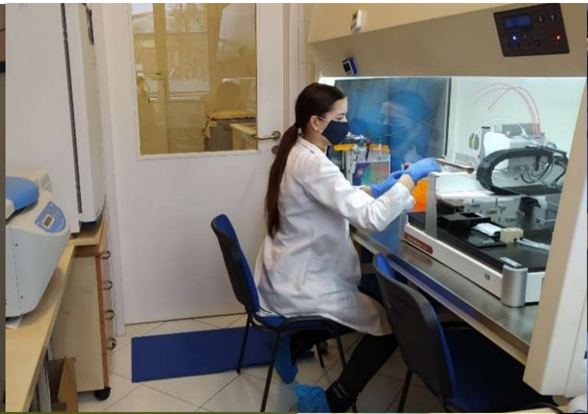
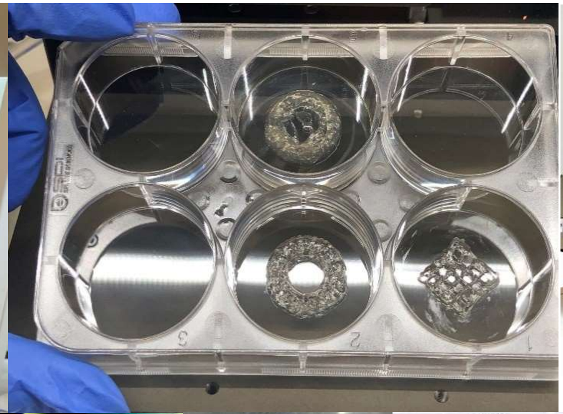
Tumour metabolism research - in vitro from 2D to 3D models,
Anti-metabolic treatments results could have different effects in 3D printed human tumour models

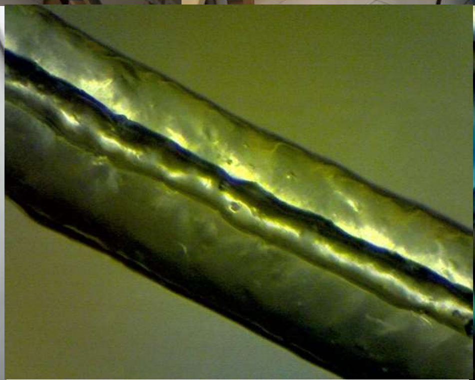
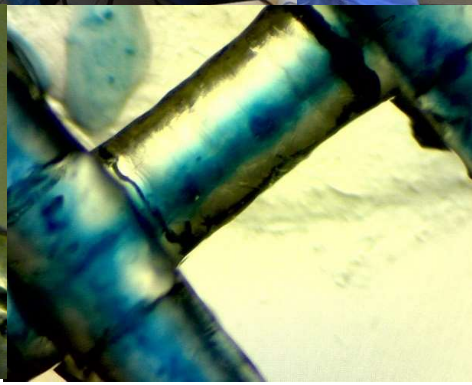


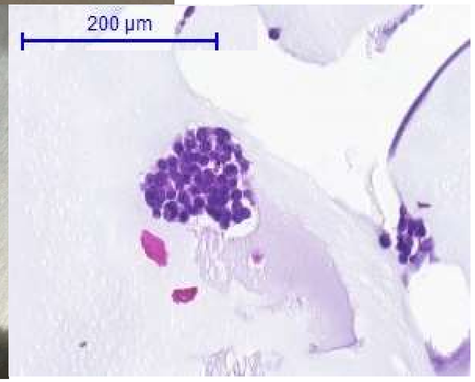


New 3D Bioprinter Unit in our tissue and cell culture laboratory



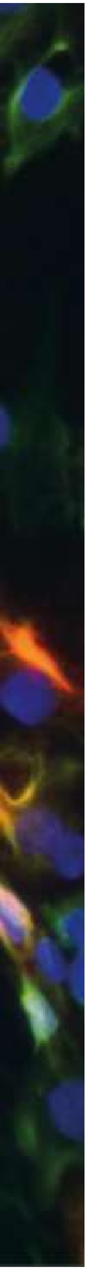


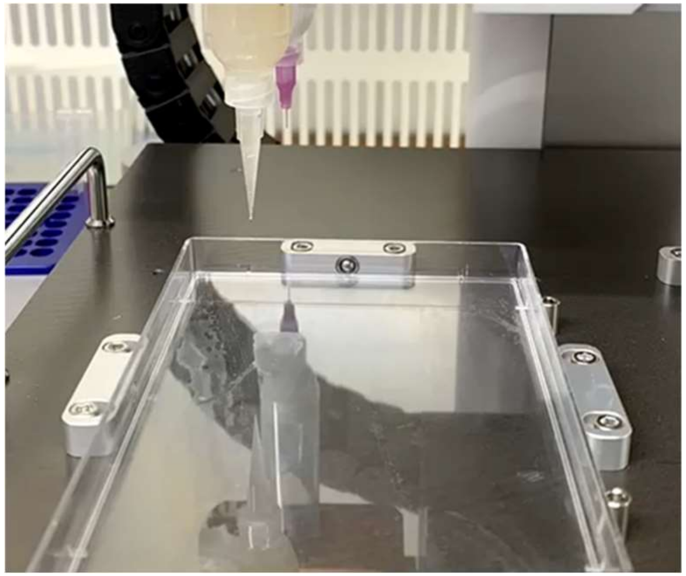


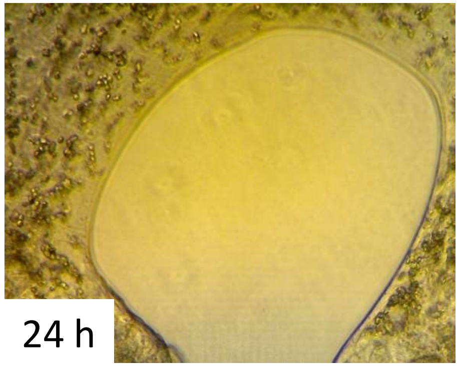


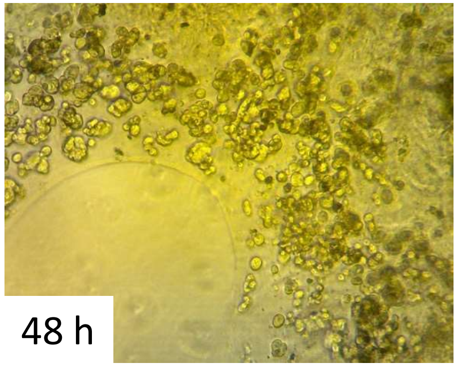

Cell compatibility test and evaluation of the printed materials using pathomorphological processes
Tested own bioink recepies

