Institute of Biotechnology
Berlin University of Technology

Berlin University Alliance

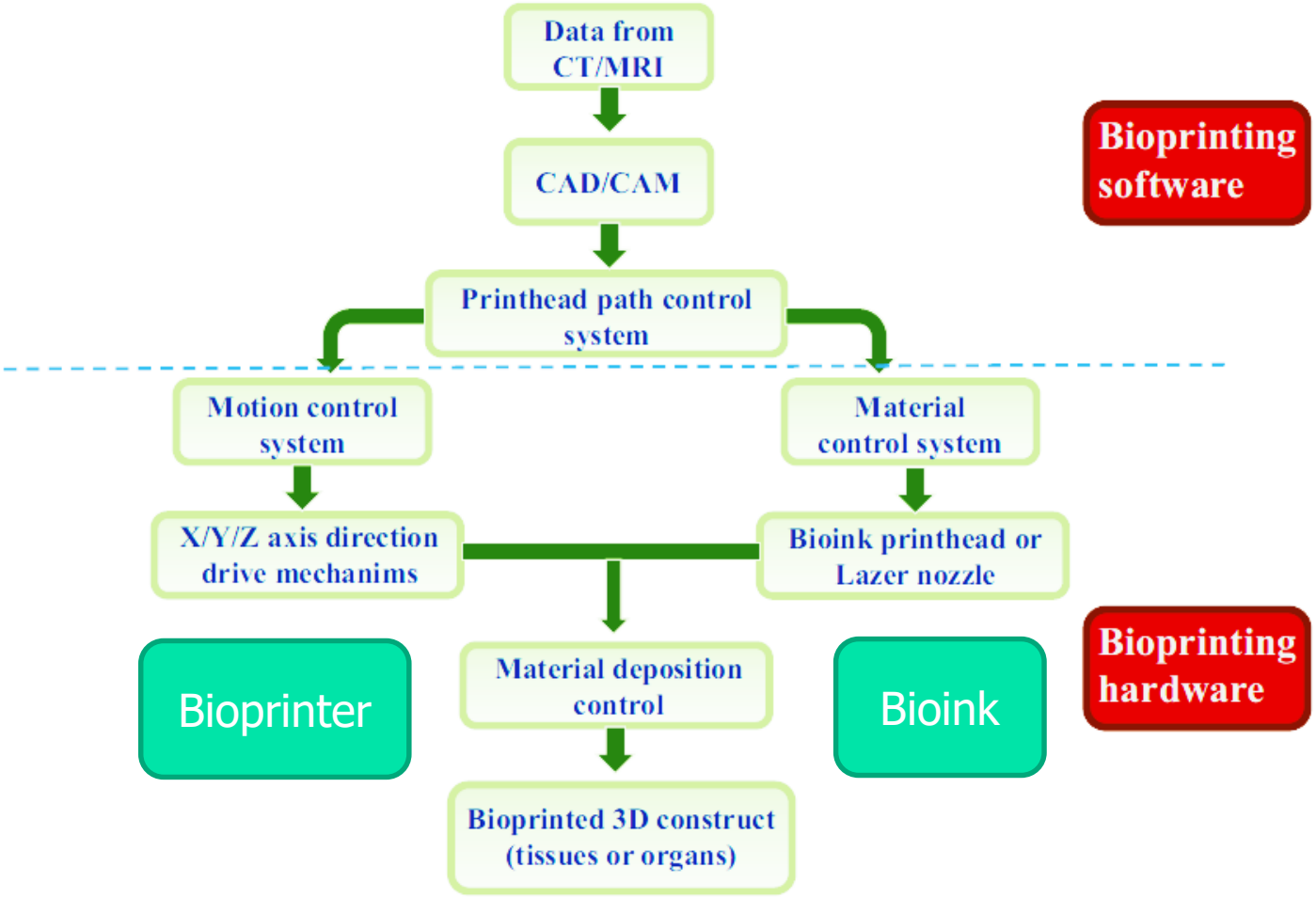
Bioprinting of 3D Organ Models for Virus and Cancer Research

Jens Kurreck

Overview

- Bioprinting Technology
- Influenza infection of bioprinted lung model
- Adenovirus infection of bioprinted liver model
- Outlook: Bioprinting in cancer research
- Outlook: Clean bioprinting

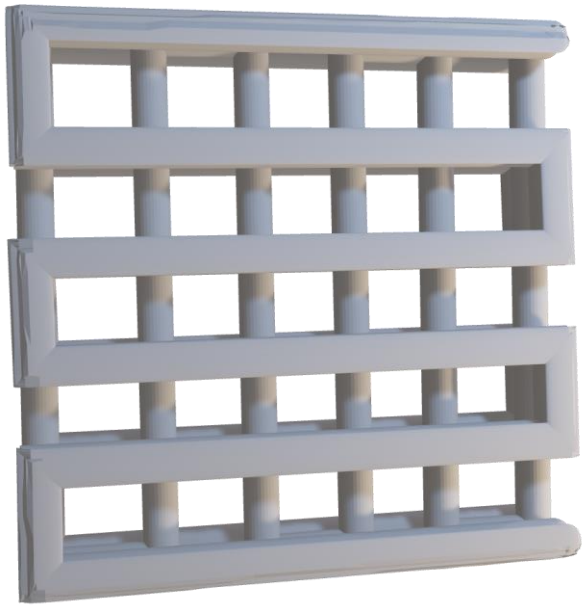
Workflow 3D Bioprinting

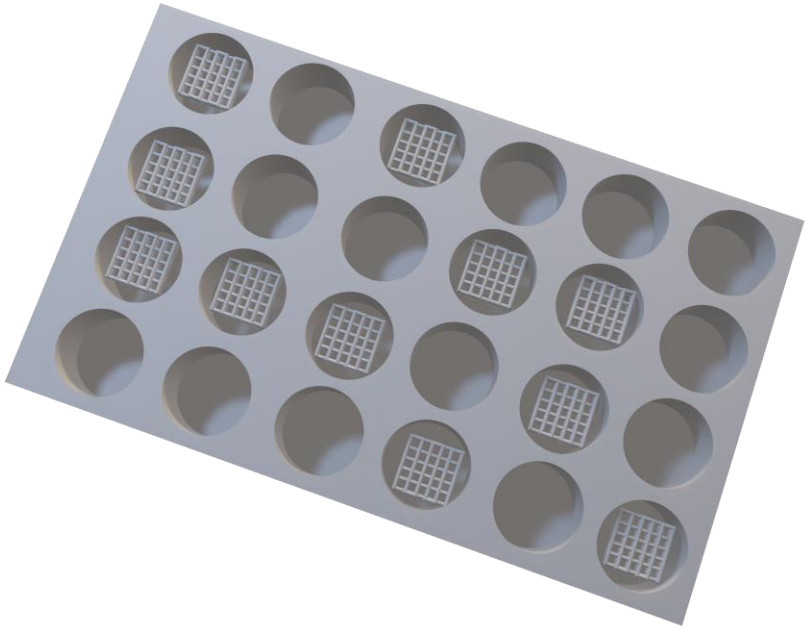


Li et al. (2016) J. Transl. Med. 14, 271.

Step 1: Design of 3D Model

- Design of 3D Model by Coputer-Aided Design (CAD), e. g. Rhino.



