

3D(Bio)printed infection models of human lung cells and skin hair follicles to study nanoantibiotics

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CRS Annual Meeting and Exposition
Philadelphia, 14-18 July 2025

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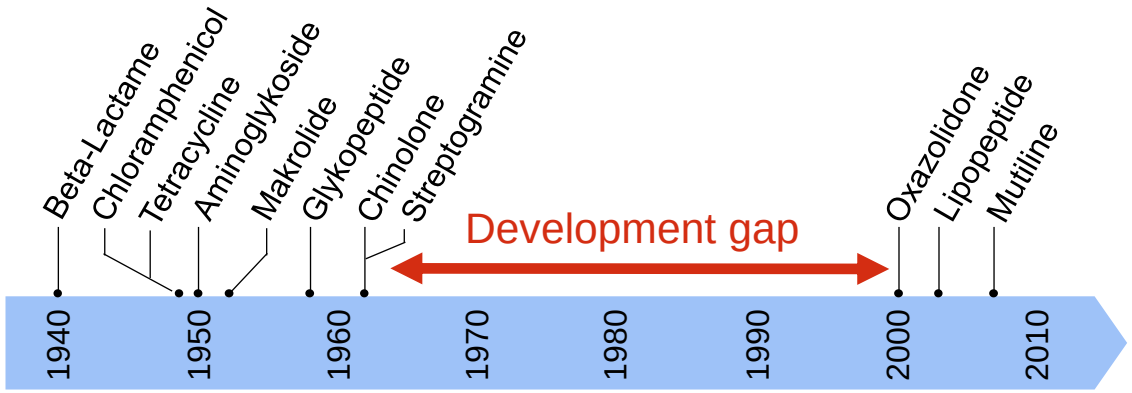
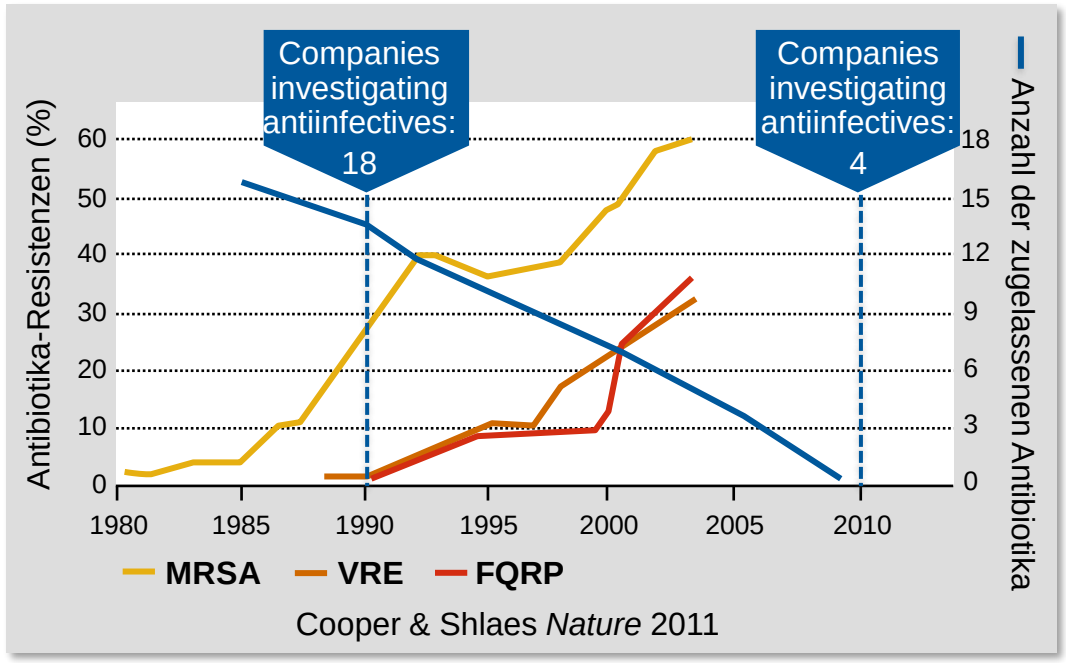
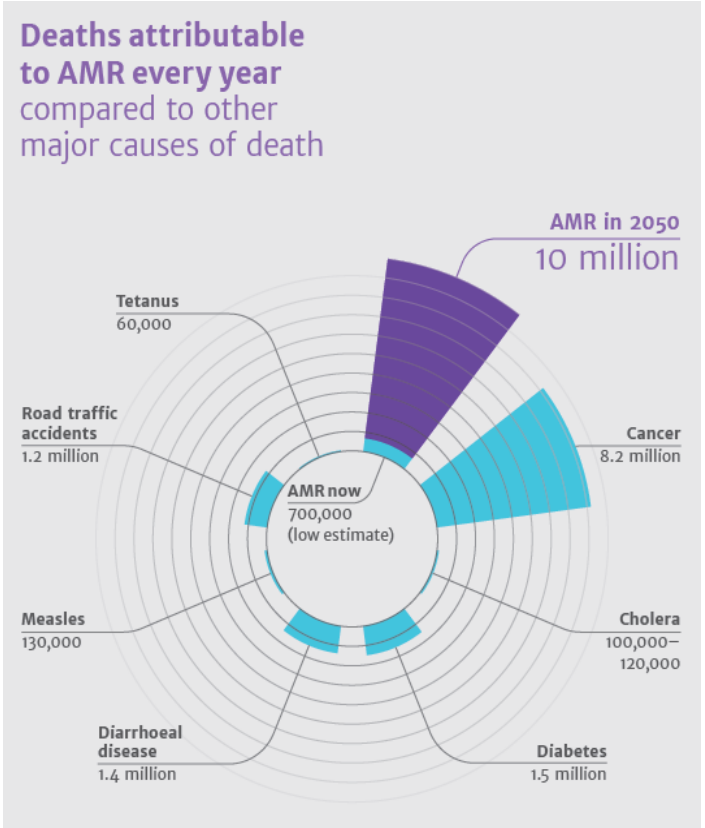
HIPS HELMHOLTZ Institut für Pharmazeutische Forschung Saarland