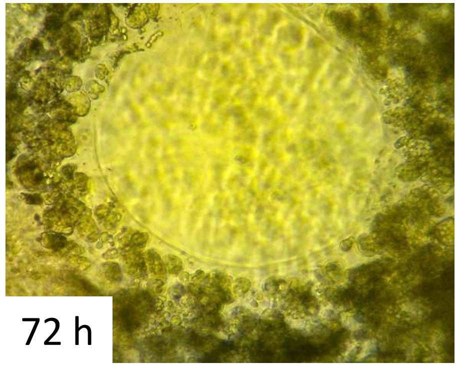
After bioprinting and long-term culturing of breast cancer cells – tissue like structures, lobulars structures were formed in vitro



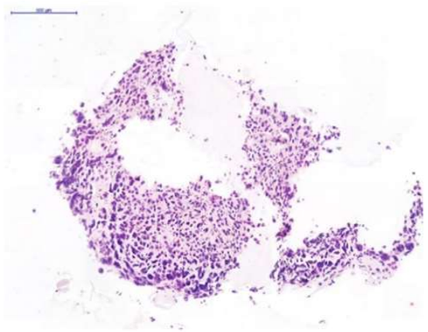


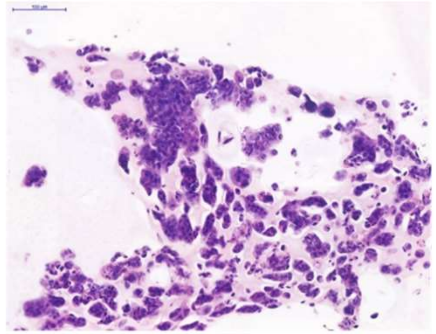




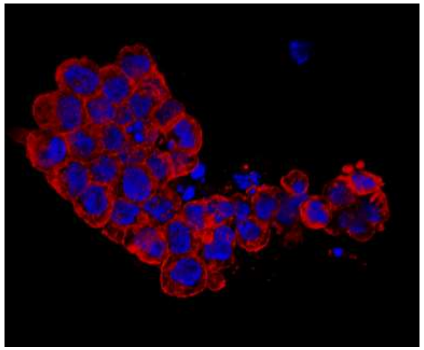


Protocols for
Proliferation tests (MTT, SRB, calcein assays)
cell recovery or
other biochemical,
molecular biology,
metabolite concentration,
protein expression and
IHC studies

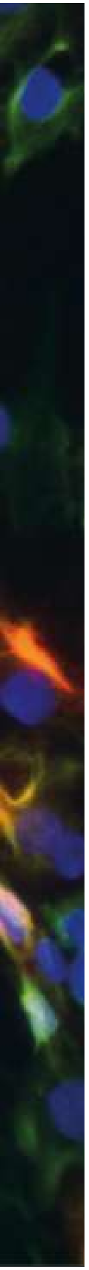




after 2 weeks



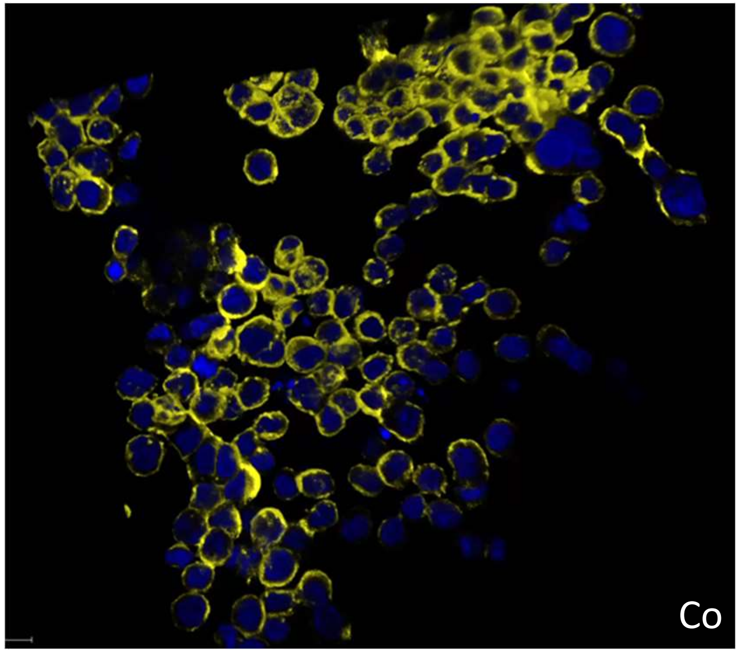
after 1 week lumen formation
in bioprinted human ZR-75.1 rafts
(ZR-75.1 luminal breast cancer cell line)



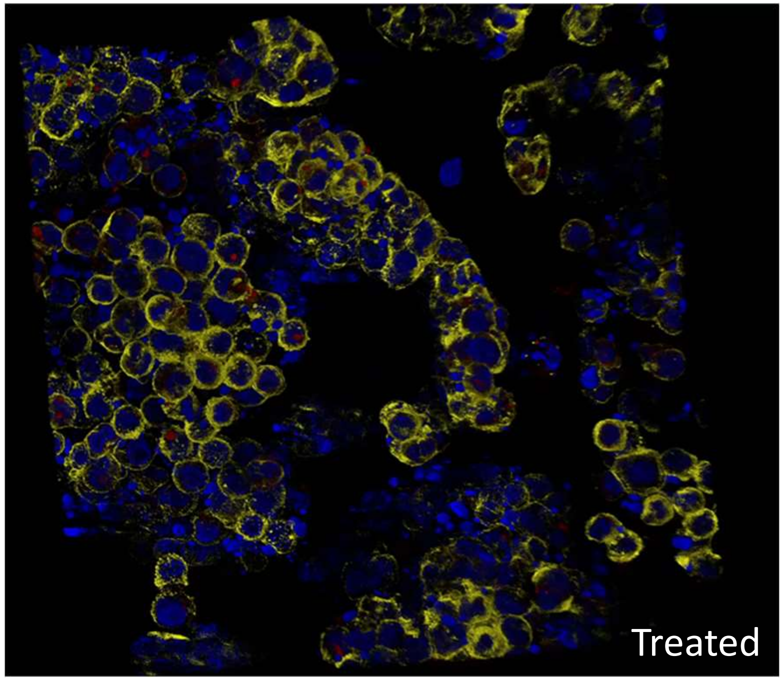
We have started to map the metabolic differencies of

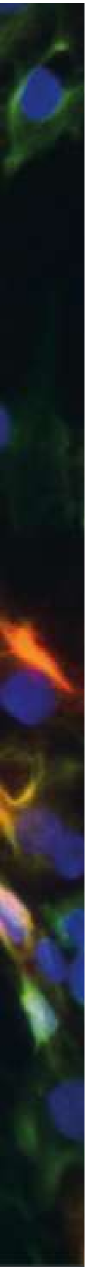
- 2D, different 3D *in vitro* cell culturing (including spheroids, organoids and 3D Bioprinted tumour tissues),
- *In vivo* tumour xenograft models and