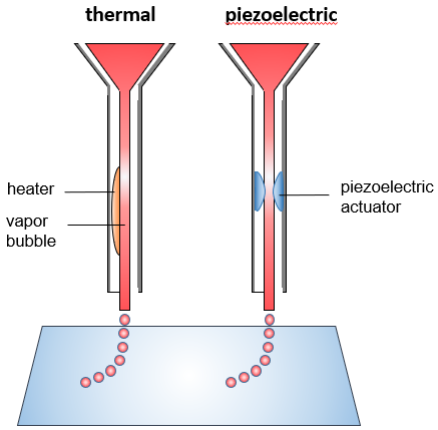


- CAD software generates models in the STL (Standard Triangle Language) format. Subsequently a slicer software converts the STL file into a language understandable to the printer, the g-Code. The object is converted into a stack of thin flat layers and the description of the movements to be made by the printer.

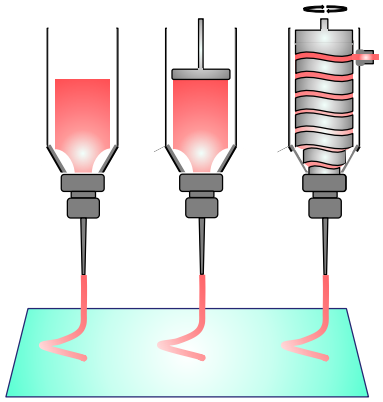
Technologies for Bioprinting

- Numerous technologies are being used in bioprinting. Each has specific advantages and disadvantages.

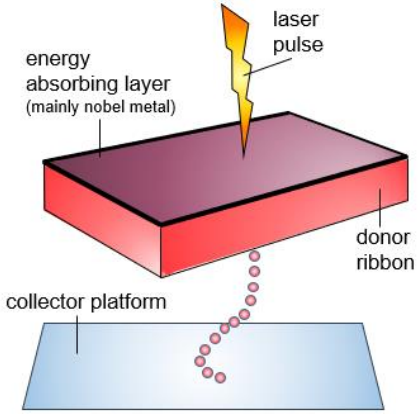
Ink-jet bioprinting

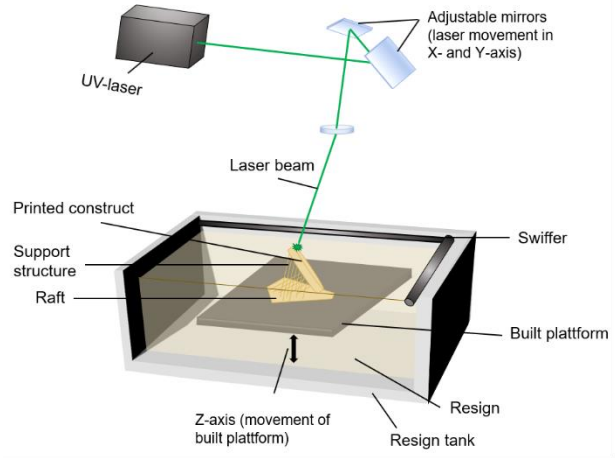


Microextrusion bioprinting



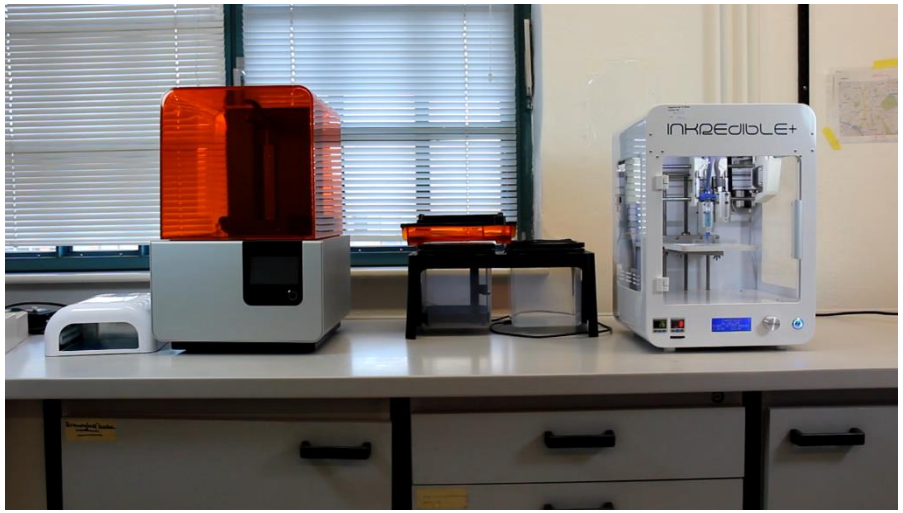
Laser-induced forward transfer (LIFT)

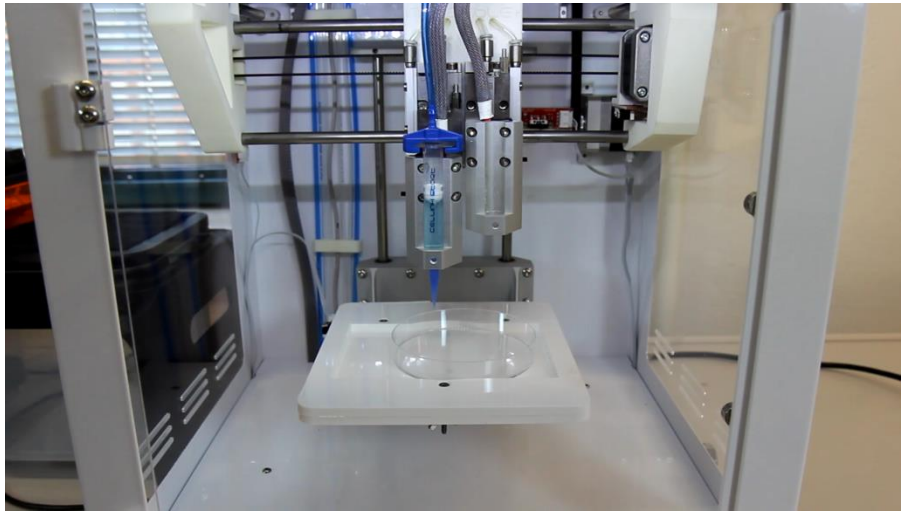




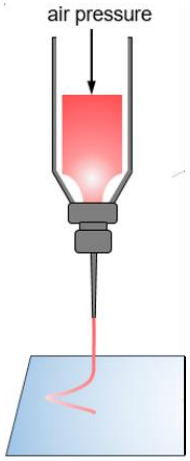
Stereolithography

Extrusion Printer





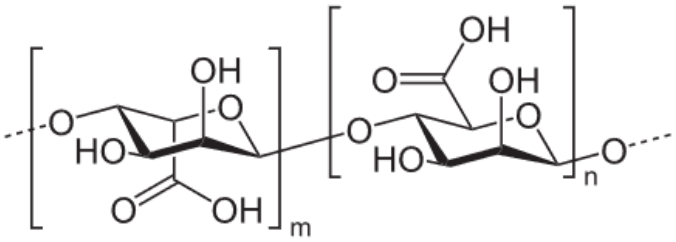
- Viscous solutions with cells are printed by pressure from extruders
- Advantages: Numerous materials, mild conditions
- Disadvantages: Limited stiffness and resolution



Bioink

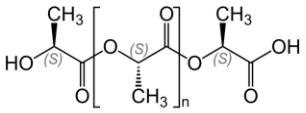
- Bioinks are soft biomaterials loaded with living cells.
  - Printability
  - Stability of printed constructs
  - Biocompatibility

Natural hydrogels

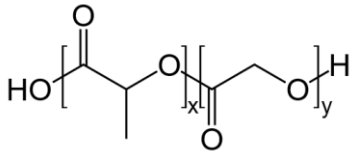


Agarose
Alginate, Gelatin etc.

