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Where are we heading with New Approach Methodologies?

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3R-Center for In Vitro Models and Alternatives to Animal Testing & µOrganoLab, University of Tubingen

13.11.2025

Photo: Jonne Renvall/University of Tampere

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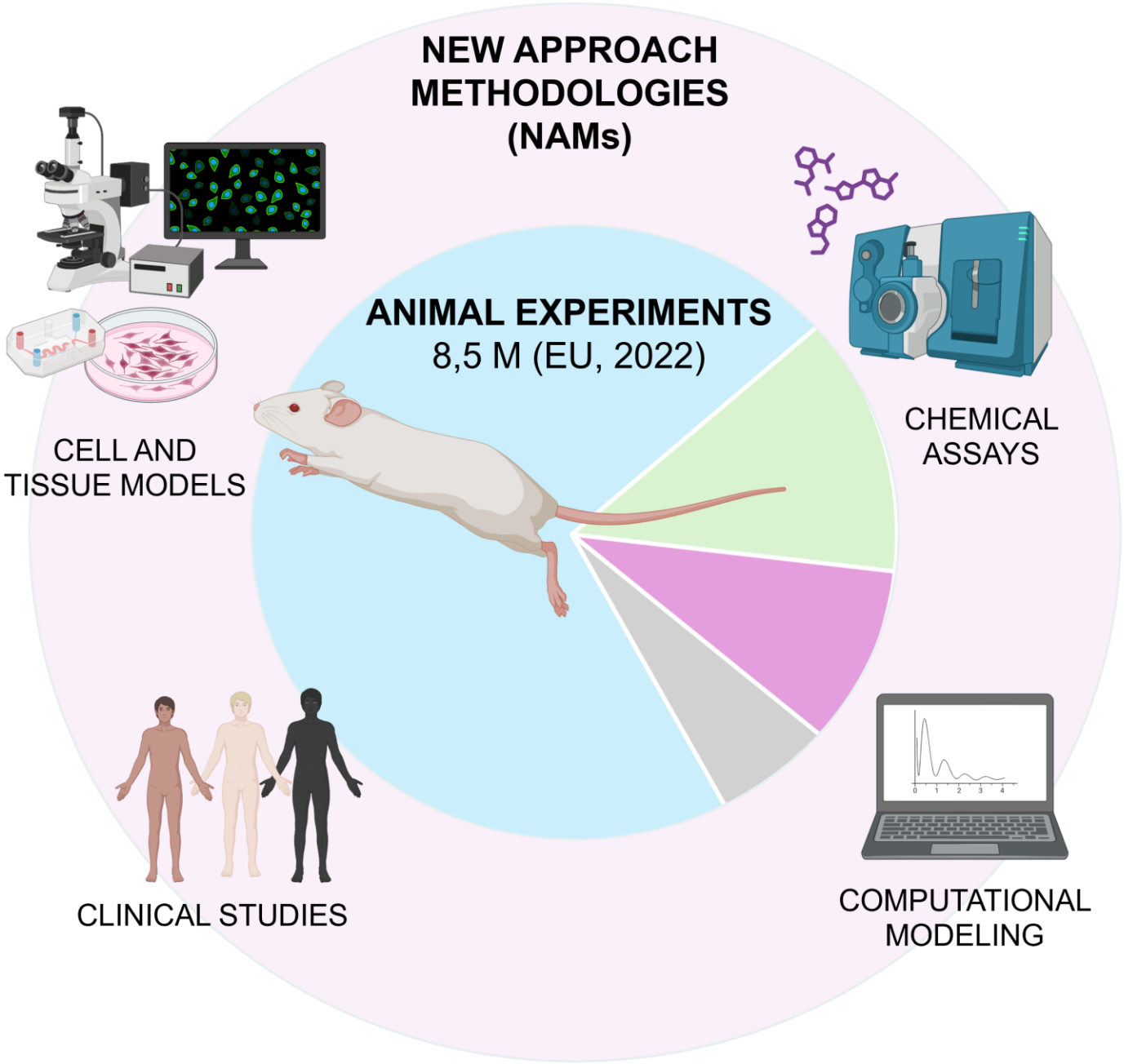

Animal experiments

- Basic research 72 %
- Drug and chemical testing 13 %
- Education and nature conservation 9 %
- Routine production (blood products, antibodies) 6 %

Image made by BioRender.com

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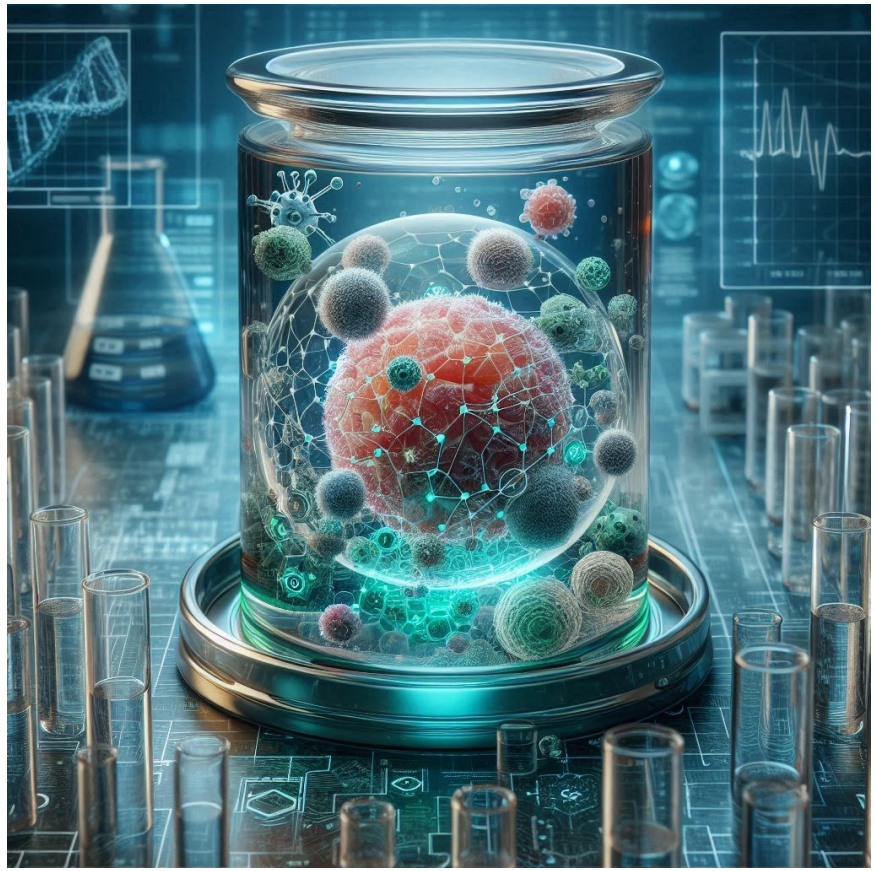
Cell and tissue models