Confocal microscope images stained with LC3 and or phalloidin + Hoechst



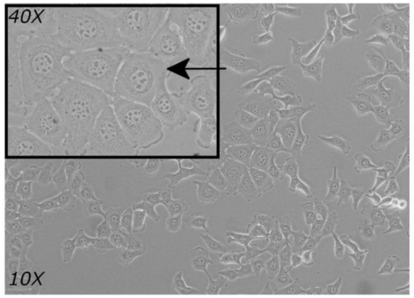
ZR-75.1
10 days
rafts



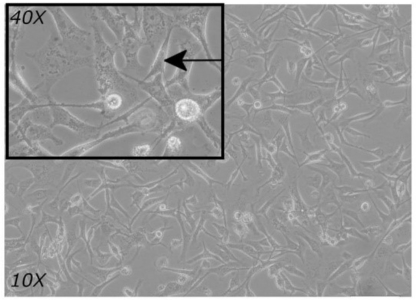


In vitro tissue formation

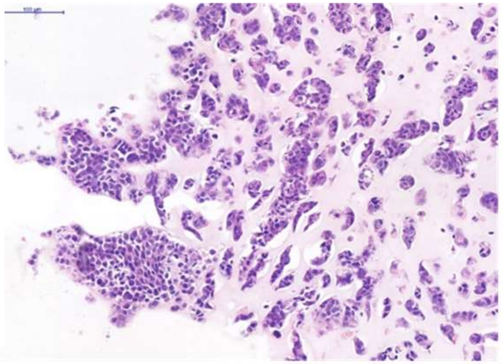
MCF7



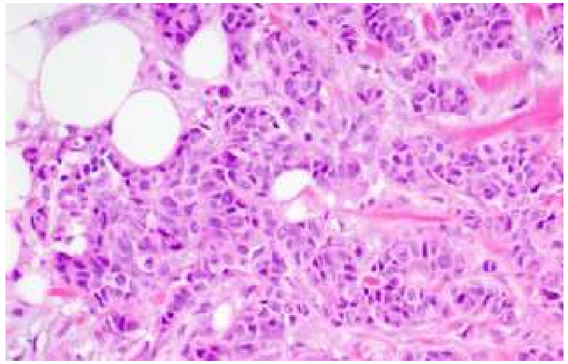
MDA-MB-231

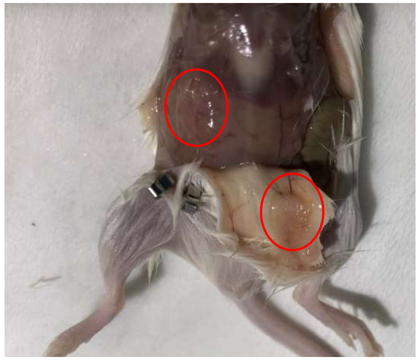


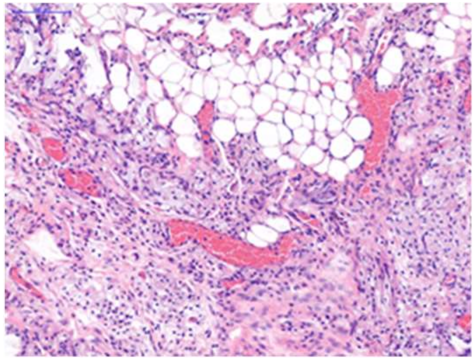
Bioprinted and *in vitro* growing rafts



Human biopsy

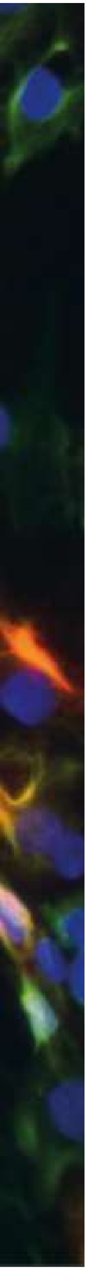




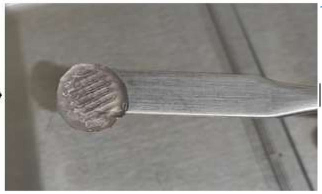


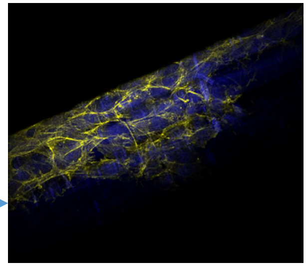
In vivo growing xenotransplanted previously bioprinted raft and its vascularisation

Conventionally non-xenotransplantable breast and lung cancer cells can be transplanted to SCID mice using bioprinted and cultured rafts (potentially useful for patient-derived xenograft models)



Different applications

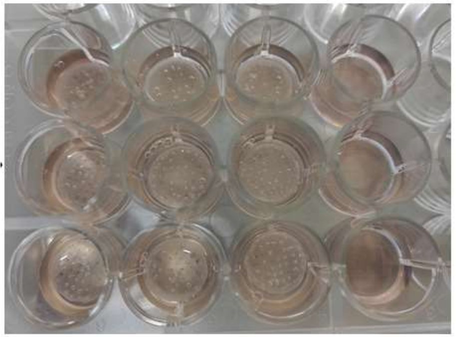




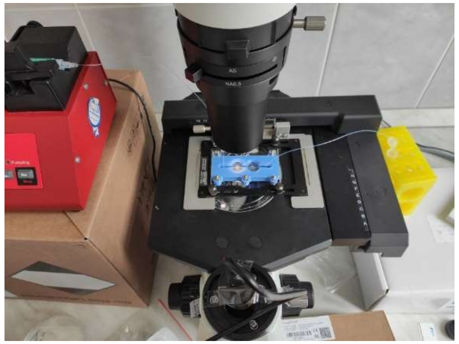
fibroblasts directly printed and grown on surgical fiber

Easy handling, thicker – more layers, aviod hypoxic conditions

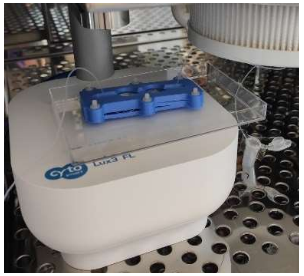
Morphological studies with Fluorescent labelling

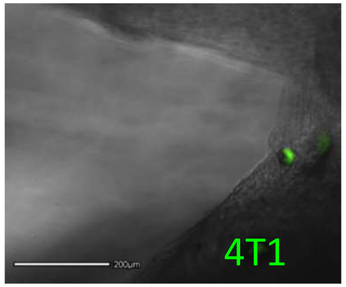


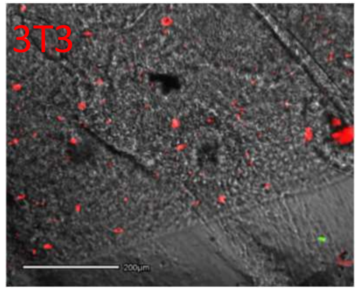
Proliferation, apoptosis assays
Drug toxicity studies