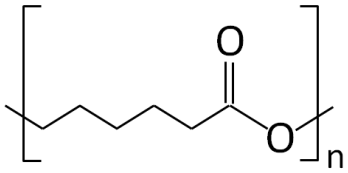
Synthetic hydrogels



Poly-lactic acid (PLA)

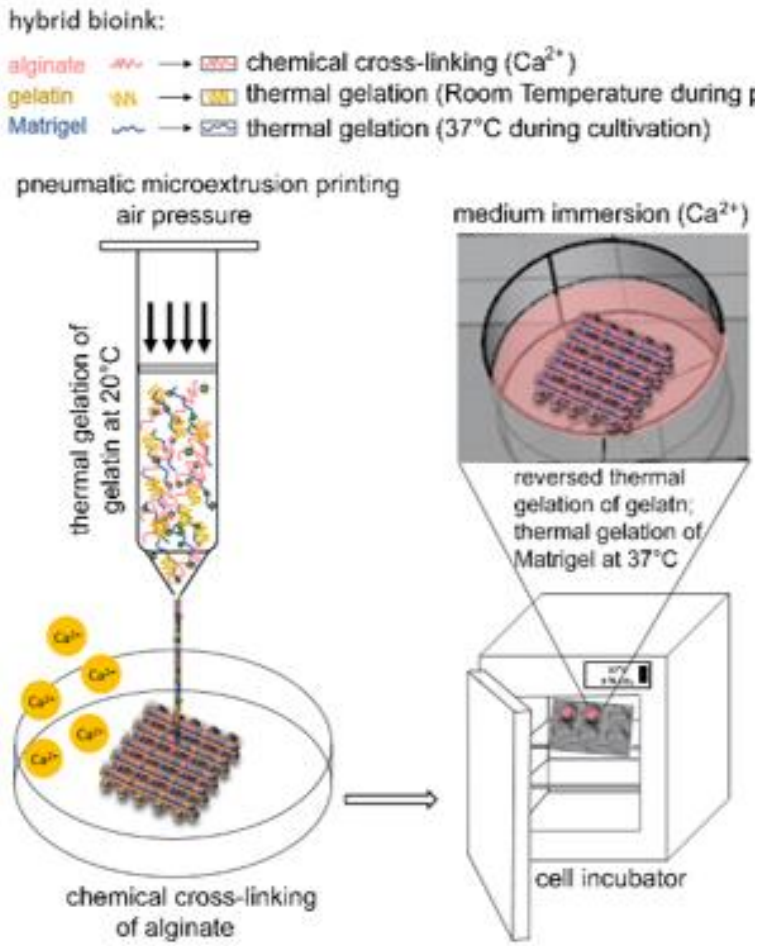


Poly(lactic-co-glycolic acid) (PLGA)



Poly-caprolactone (PCL)

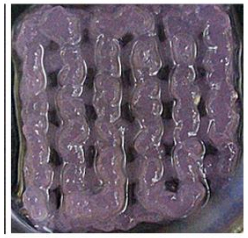
Extrusion Bioprinting

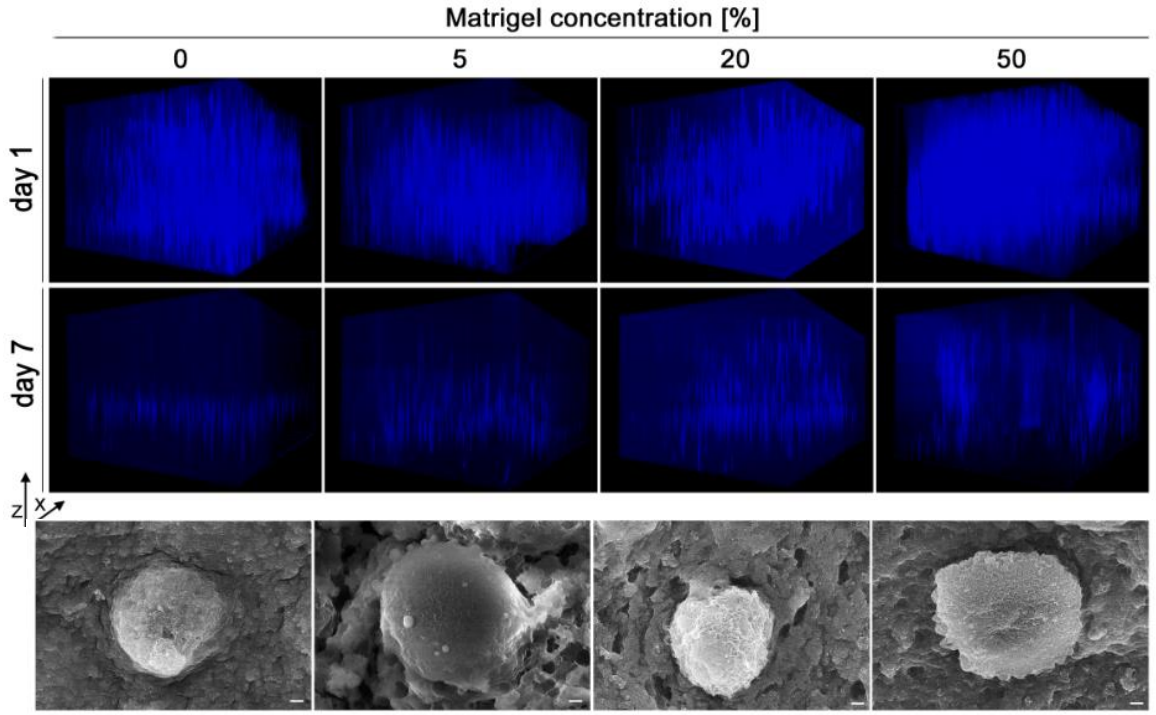


Overview

- Bioprinting Technology
- **Influenza infection of bioprinted lung model**
- Adenovirus infection of bioprinted liver model
- Outlook: Bioprinting in cancer research
- Outlook: Clean bioprinting

Optimization of Bioink for Lung Cells (A549)