In vitro model with cells and a cell culture flask. Generated with AI

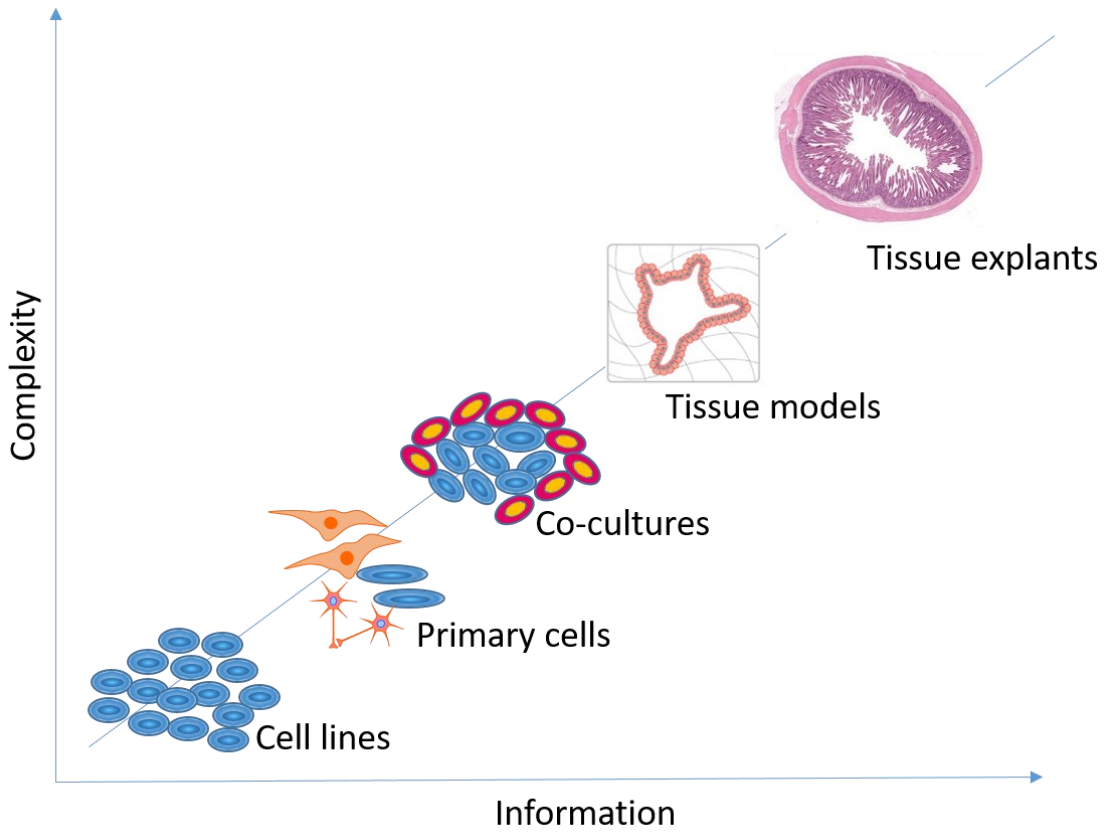


Image from Zeltner & Studer 2015

"*In vitro* models are experimental systems used to study biological processes outside their natural context, typically in a controlled laboratory environment." (copilot)

Immortalized cell lines, primary cells, stem cells, cell models (2D and 3D cell models, organoids and spheroids, organ-on-chips), *ex vivo* models, micro-organisms

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Why are cell and tissue models needed?

- **Basic research**: studying biological processes and mechanisms, eg. developmental biology
- **Translational research**: improved understanding of diseases. Pathological conditions are modelled by recreating the disease in laboratory environment
- → mechanisms or progression of the disease is studied
- **Testing safety and efficacy of compounds**: drugs, chemicals, medical devices, food components
- **Regenerative medicine**: expanding healthy cells in laboratory to replace damaged tissue (transplantation, cell delivery)
- **Reducing and replacing animal models**: final goal of the Directive 2010/63/EU is to phase out animal experiments "as soon as it is scientifically possible"

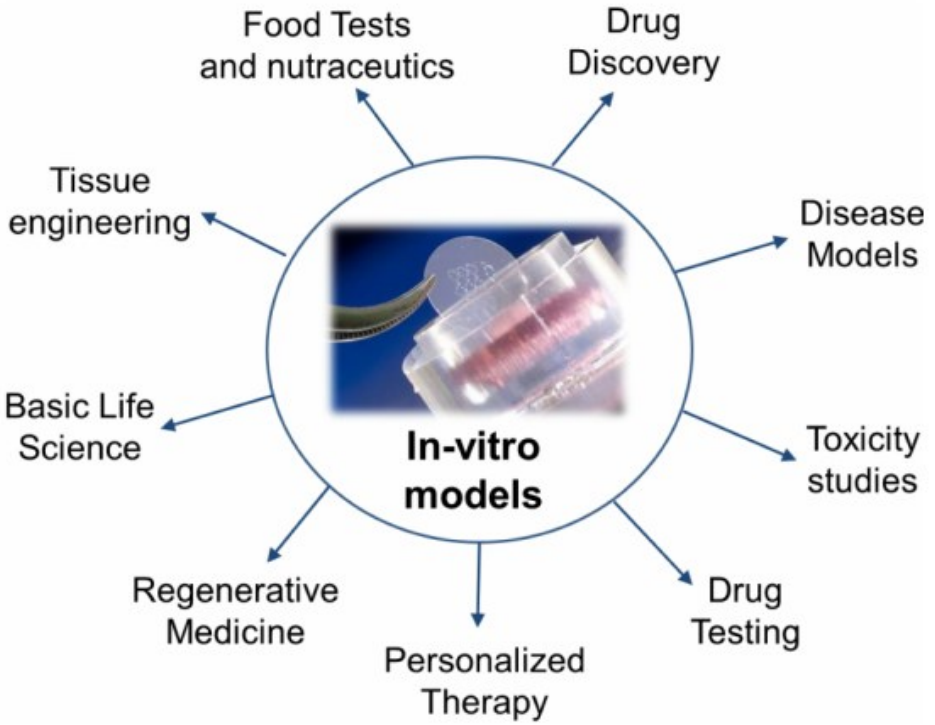
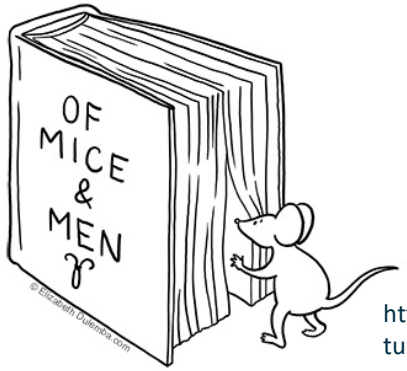
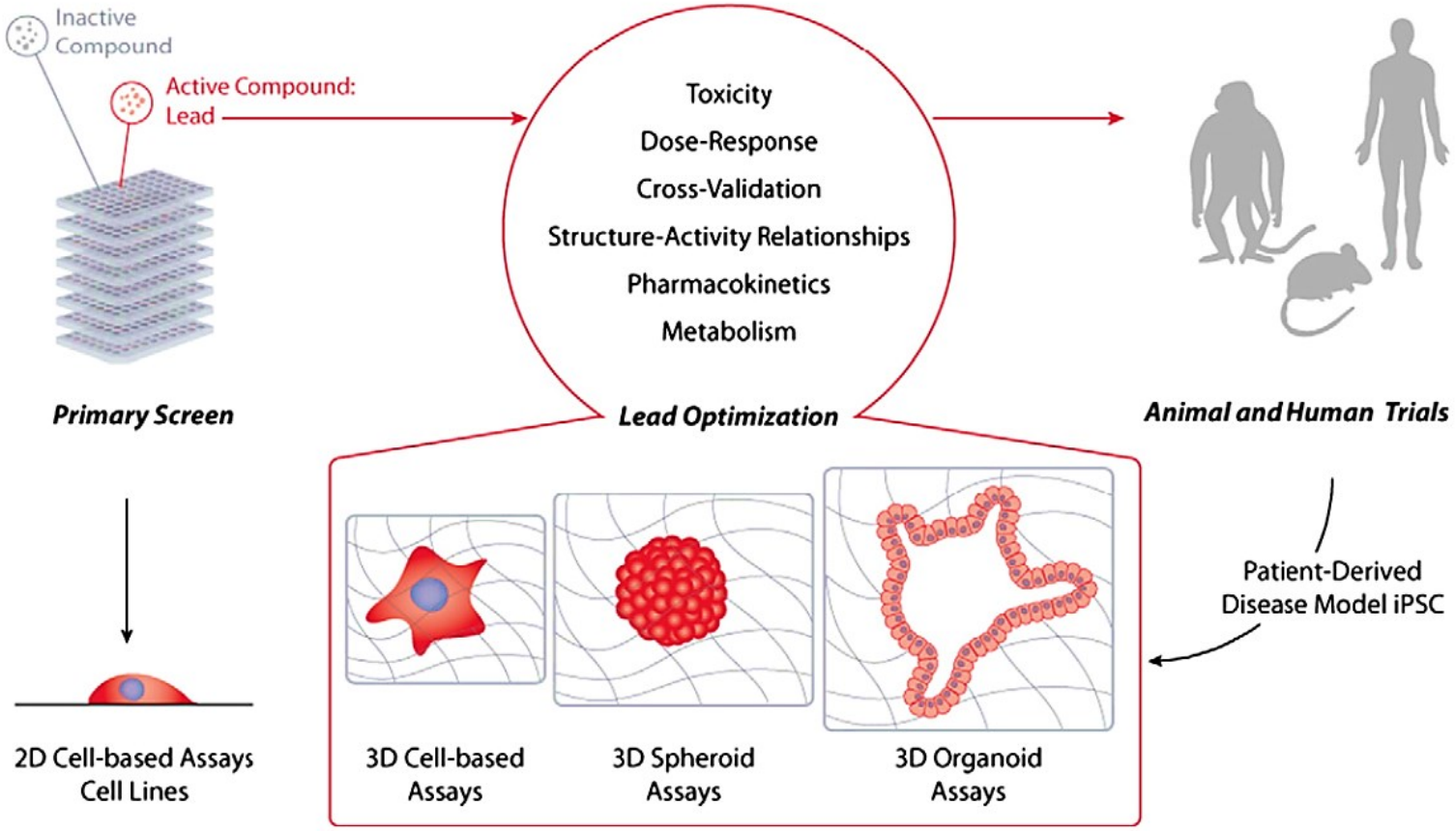