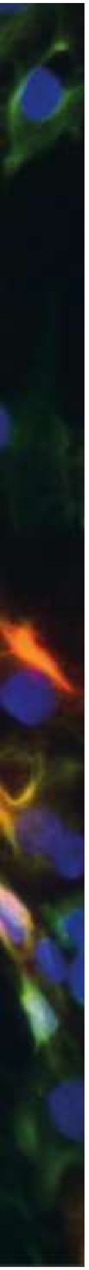


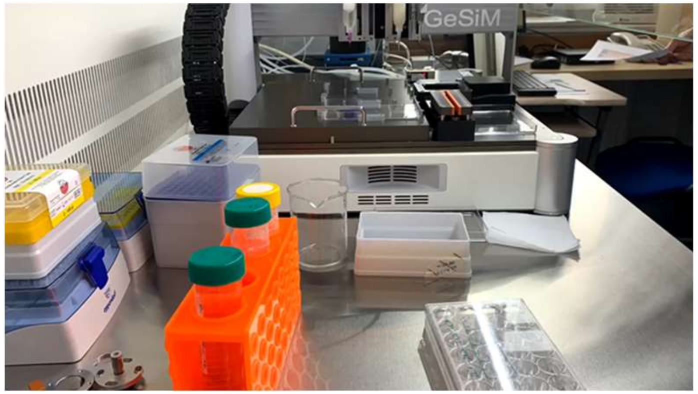
Microfluidic plates
Tumour-on- chip studies
Migration – metastasis studies







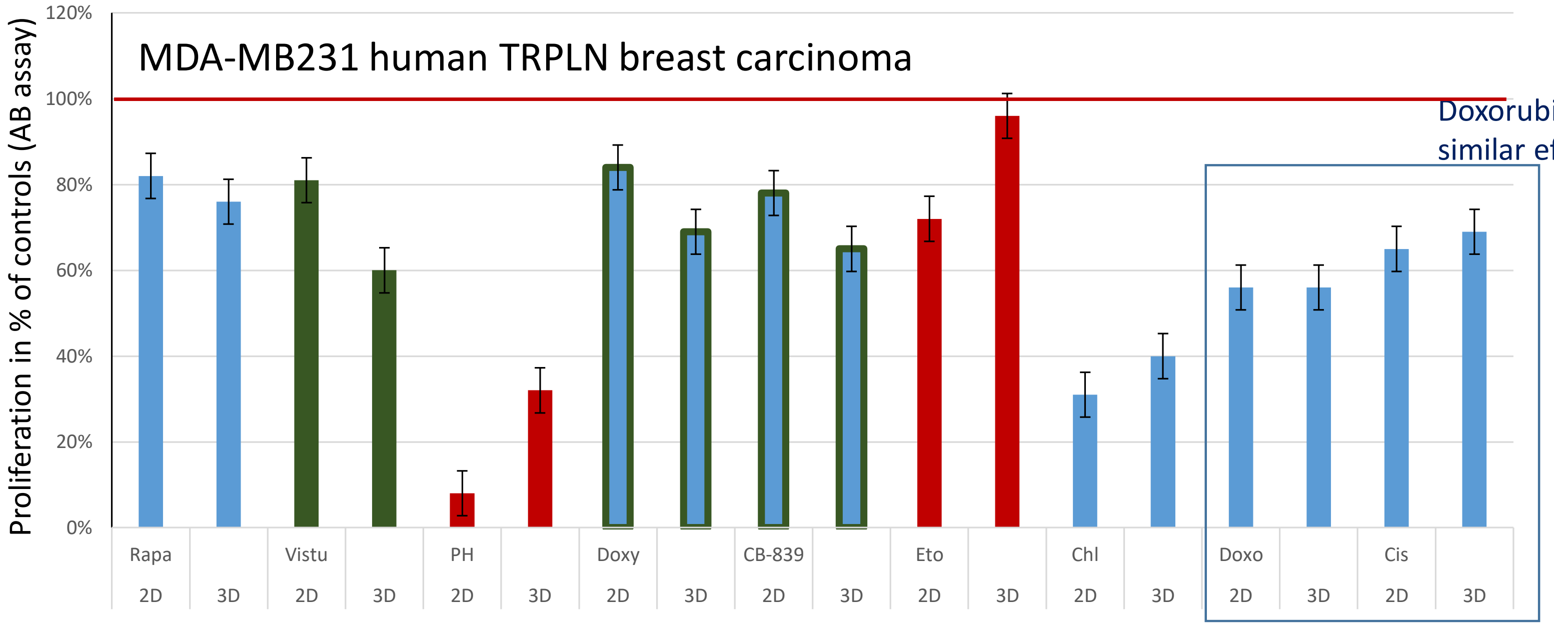




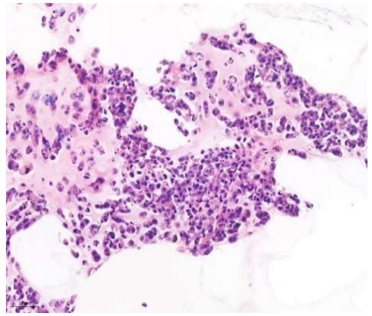
Metabolic inhibitor sensitivity of MDA-MB231 bioprinted rafts

Proliferation tests:
- mAB → Metabolic activity NAD+ H+ és NADP+H+
- mSRB → Total protein
- mBradford → Total protein
- calcein → Viability of cells

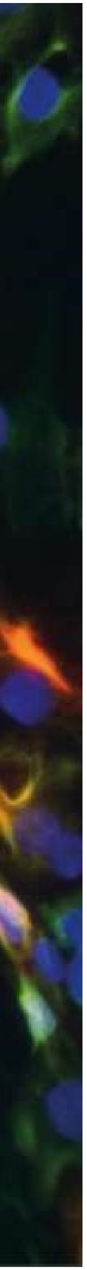
AB



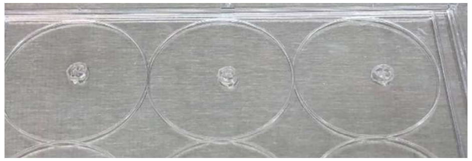
Doxorubicin and cisplatin have similar effects