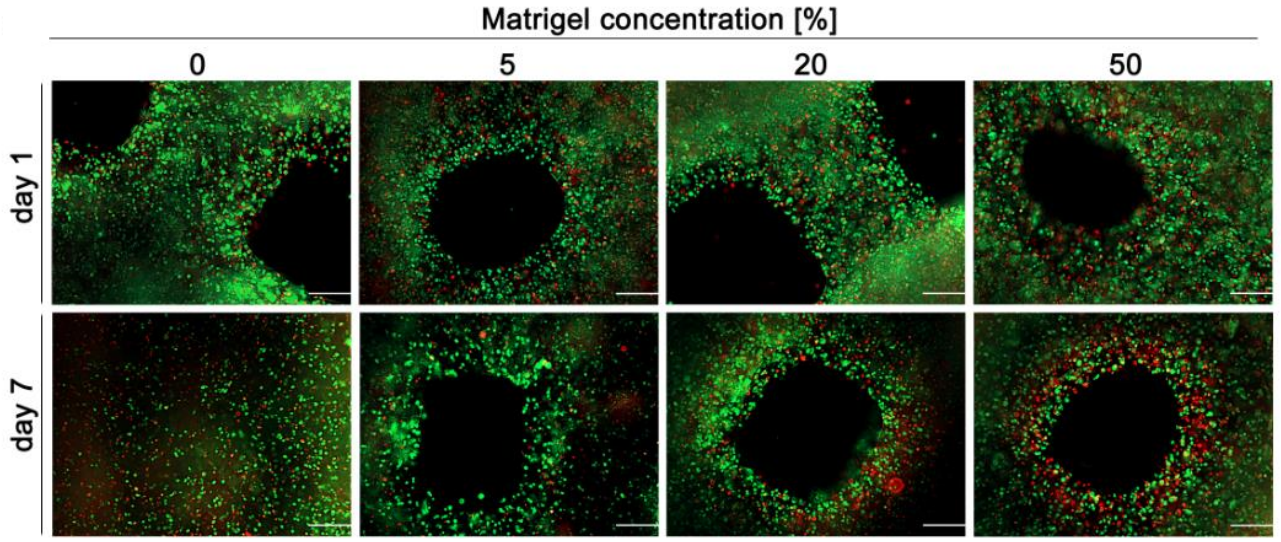


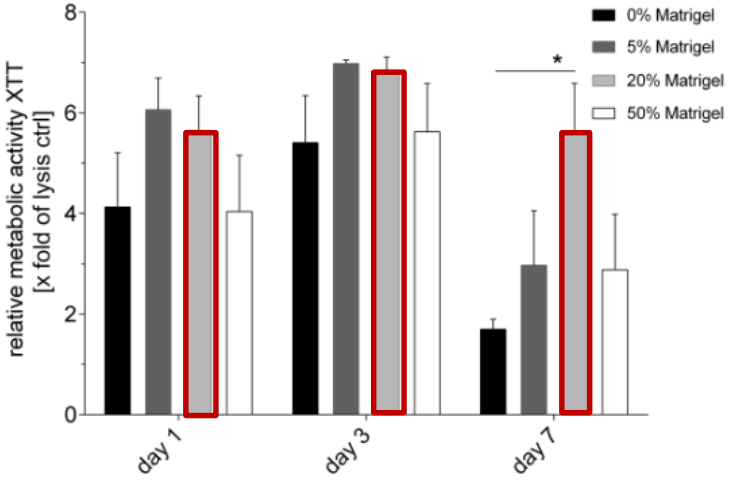


- Ink composed of 2% alginate, 3% gelatin and 20% Matrigel resulted in good 3D distribution of the cells (up to 7 days) and good porosity of the matrix.

Berg, Hiller, Kissner, Qazi, Duda, Hocke, Hippenstiel, Elomaa, Weinhart, Fahrenson, Kurreck (2018) Scientific Reports. 8, 13877.

Cell Viability



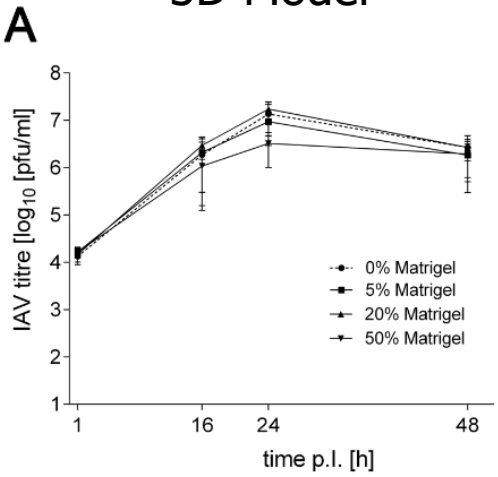


- 2% alginate, 3% gelatin and 20% Matrigel also revealed optimal cell viability

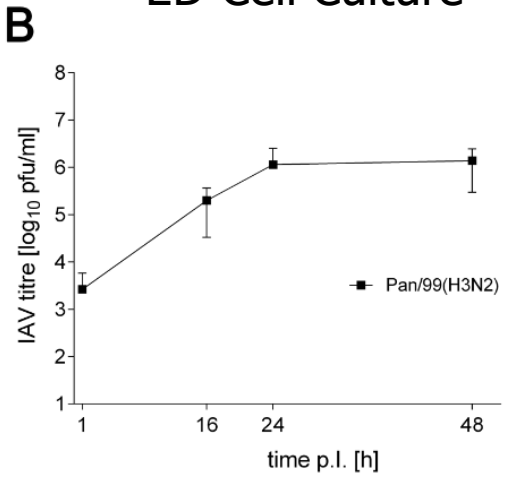
Berg, Hiller, Kissner, Qazi, Duda, Hocke, Hippenstiel, Elomaa, Weinhart, Fahrenson, Kurreck (2018) Scientific Reports. 8, 13877.

Infection of 3D Model with Influenza A Virus

3D Model

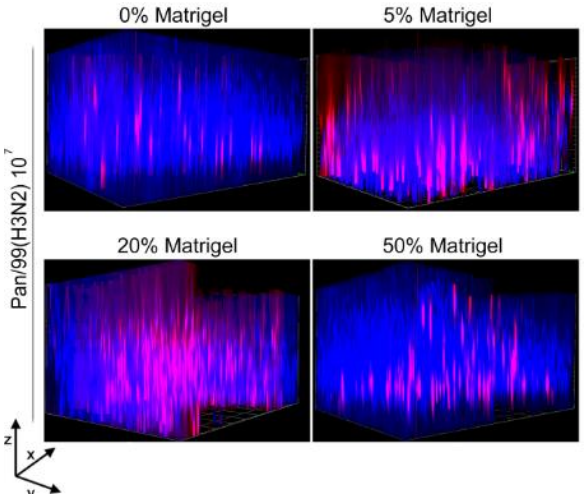


2D Cell Culture

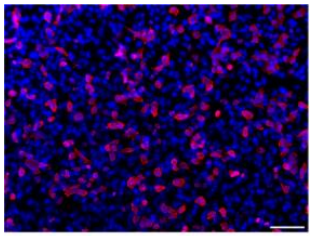


- Influenza A virus replicates in 3D lung model.

3D Model



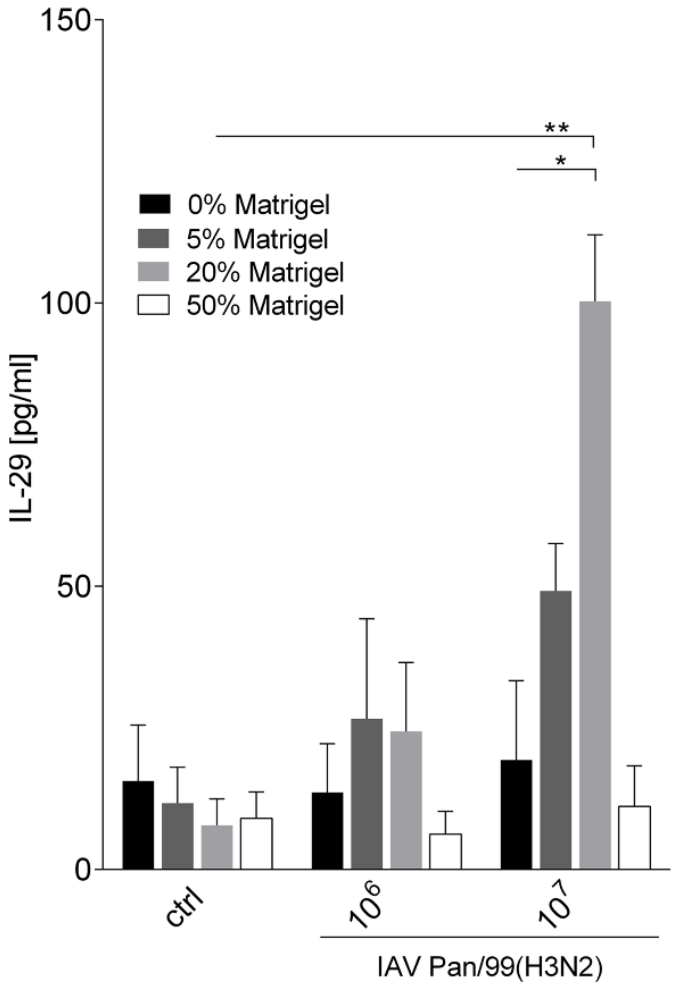
2D Cell Culture



- Clustered pattern of infection as seen in the lung which contrasts the even distribution in 2D cell culture.