Image from Mattei et al. 2014. Design Criteria for Generating Physiologically Relevant In Vitro Models in Bioreactors.



https://dulemba.blogspot.fi/2017/01/coloring-page-tuesday-mice-and-men.html

Advanced cell models can accelerate and improve drug development



- Drug development takes 10–15 years
- 90 % failure rate for drug candidates in phase I human clinical trial
- Mean cost of developing a new drug is 172.7 M$ (at 2000-2018)
  → increased to 515.8 M$ when cost of failures were included
  → R&D costs increased from 11.9% to 17.7% from 2008 to 2019

Image modified from Ranga et al 2014

Sertkaya et al. 2024. JAMA Netw Open 7:e2415445. doi: 10.1001/jamanetworkopen.2024.15445.

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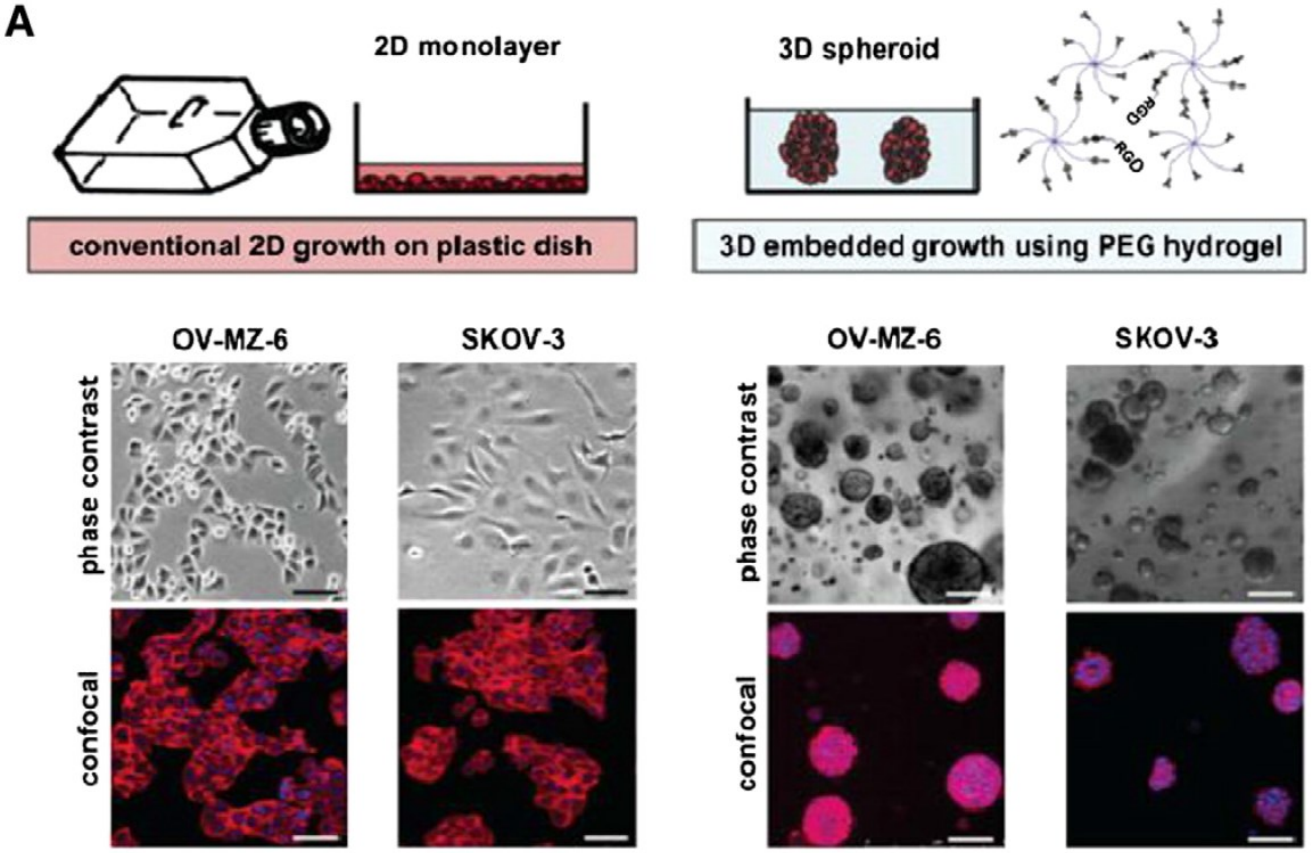
From 2D cell culture to 3D tissue models

- Traditionally, cells are grown in plastic bottles in planar 2D cultures
  - conditions mimic human body with +37°C, 5% CO_2 and 20% O_2
- In the body, cells are in 3D environment, in contact with other cell types, extracellular matrix and interact with neurons and blood vessels
- When building *in vitro* models, 3D tissue-spesific environment is needed
- With improved physiological mimicry, we get closer to the *in vivo* situation and can reflect the tissue functions with improved accuracy
  - control, manipulation, monitoring



Cancer cell line morphology and proliferation are clearly different between conventional 2D plastic culture and within synthetic PEG hydrogel (Actin filaments stained with rhodamine phalloidin , nuclei with DAPI), Ranga et al 2014

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Tampere University

Organ-on-Chip technology

- Organ-on-Chip (OoC) = platform mimicking tissue functions by combining cell biology with microtechnology
  - chip platform with fluidic flow
  - living biological entity
  - stimulation and control
  - analysis
- Control of environmental properties (temperature, pH, CO_2, O_2) and biological processes
  - Cell-cell and cell-environment interactions
  - Real-time physiological responses and their monitoring
- Tissue interactions and even systemic effects by combining OoCs in a “Body-on-Chip”
- vascularization, innervation, immune system