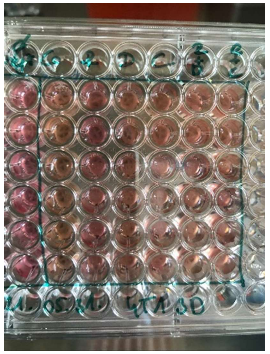


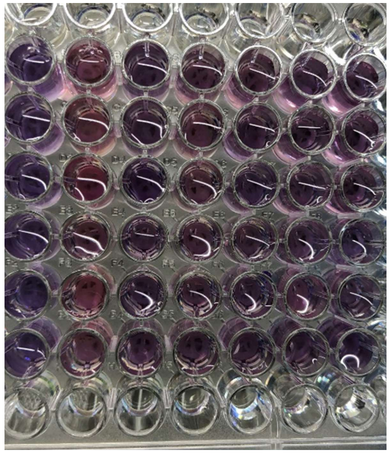
The effects of metabolic inhibitors are different in 2D cultures and 3D bioprinted rafts

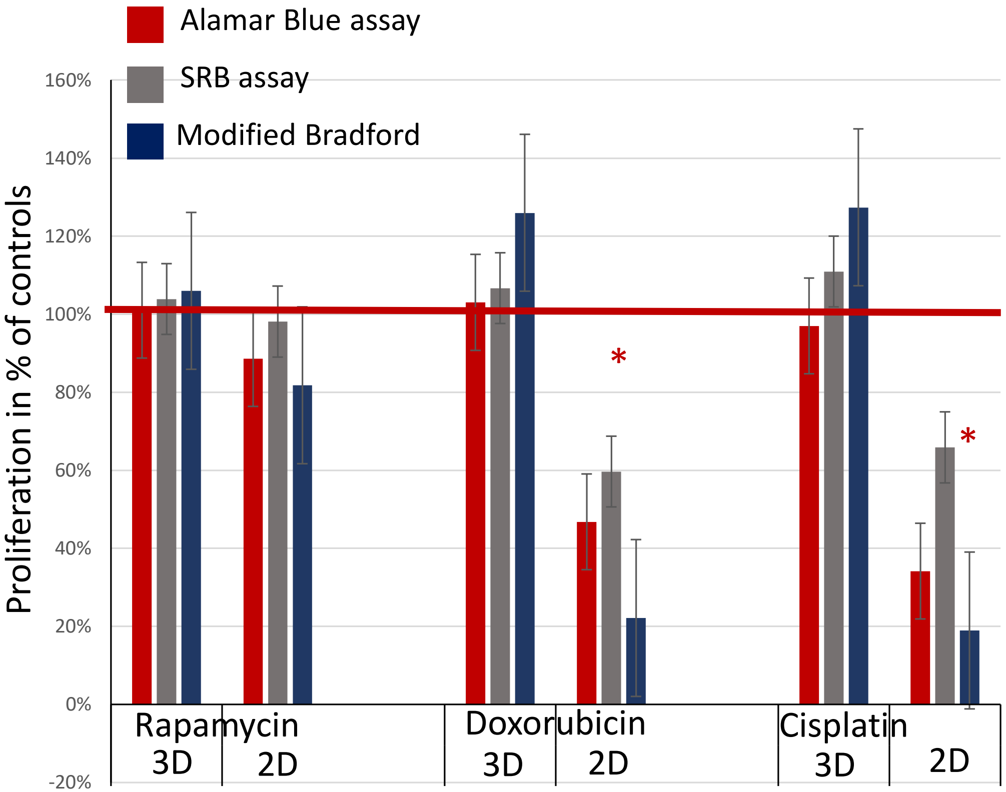


Mouse 4T1 breast cancer cell line
in vitro mTORI and doxorubicin, cisplatin sensitivity





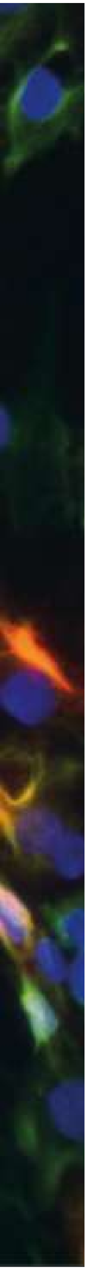


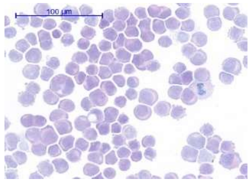


In **3D bioprinted** *in vitro* rafts, the cells were **resistant** to rapamycin, doxorubicin and cisplatin

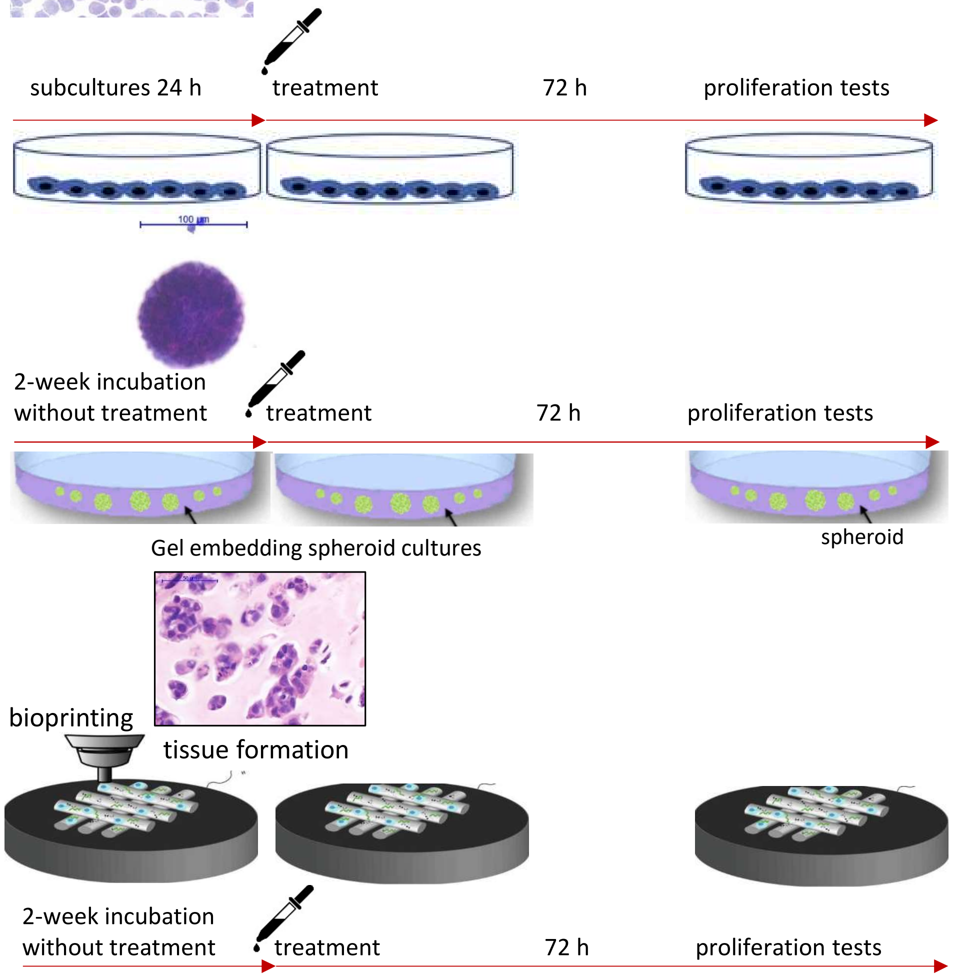
In **2D cell** cultures the majority of the studied cells are **more sensitive** in different drug treatments, than in 3D bioprinted raft cultures