Berg, Hiller, Kissner, Qazi, Duda, Hocke, Hippenstiel, Elomaa, Weinhart, Fahrenson, Kurreck (2018) Scientific Reports. 8, 13877.

Infection of 3D Model with Influenza A Virus



- Infected cells in printed 3D model produce IL-29, i.e. the model supports a proinflammatory response.

Berg, Hiller, Kissner, Qazi, Duda, Hocke, Hippenstiel, Elomaa, Weinhart, Fahrenson, Kurreck (2018) Scientific Reports. 8, 13877.

Overview

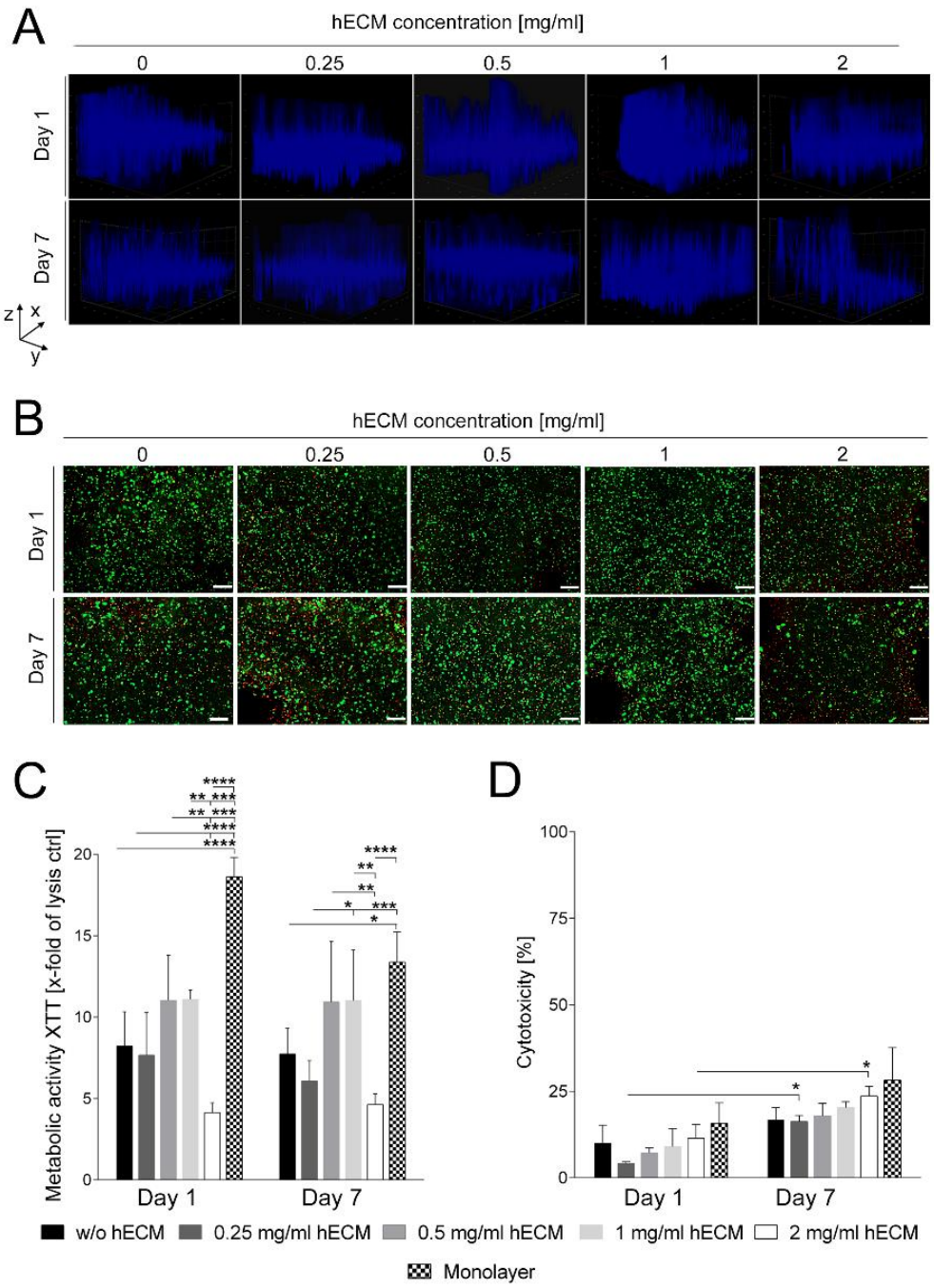
- Bioprinting Technology
- Influenza infection of bioprinted lung model
- **Adenovirus infection of bioprinted liver model**
- Outlook: Bioprinting in cancer research
- Outlook: Clean bioprinting

Replacement of Matrigel

- Matrigel is an extracellular matrix (ECM) harvested from mouse Engelbreth Holm-Swarm sarcoma. To reduce suffering of animals and to avoid species-specific differences, lung ECM from a human donor was used.
- The bioink was optimized for a liver model with HepaRG cells.

Matrigel = Extracellular matrix from Engelbreth Holm-Swarm sarcoma

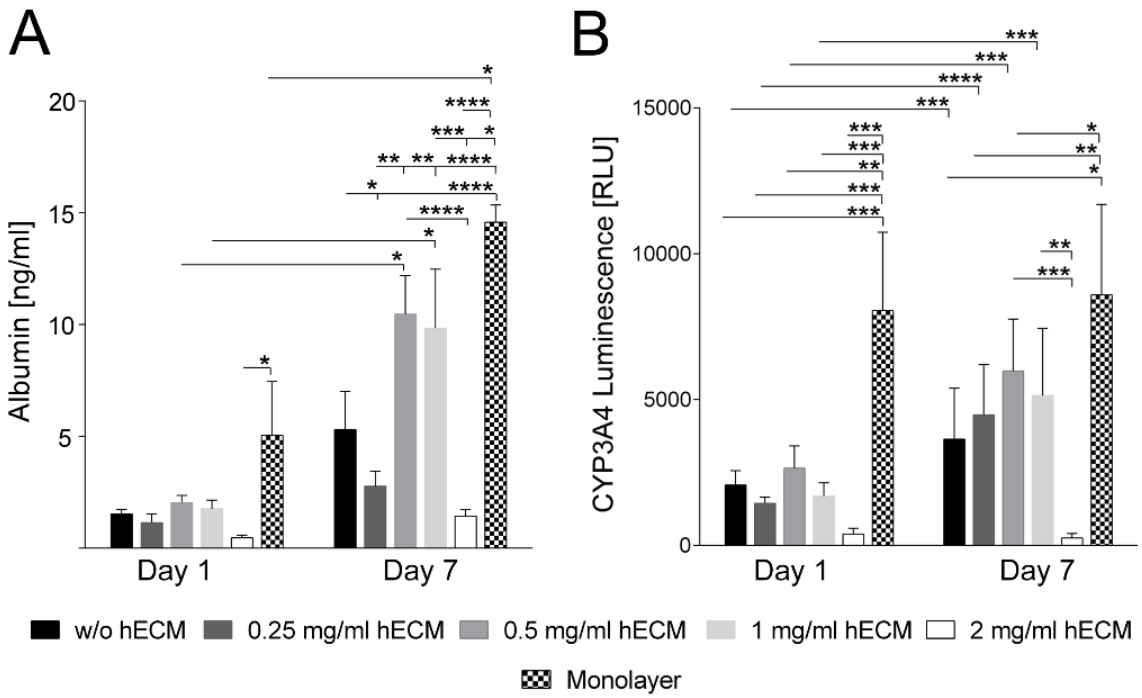
Bioink for Liver Model



- 0.5 – 1 mg hECM in 2% alginate and 3% gelatin were found to be optimal for:
  - 3D cell distribution
  - Life/dead ratio
  - Metabolic activity
  - Low cytotoxicity

Hiller, T.; Berg, J.; Elomaa, L.; Röhrs, V.; Ullah, I.; Schaar, K.; Dietrich, A. C.; Al-Zeer, M. A.; Kurtz, A.; Hocke, A. C.; Hippenstiel, S.; Fechner, H.; Weinhart, M.; Kurreck, J. (2018) Int. J. Mol. Sci. 19 2018, 3129.