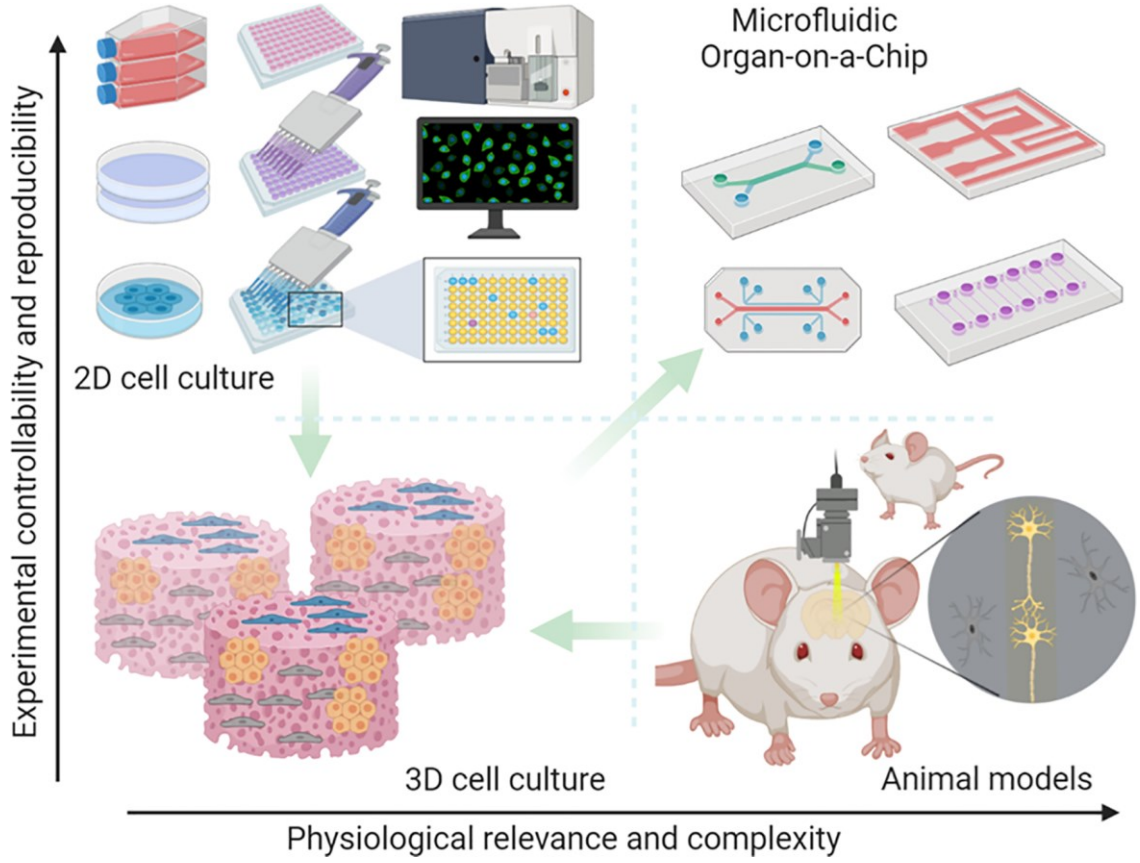

Image from Ma et al. 2021. Organ-on-a-Chip: A new paradigm for drug development

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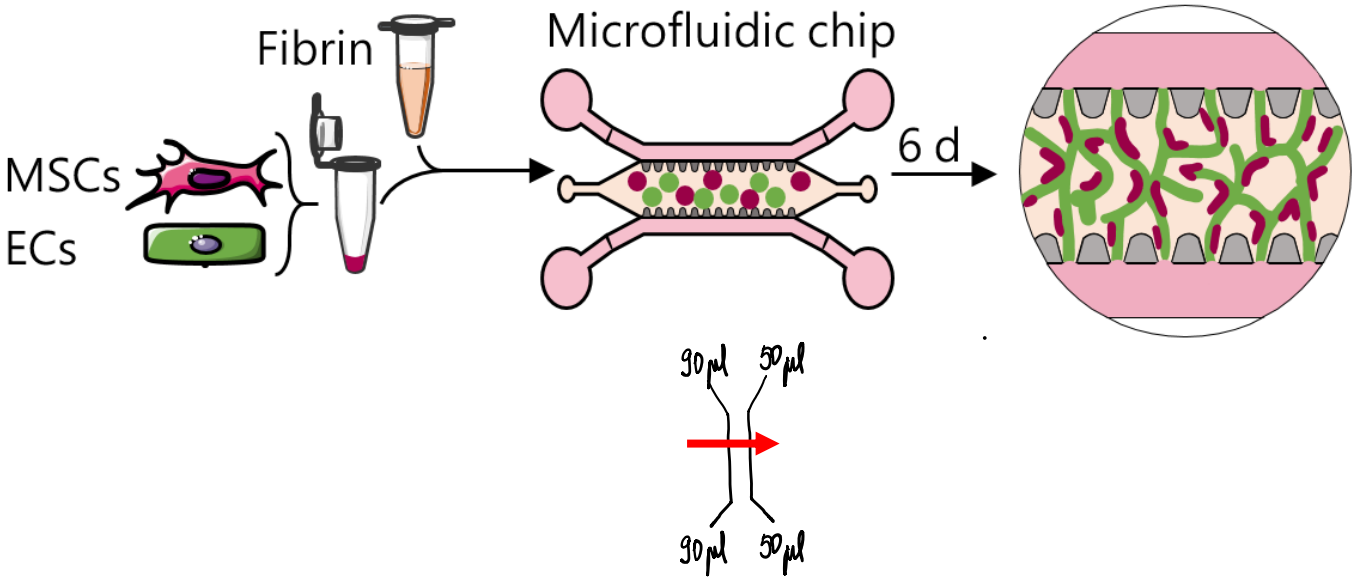
Microvasculature-on-chip

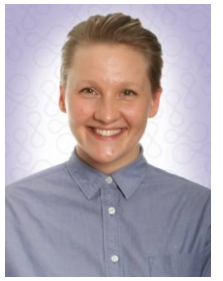
Front Bioeng Biotechnol. 2022 Feb 8:10:764237. doi: 10.3389/fbioe.2022.764237. eCollection 2022.

Vasculogenic Potency of Bone Marrow– and Adipose Tissue–Derived Mesenchymal Stem/Stromal Cells Results in Differing Vascular Network Phenotypes in a Microfluidic Chip

Anastasiia Mykuliak [1 2], Alma Yrjänäinen [1 2], Antti-Juhana Mäki [3], Arjen Gebraad [1 2], Ella Lampela [1 2], Minna Kääriäinen [4], Toni-Karri Pakarinen [5], Pasi Kallio [3], Susanna Miettinen [1 2], Hanna Vuorenpää [1 2]