Animal experiments are ongoing to test the *in vivo* sensitivity

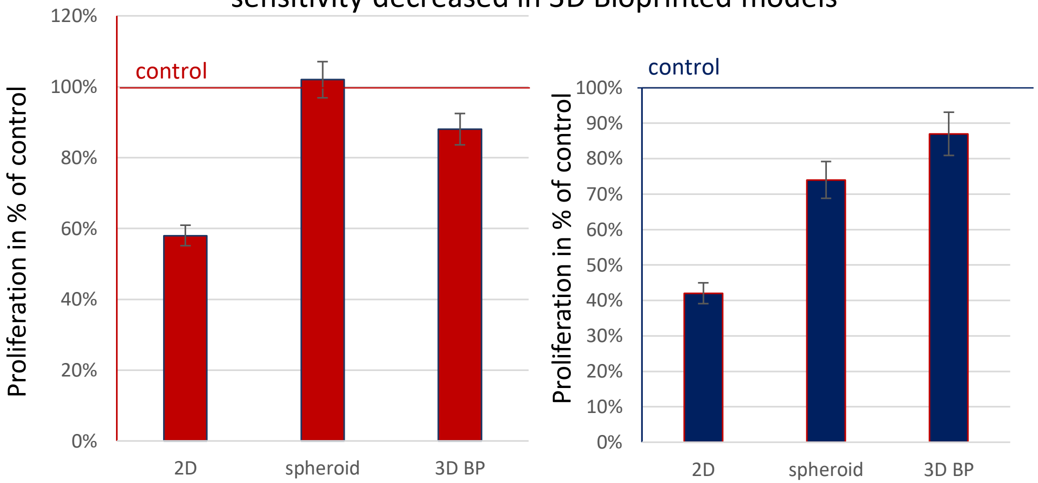




In vitro rapamycin and doxorubicin sensitivity in human ZR-75.1 cells

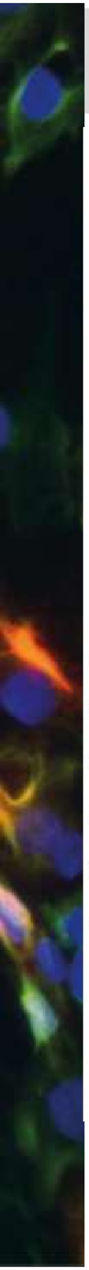


doxorubicin and **rapamycin**
sensitivity decreased in 3D Bioprinted models



Proliferation capacity was detected by Alamar Blue assay in human breast cancer cell line

Altered cellular metabolism in different *in vitro* cell cultures systems versus *in vivo* xenografts



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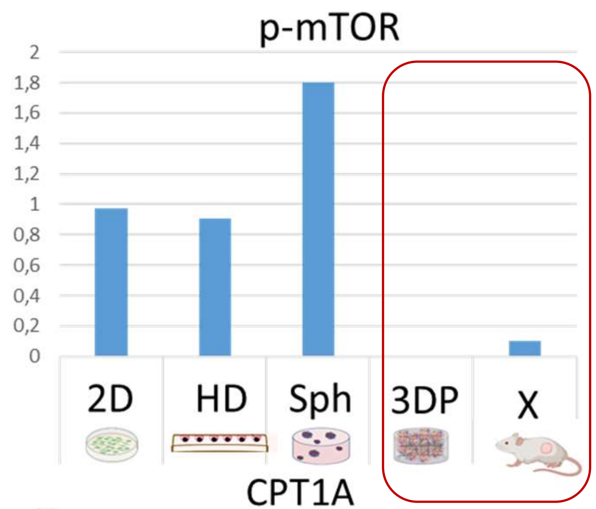
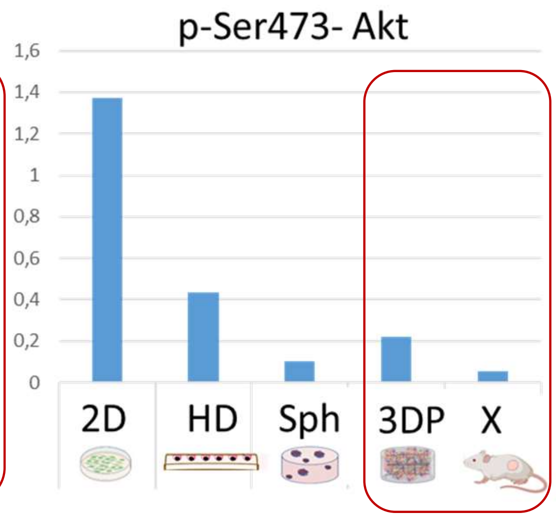
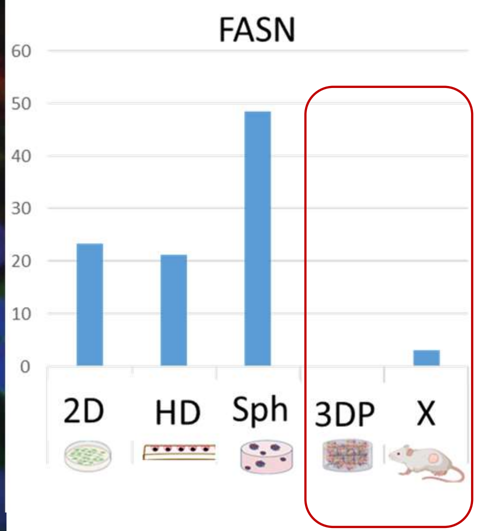
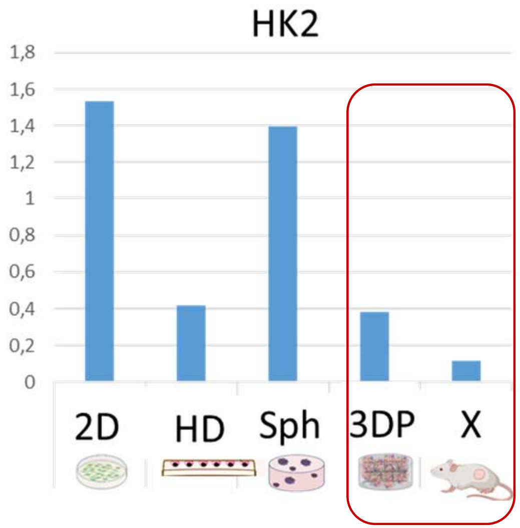
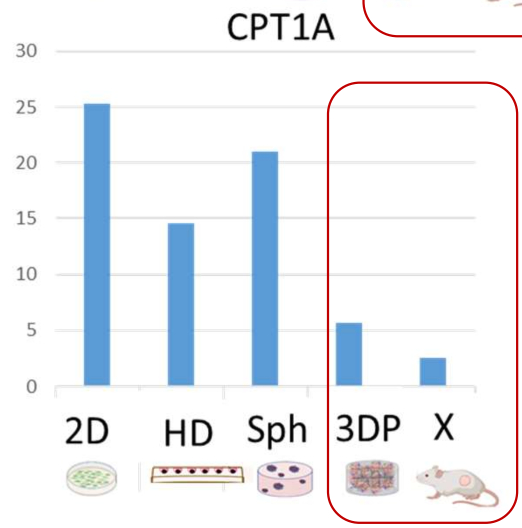