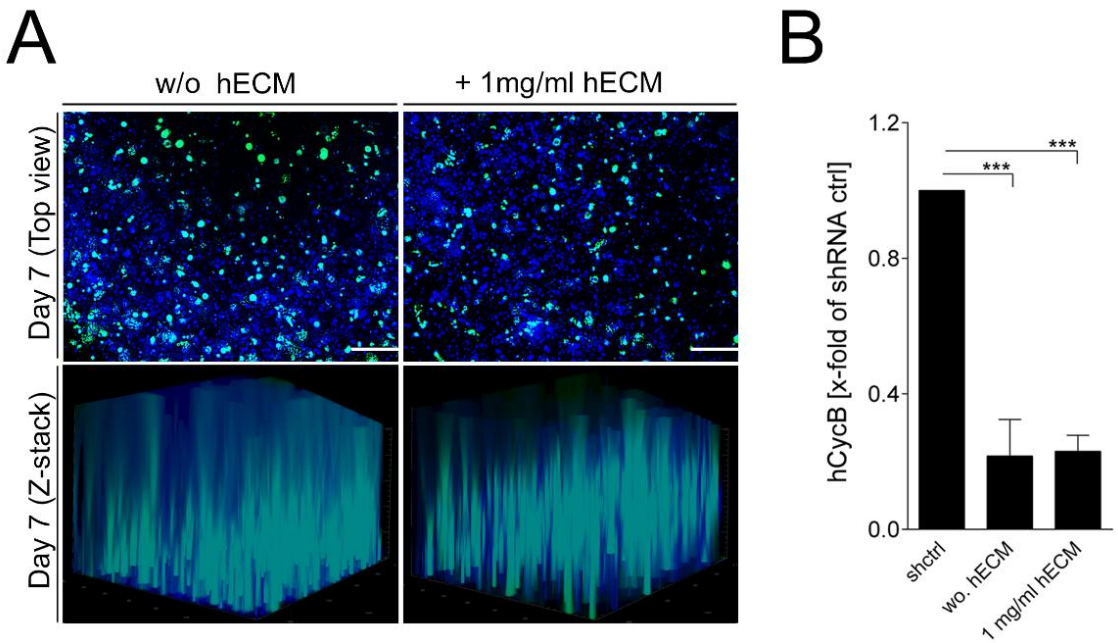
Bioink for Liver Model



- Typical markers for the liver were induced in the liver model:
  - Albumin secretion
  - CYP3A4 expression

Hiller, T.; Berg, J.; Elomaa, L.; Röhrs, V.; Ullah, I.; Schaar, K.; Dietrich, A. C.; Al-Zeer, M. A.; Kurtz, A.; Hocke, A. C.; Hippenstiel, S.; Fechner, H.; Weinhart, M.; Kurreck, J. (2018) Int. J. Mol. Sci. 19 2018, 3129.

Bioink for Liver Model

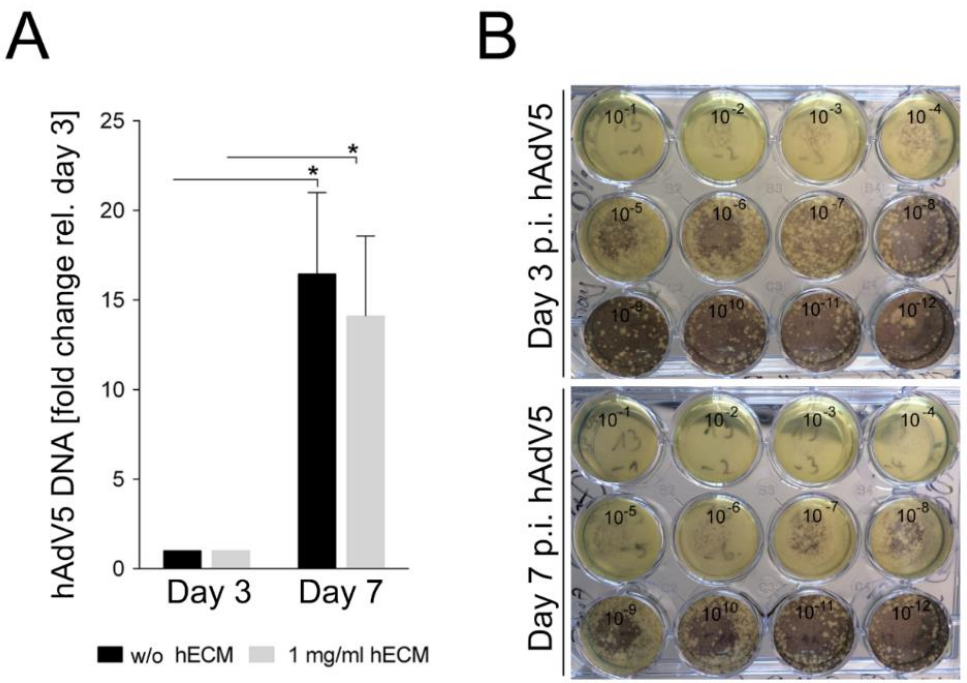


- Adeno-associated virus (AAV) vectors are the most promising vehicles for gene transfer.
- The liver model was efficiently transduced by AAV vectors.
- Delivery of an shRNA-expression cassette resulted in efficient knockdown of the target gene.

Hiller, T.; Berg, J.; Elomaa, L.; Röhrs, V.; Ullah, I.; Schaar, K.; Dietrich, A. C.; Al-Zeer, M. A.; Kurtz, A.; Hocke, A. C.; Hippenstiel, S.; Fechner, H.; Weinhart, M.; Kurreck, J. (2018) Int. J. Mol. Sci. 19 2018, 3129.

Bioink for Liver Model

- Human adenovirus 5 causes severe, often fatal liver infections in immunocompromised patients.
- Virus replicated efficiently after infection of printed liver model. Proof-of-concept that the liver model is suitable to study adenovirus infection.