Anastasiia Mykuliak, Doctoral researcher

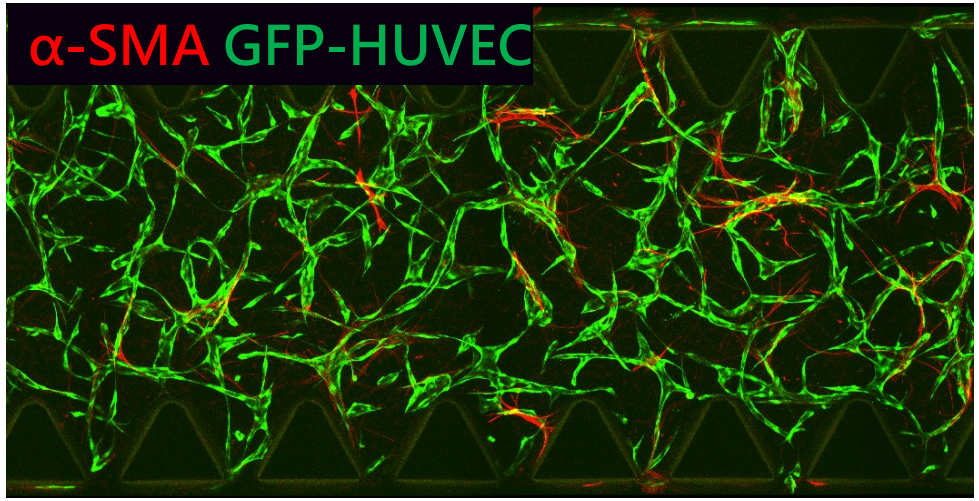


Alma Yrjänäinen, Doctoral reseacher

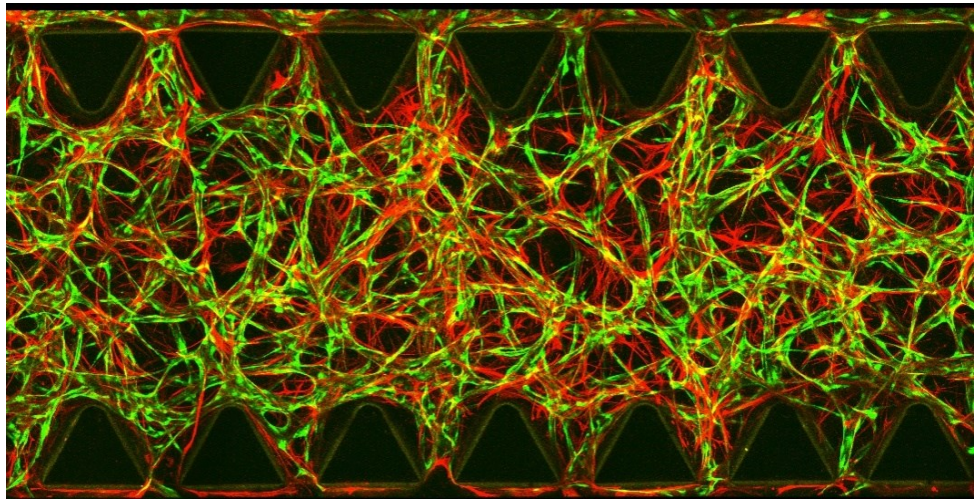
Susanna Miettinen, Prof., Adult Stem Cell Group leader

Hanna Vuorenpää, Postdoctoral researcher

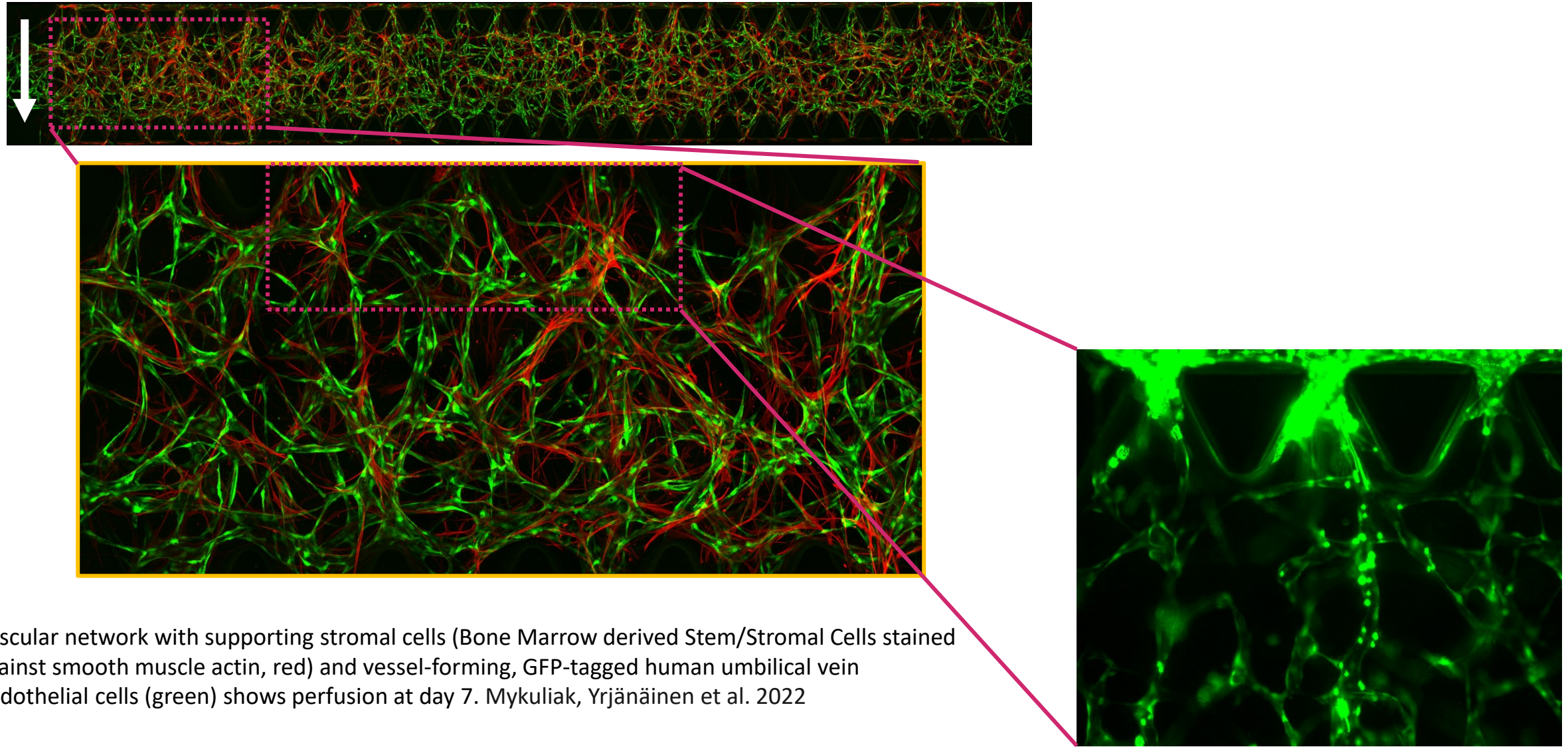
EC-ASC



EC-BMSC



Perfusable vascular network forms the basis for Organ-on-Chip



Vascular network with supporting stromal cells (Bone Marrow derived Stem/Stromal Cells stained against smooth muscle actin, red) and vessel-forming, GFP-tagged human umbilical vein endothelial cells (green) shows perfusion at day 7. Mykuliak, Yrjänäinen et al. 2022

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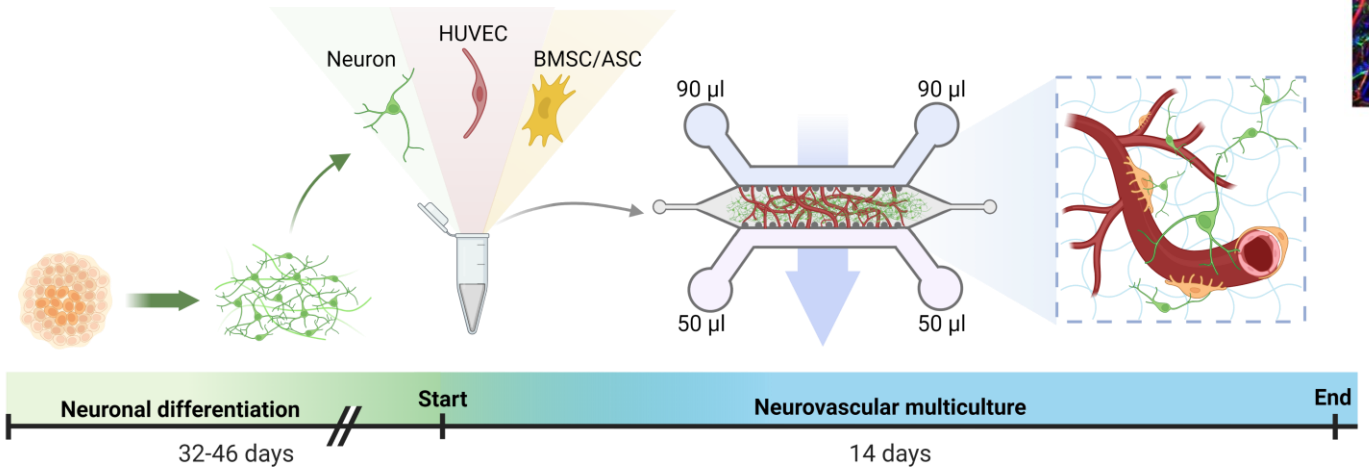
Neuro-vascular network on-chip

Cell Commun Signal. 2023 Jun 14;21(1):132. doi: 10.1186/s12964-023-01159-4.