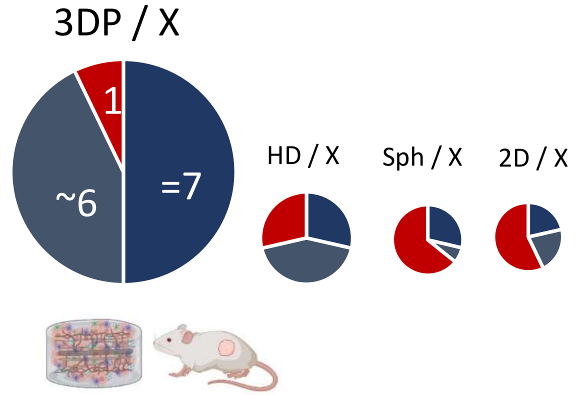




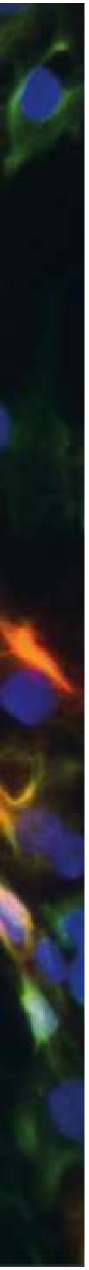




Expression pattern matching

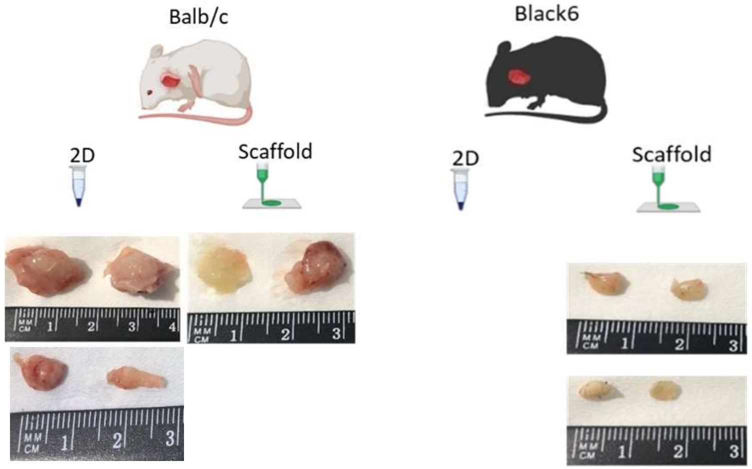


Based on metabolic protein expression, the best matching model could be the 3D Bioprinted raft



Xenografts from 3D Bioprinted rafts

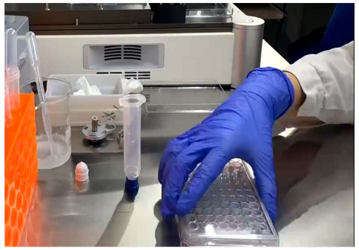
4T1 breast cc. (BALB/c)



Human ZR75.1

50 000 -100 000 cells/SCID

2,5-10 M cells/SCID

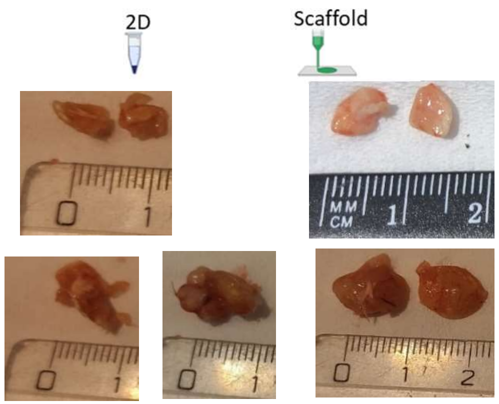


4 – max. 6 weeks

Immediatly
(slow growing ~2 months)
1-2 weeks culturing before xenotransplantation ~3-4 weeks

Previously non-xenotransplantable cells, started to growth in mice after using 3D Bioprinted raft implantation (e.g. human SCLC)

human ZR-75.1

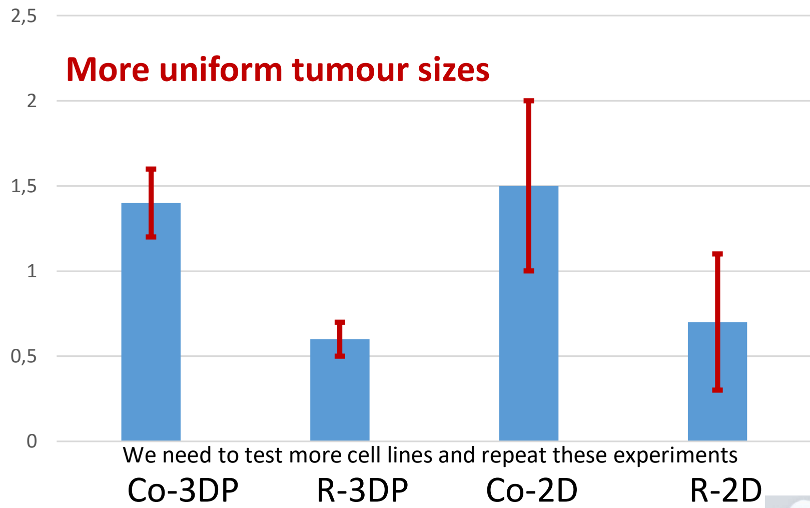


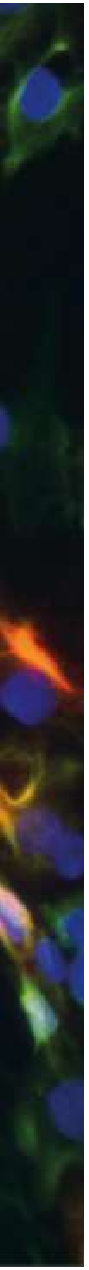
4 weeks

1 week *in vitro*
3 weeks *in vivo*

6 weeks

1 week *in vitro*
5 weeks *in vivo*





Advantages/Disadvantages – using 3D Bioprinted tumour models *in vitro* or in xenotransplatation