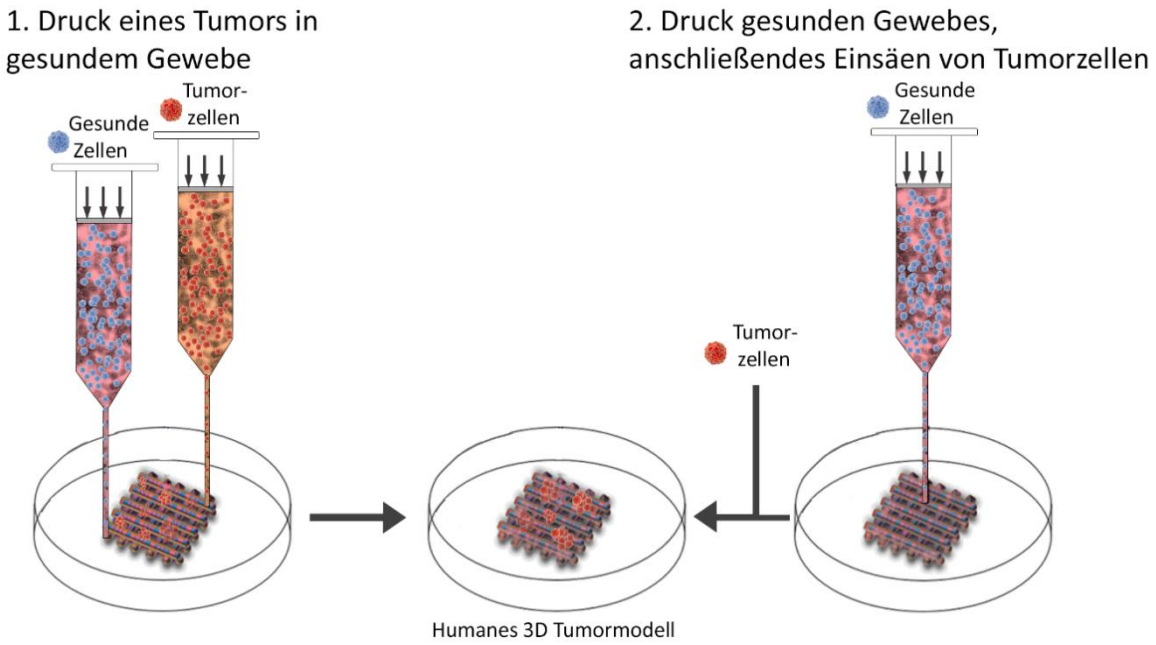
Hiller, T.; Berg, J.; Elomaa, L.; Röhrs, V.; Ullah, I.; Schaar, K.; Dietrich, A. C.; Al-Zeer, M. A.; Kurtz, A.; Hocke, A. C.; Hippenstiel, S.; Fechner, H.; Weinhart, M.; Kurreck, J. (2018) Int. J. Mol. Sci. 19 2018, 3129.

Overview

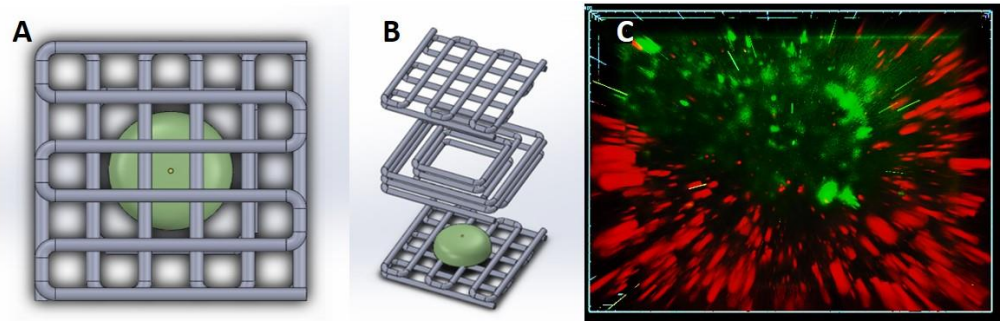
- Bioprinting Technology
- Influenza infection of bioprinted lung model
- Adenovirus infection of bioprinted liver model
- **Outlook: Bioprinting in cancer research**
- Outlook: Clean bioprinting

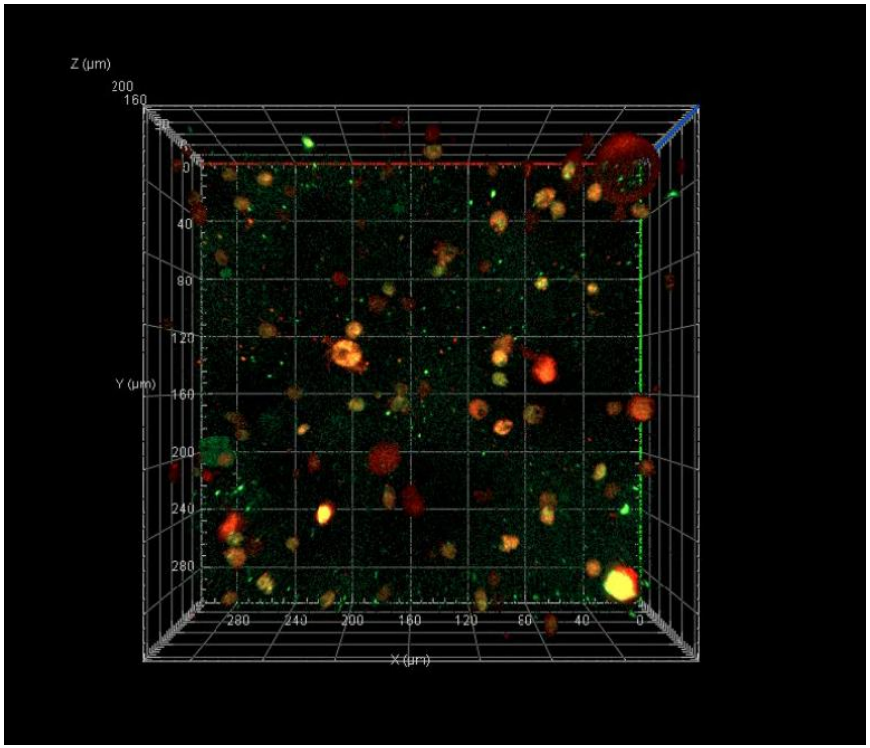
Outlook Cancer Research

- BMEL database on animal experiments: More than 200.000 animals are used annually for cancer research (10% of all test animals).
- According to our database searches, 3D bioprinting has hardly been used for cancer research yet.
- Plan: Bioprinting of tumor model.