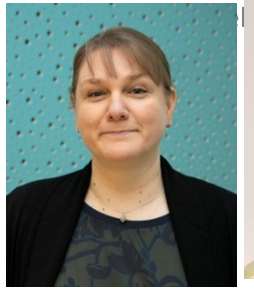
Simultaneous induction of vasculature and neuronal network formation on a chip reveals a dynamic interrelationship between cell types

Lotta Isosaari [1 2 3], Hanna Vuorenpää [2 3], Alma Yrjänäinen [2 3], Fikret Emre Kapucu [1], Minna Kelloniemi [4], Toni-Karri Pakarinen [5], Susanna Miettinen [2 3], Susanna Narkilahti [6]



Lotta Isosaari, Doctoral researcher

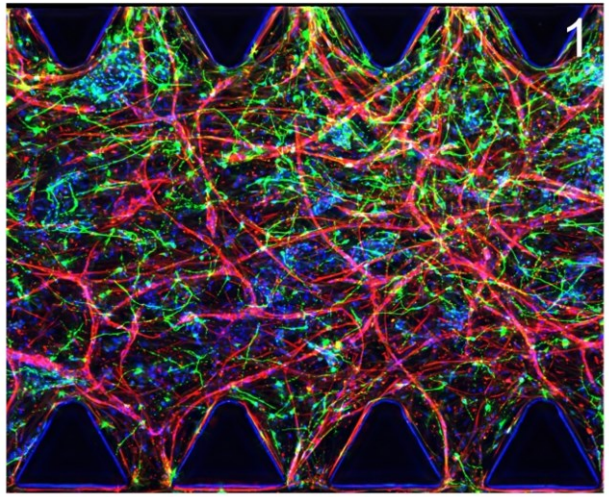
Hanna Vuorenpää, Postdoctoral researcher



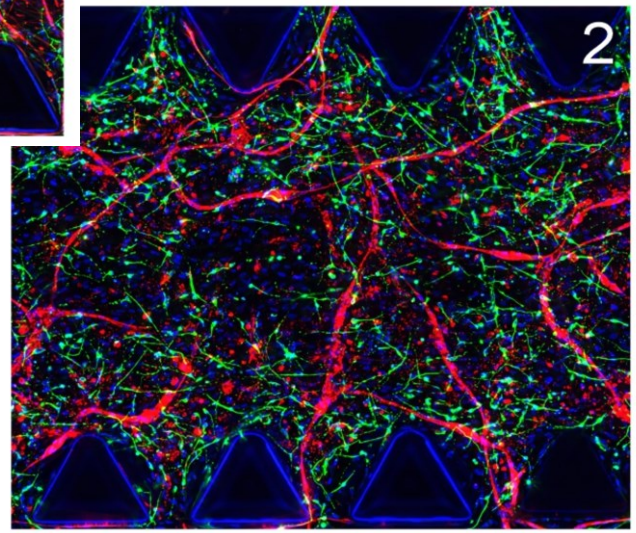
Susanna Miettinen, Prof., Adult Stem Cell Group leader

Susanna Narkilahti, Neuro Group leader

EC-BMSC-neuro multicultures



EC-ASC-neuro multicultures



Nuclei (DAPI)
Neurons (MAP-2+βtubIII)
Endothelial cells (CD31)

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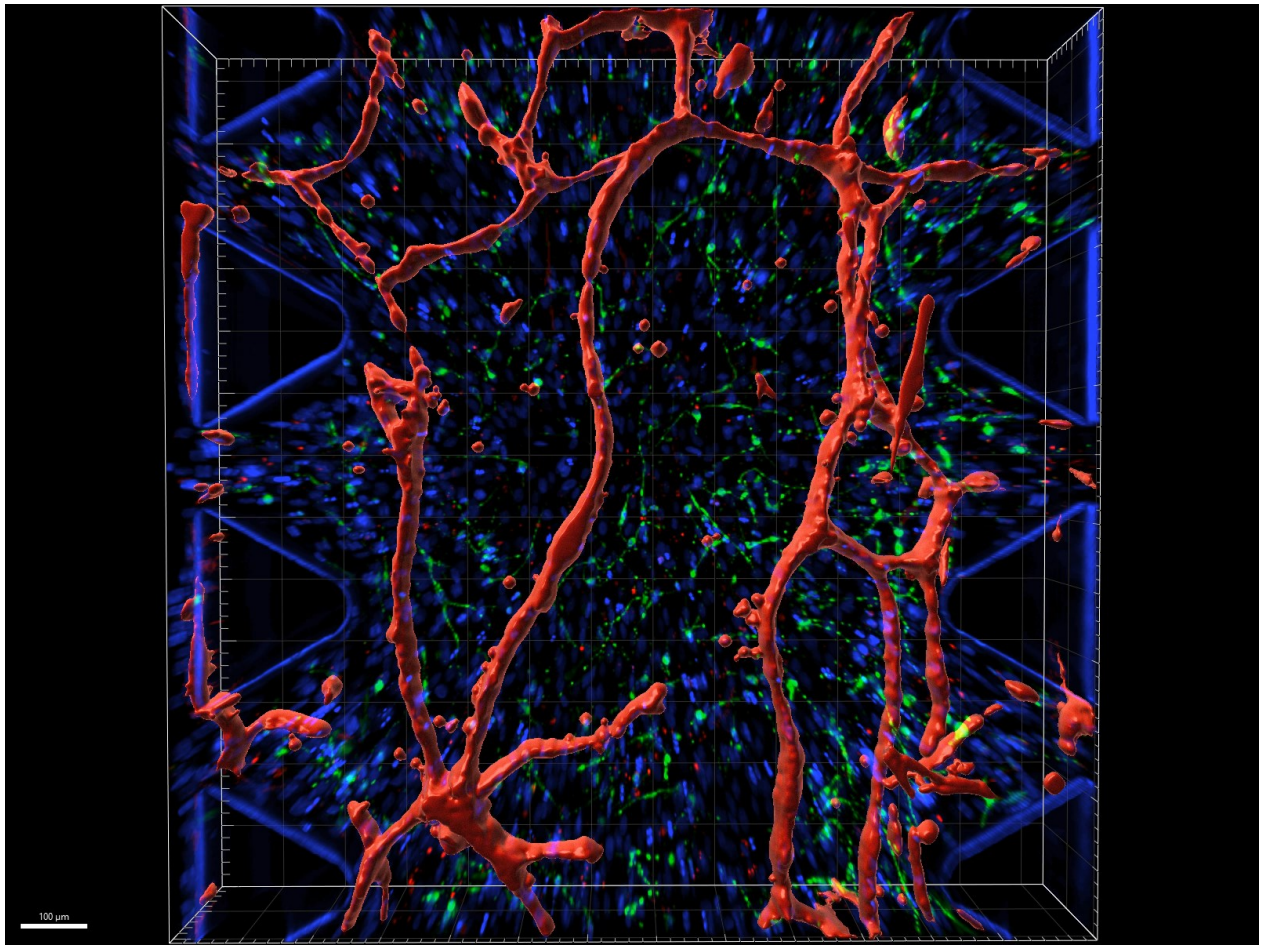
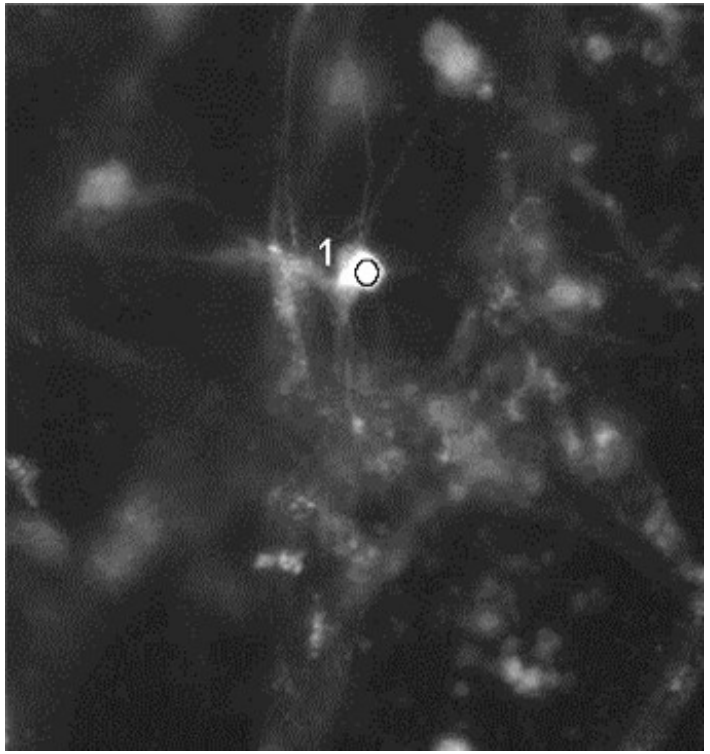
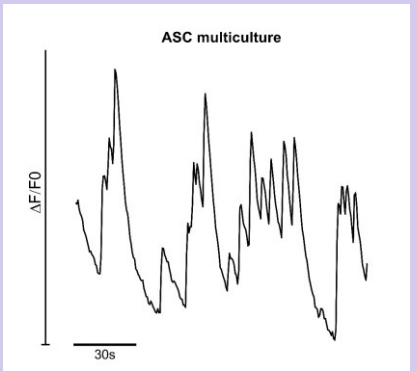
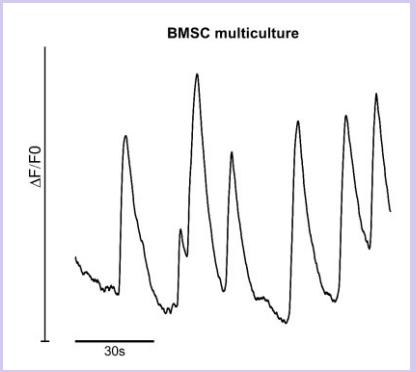
Neurons interact with the vascular network and remain functional