Advantages

Using *in vitro* rafts in basic research tests reduces the number of *in vivo* experiments (replacement)

Give real 3D culture *in vivo*

Help lower the cell demand in xenotranplantation

Help lower the variability in tumour size

previously non-grafting tumour cells can be xenotransplanted and used in *in vivo* models

Disadvantages

You need Bioprinter (it is expensive)

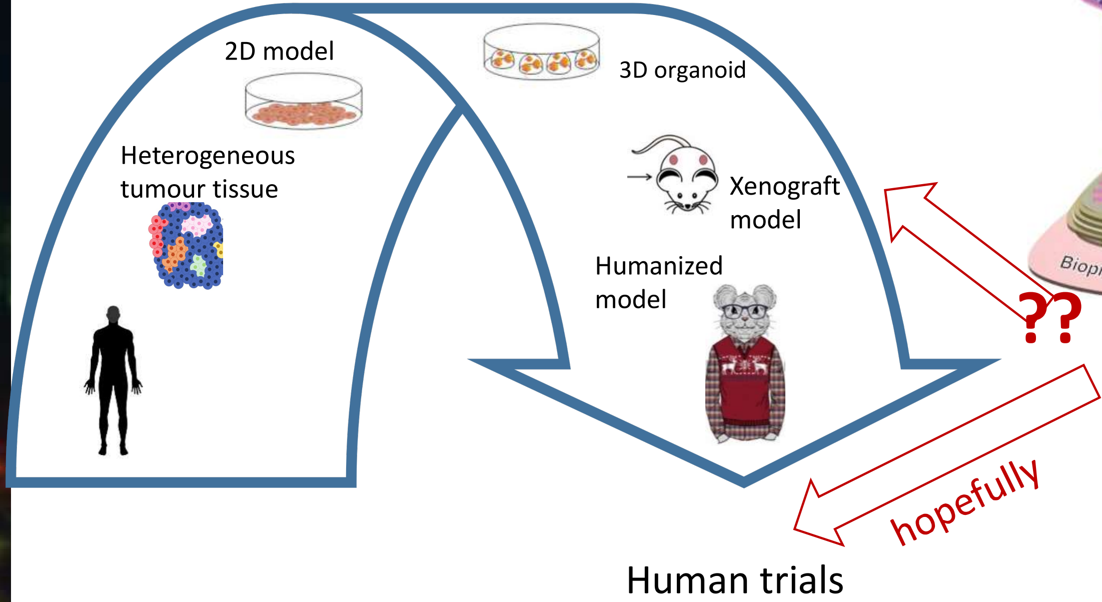
Longer *in vitro* and *in vivo* experiments (it could advantage, as well – e.g. tumour evolution experiments)

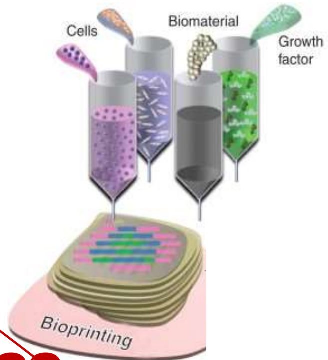
The engraftments are a bit more complicated (small operation) then subcutanous injections

IT COULD BE USEFUL TO - REPLACE A PORTION OF ANIMAL EXPERIMENTS
FOR - BETTER DRUG PRE-SELECTION IN STUDIES

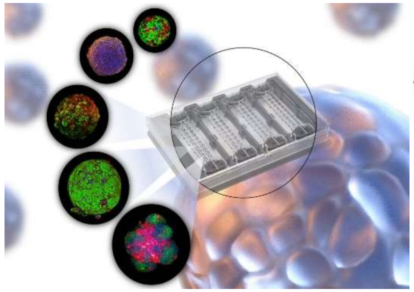


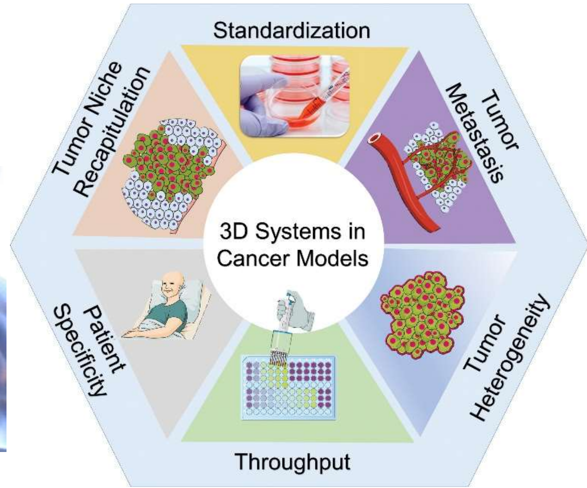






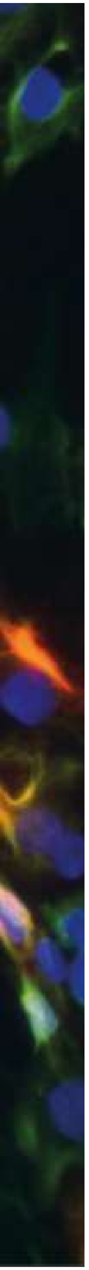
3D Bioprinted human tumour rafts
Real *in vitro* 3D models
higher standardisation
represent tumour heterogeneity
use in high throughput screening technology
use in personalised drug selection
Its combination with body/organ-on-chip model
in better *in vitro* models
Combining with PDX better *in vivo* models





Our goals to find **- the already *in vitro* neglected metabolic factors potential new metabolic targets and**
- testing "new or old" drugs with less side-effects for personalised therapy in different models

We need better ***in vitro* model systems**
and development in well-known *in vivo* carcinogenesis, tumour progression and drug tests before human trials



*"We live longer and healthier lives than ever before. Whilst there have been remarkable improvements in the human environment, and **animal research has played a major part in developing improvements in human health.**"* - Professor Robert Winston

Mainstream medical and scientific organisations and leading scientists all agree that **animal research is essential for medical progress, but we try to do our best to reduce and optimise our model systems**.
I hope that the presented and shown **3D Bioprinting and using 3D Bioprinted tumour models could be an additional, new tools** in these developments.

Thank you

for

Your attention

The help of my colleagues and our supports

Nemzeti Kiválóság Program

AZ NKFI ALAPBÓL MEGVALÓSULÓ PROGRAM

MAGYAR TUDOMÁNYOS AKADÉMIA 1825

NEMZETI KUTATÁSI, FEJLESZTÉSI ÉS INNOVÁCIÓS HIVATAL