Outlook Cancer Model





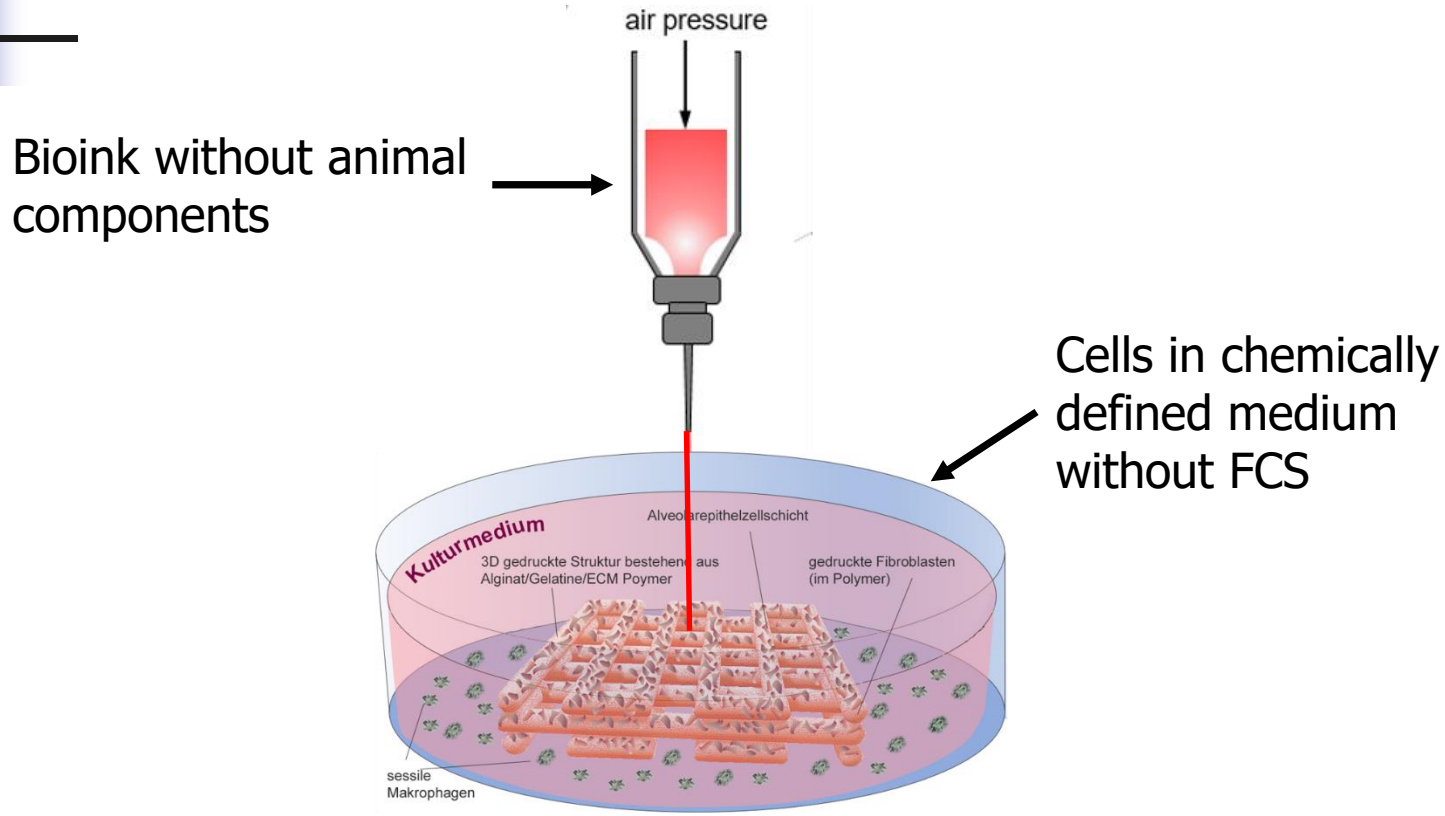
Overview

- Bioprinting Technology
- Influenza infection of bioprinted lung model
- Adenovirus infection of bioprinted liver model
- Outlook: Bioprinting in cancer research
- **Outlook: Clean bioprinting**

Outlook: Clean Bioprinting

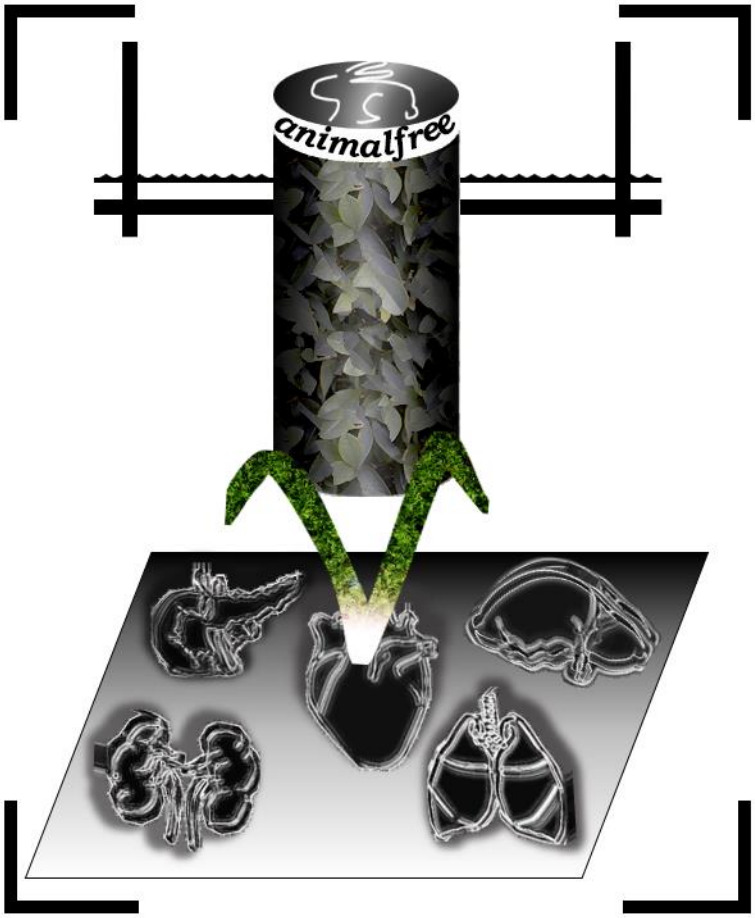
- Bioprinting of humanized organ models is widely (and correctly) considered a promising alternative to animal experiment; however, virtually all bioprinting approaches include components of animal origin:
  - Cells in Fetal Calve Serum
  - Matrigel
  - Gelatine or collagen in the bioink

Clean Bioprinting



- We propose clean bioprinting as a new concept to generate organ models without animal components (no FCS, not gelatin/collagen, no Matrigel).
- This approach not only contributes to animal welfare, it also prevents ambiguous results due to chimeric human/animal systems.

Logo: Clean Bioprinting



Summary

- 3D Printing is a powerful technology to generate object with high spacial resolution.
- Bioprinting can be used to produce organ models.
- 3D organ models can not only replace animal experiments, but they also provide the advantage that they are humanized by the use of human cells.
- We have generated bioprinted 3D models for the lung and the liver with good physiological properties (cell viability, marker expression etc.).
- Both models have been used for infection studies with influenza and adenovirus, respectively. The viruses infect the models and replicate.

Acknowledgements

Group at TUB

Munir Al-Zeer
Johanna Berg
Alexandra Bettinelli
Babette Dieringer
Ann-Christin Dietrich
Mona Fechler-Bitteti
Henry Fechner
Anja Geisler
Lisanne Heimann
Thomas Hiller
David Höppe
Bernd Krostitz
Kai Kurczyk
Alexander Mensch
Julia Moradi
Viola Röhrs
Sebastian Schlüter
Konrad Schmidt
Ida Shaef
Beatrice Tolksdorf
Zia Weber
Anita Wehner
Dongwei Wu
Mathias Ziersch

Ongoing Collaborations

CHARITÉ UNIVERSITÄTSMEDIZIN BERLIN

Andreas Hocke
Stefan Hippenstiel
Katja Hönzke

Freie Universität Berlin

Laura Elomaa
Marie Weinhart
Sarah Hedrich
Christoph Weise

Berlin-Brandenburg School for Regenerative Therapies

Taimoor Quazi
Georg Duda

ROBERT KOCH INSTITUT

Thomas Bock

BioNukleo

Anke Wagner
Heba Yehia

berlin

Anja Wagemanns
Lilian Heim
Mario Birkholz

GRUNENTHAL

Markus Valdor
Thomas Christoph

Funding

DFG Deutsche Forschungsgemeinschaft

BfR Bundesinstitut für Risikobewertung

Bundesministerium für Bildung und Forschung

set Stiftung zur Förderung der Erforschung von Ersatz- und Ergänzungsmethoden zur Einschränkung von Tierversuchen

DAAD Deutscher Akademischer Austauschdienst German Academic Exchange Service

FCI FONDS DER CHEMISCHEN INDUSTRIE

Wilhelm Sander-Stiftung fördert medizinische Forschung

GRUNENTHAL

Ärzte gegen Tierversuche e.V.