Isosaari et al. 2023. Cell Commun Signal.

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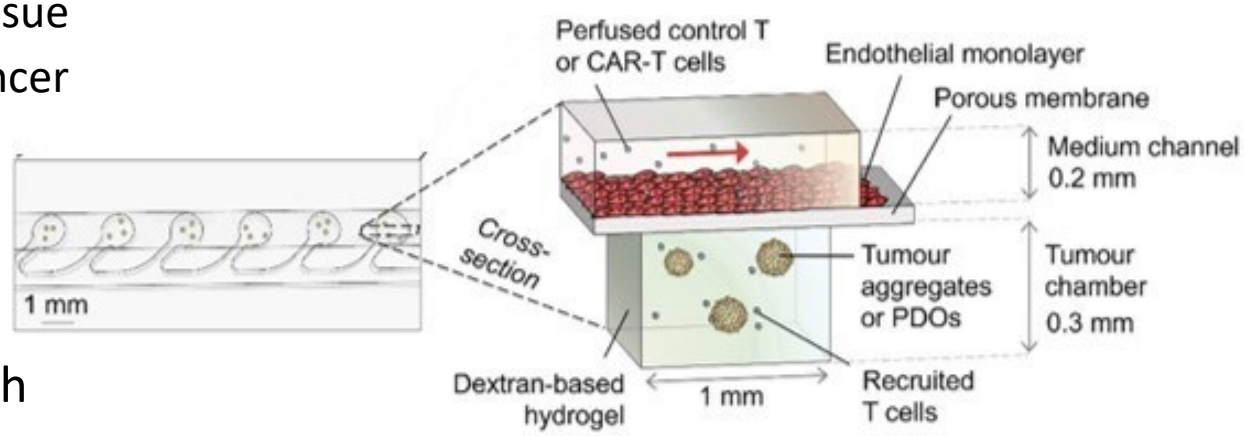
Tumor-on-Chip with immune response

- Breast cancer-on-chip model with integrated endothelial barrier
  - breast cancer cells lentivirally transduced to express GFP
  - human microvascular endothelial cells isolated from skin tissue
  - CAR-T cells (genetically engineered T cells to attack own cancer cells) from donor blood mononuclear cells
  - patient-derived organoids established from breast cancer patients
- Model recapitulates the initial events in CAR-T cell administration in patients → CAR-T cell perfusion through the vasculature and extravasation towards the tumor
- Model captures the phases over the following week → CAR-T cell infiltration and lysis of tumor cells + cytokine release
- Testing pharmacologic safety switch to control CAR-T cells during the therapy
- Integration of metastatic breast cancer patient organoids to demonstrate their applicability in the model and CAR-T cell response



Tissue-engineered vascular models are used to provide mechanistic insight into tumor behaviour, extravasation and therapeutic delivery. Maulana et al. 2024. Breast cancer-on-chip for patient-specific efficacy and safety testing of CAR-T cells. Cell Stem Cell. doi: 10.1016/j.stem.2024.04.018.

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Global activities in implementing NAMs

- **USA**: Food and Drug Adminstration (FDA) Modernization Act 2.0 authorized the use of NAMs to support new drug application and removed the requirement to use animal studies (2022)
    - In the long-term, FDA aims to make animal studies an exception rather than norm in preclinical safety/toxicology testing
- **European Union**: EU Commission roadmap for phasing out animal testing in chemical safety assessments
    - short- and long-term actions, including accelerating the development and regulatory acceptance of NAMs in chemical safety assessment
    - the roadmap is planned to be published in the first quarter of 2026
- **UK:** plan to phase out animal experiments sets out specific commitments
    - To end regulatory testing on skin and eye irritation and skin sensitization using animals by the end of 2026
    - By 2027, researchers are expected to end tests of the strength of botox on mice
    - By 2030, reduce pharmacokinetic studies on dogs and non-human primates

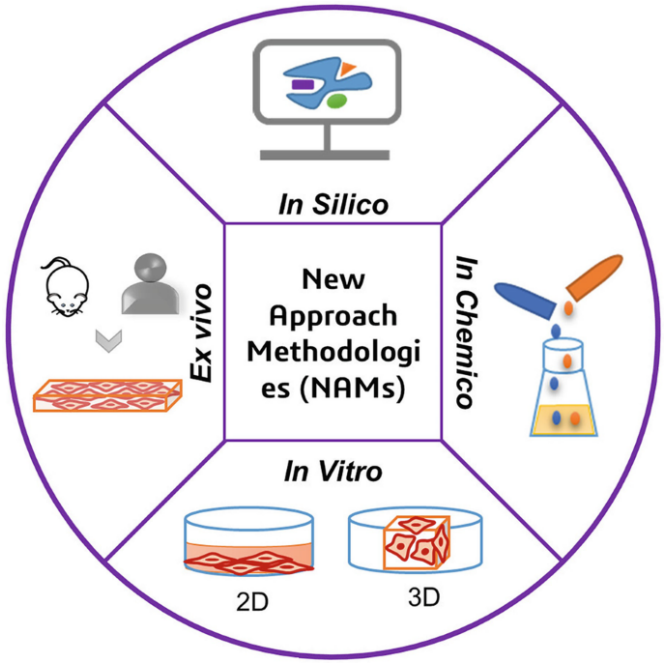

Image from Kasoju & Kripasagari 2024. Animal Models In Research, pp 47–76. New Approach Methodologies (NAMs): Rethinking Preclinical Evaluation of Pharmaceuticals and Medical Devices Beyond Animal Models.

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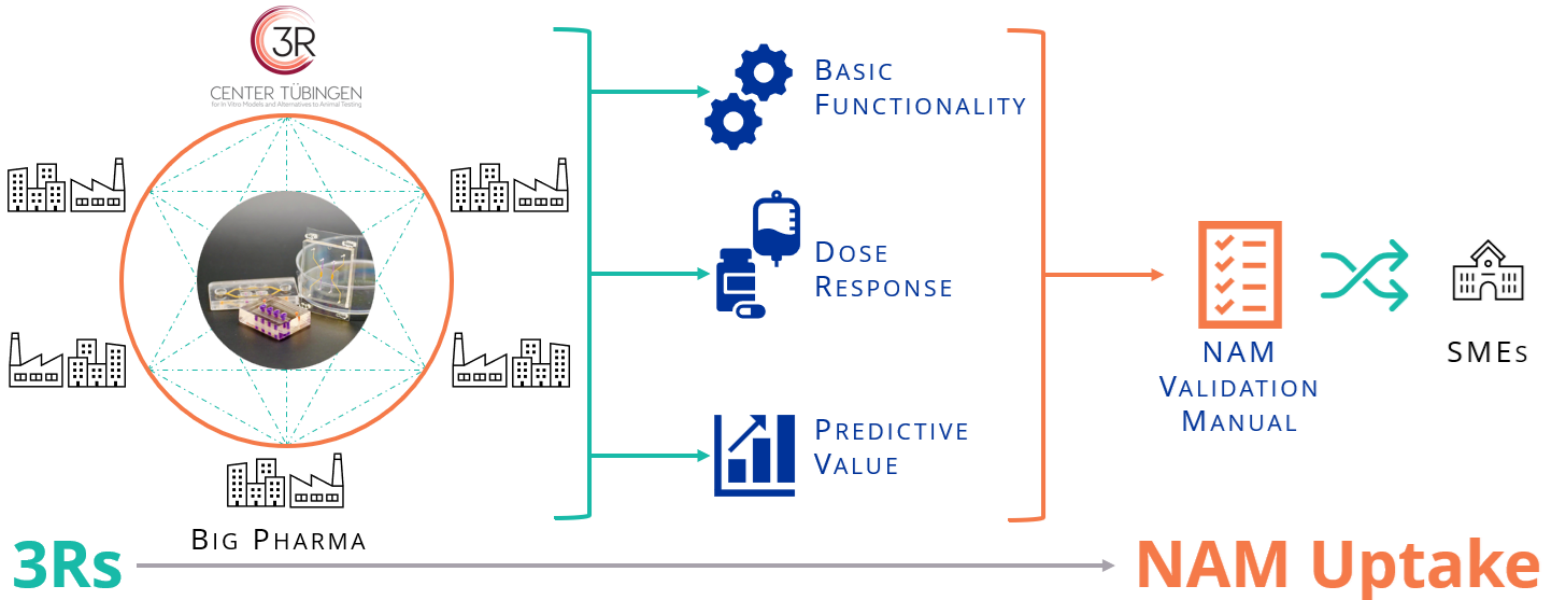
Industrial adaption of NAMs

Objective

Demonstrate stability, reproducibility and predictive value of Organ-on-Chip models to generate best practices for broader industry adoption.

How?

Multi-site functional pre-validation of Organ-on-Chip models with industry partners. SMEs with prior Organ-on-Chip experience (as users or technology providers) are invited for the study as well as pharma companies interested in NAM validation.



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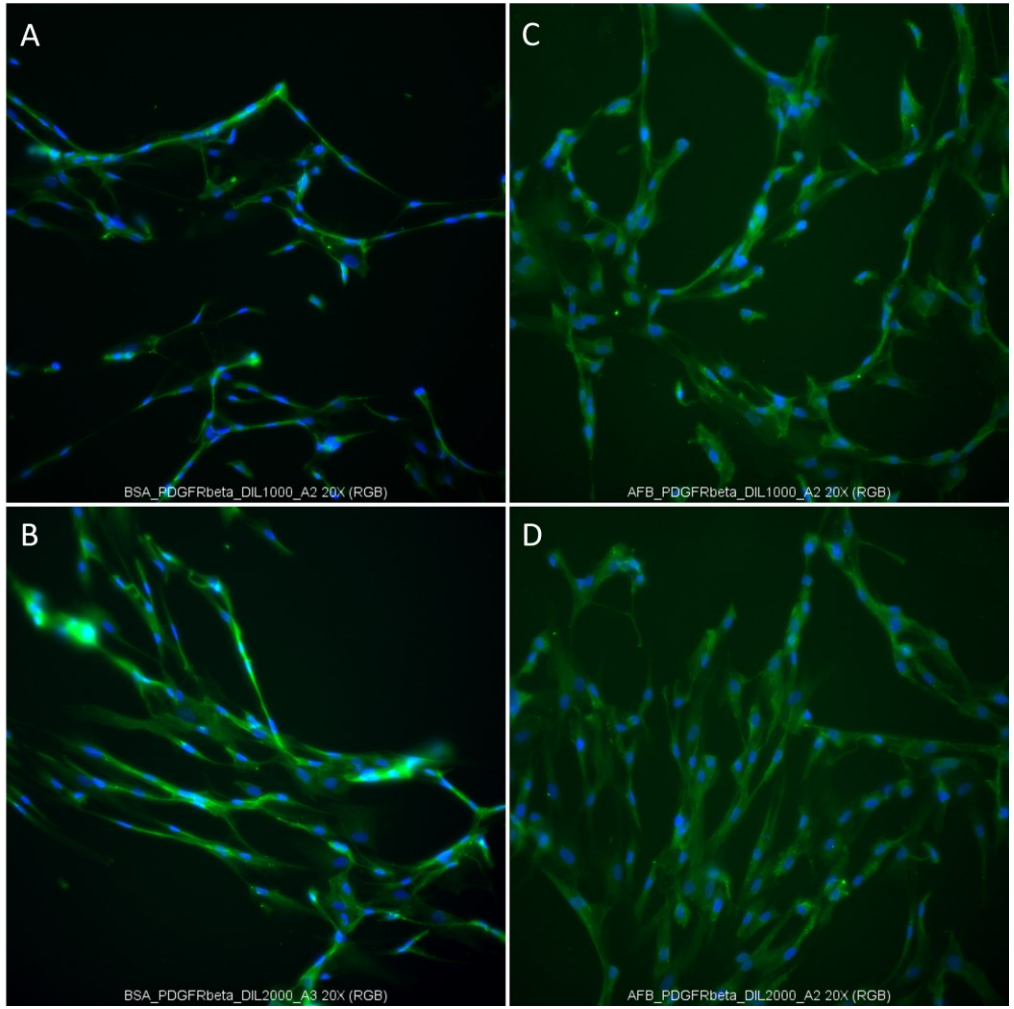
Replacing animal-derived reagents in NAMs -example of antibodies



An animal-free antibody (against platelet growth factor receptor β) was tested on adipose tissue stem cells with bovine serum albumin (A,B) and with an animal-free blocker (C,D). Photo Johanna Laakkonen/University of Tampere

- Detection of target proteins relies heavily on animal-derived antibodies although issues with reproducibility and specificity are widely acknowledged
  - antibodies are collected from immunized animals
- Replacing animal-derived antibodies with animal-free antibodies in immunocytochemical staining of cells
  - funding from Juliana von Wendt Foundation for pilot project (6 months)
  - aiming to replace the most commonly used antibodies in immunocytochemical staining of cells
- Lessons learned from the project
  - completely animal-free antibodies are difficult to find
  - antibody users need to put pressure to the market
  - Replacement is possible but laborious

Where are we now with NAMs?

Shortcomings

- Lack of adequate physiological complexity
- Lack of organ interactions and systemic effects
- Lack of immunological complexity
- Low throughput
- Lack of expertise (education and training)
- Lack of regulatory readiness (reproducibility, realiability)
- Lack of industrial adoption

Benefits

- Mechanistic understanding of basic biology and diseases
- Personalized medicine applications
- Control and manipulation
- Integration of technology (micro and nanotechnology, sensors, AI)
- Human relevance leading to improved treatmens
- Ethically more sustainable
- Faster decision making
- Reducing costs

Future perspectives for New Approch Methods

- Complexity of NAMs is increasing and biology is combined with eg. patient samples, 3D imaging and microtechnology
  - building confidence in the technology
- AI and big data analytics can help to store and analyze large data sets → future role of AI likely bigger and growing rapidly
- Requirements for personalized medicine are increasing
- Public pressure to replace animal testing is shaping the (political) decision-making
- EU and UK roadmaps to phase out animal experiments, US modernization act

Image from 2nd Conference Commission roadmap towards phasing out animal testing for chemical safety assessments/25th October 2024

Thank you Björn Ekwall Memorial Foundation!

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Photo: Jonne Renvall/Tampere University