Antimicrobial resistance (AMR) - a maior challenge to human health







UK Review on Antimicrobial Resistance,
Chaired by Jim O'Neill, Dec 2014

In spite of COVID, the the societal appreciation of Infection research is very limited!

Revenue of the anti-cancer drug Glivec 2013

4.000 Mio €

772 Mio €

Total value of **all** antibiotics prescribed in Germany 2012

Annual support of AMR research EU+US

400 Mio €

Annual marketing expenses

98.000 Mio $

Annual R&D expenses of the 10 largest pharmaceutical companies

65.000 Mio $

Sources: Die Zeit, brandeins

In 2009, HIPS was founded as a new institute between HZI and UdS

Aiming at the development of novel anti-infectives to tackle AMR

UNIVERSITÄT DES SAARLANDES

SAARLAND
Großes entsteht immer im Kleinen.

HZI HELMHOLTZ Centre for Infection Research

Pharmaceutical Research

Infection Research

HIPS HELMHOLTZ Institut für Pharmazeutische Forschung Saarland

HIPS started with a handfull PI's in 2009
Now: PharmaScienceHub HIPS/UdS


A. Gurevich


O. Kalinina


A. Keller


A. Volkamer


F. Kern

Bioinformatics

Active Compounds


R. Müller


C. Fu


F. Hubrich


T. Gulder


K. Bozhüyük

Medchem


A. Titz


A. Hirsch


M. Empting

Drug Delivery


C.-M. Lehr


A. Luzhetskyy


S. Sieber


A. Kiefer


U. Kazmaier


T. Gulder


M. Nesterkina


S. Lee

Medicine

HIPS Groups
50/50 Bridge Professorships
Affiliated University Groups


C. Beemelmanns


S. Smola


R. Bals

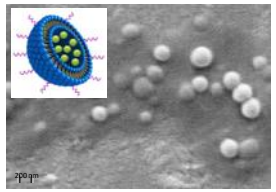

Gastro-enterologie

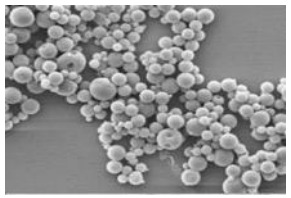
Ten new groups and 250+ collaborators on site since 2020

Drug Delivery across Biological Barriers = Central Research Theme @ HIPS-DDEL

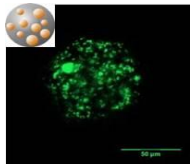
New excipients and Drug conjugates

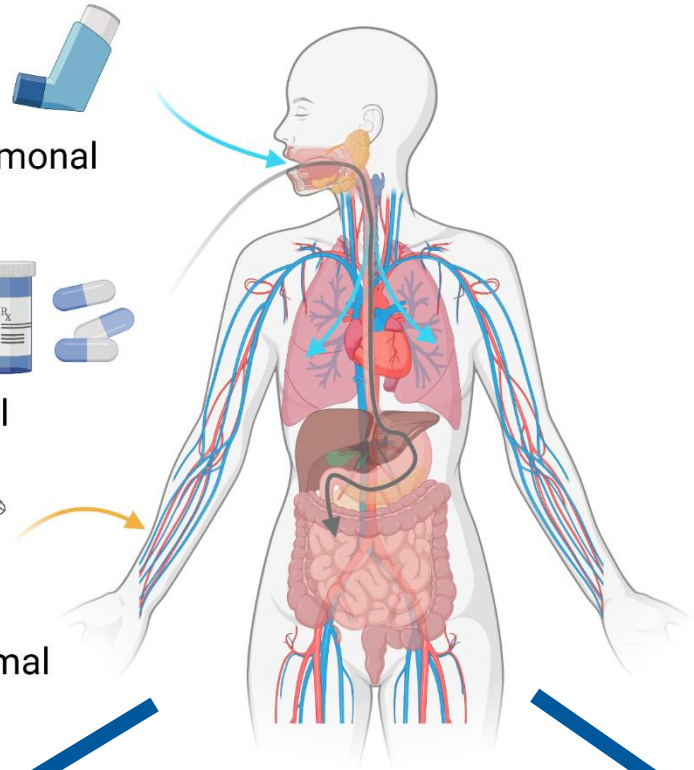
Nanotechnology



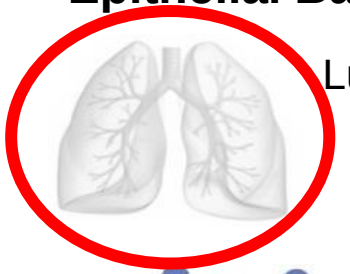


Formulation

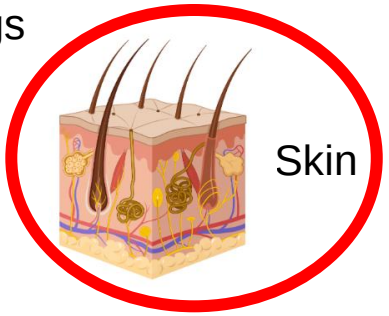




Epithelial Barriers



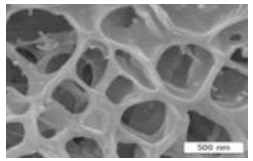
Lungs



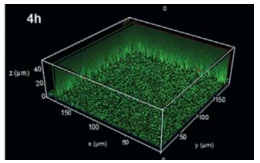
Skin

Intestinal mucosa

Non- cellular Barriers



Mucus

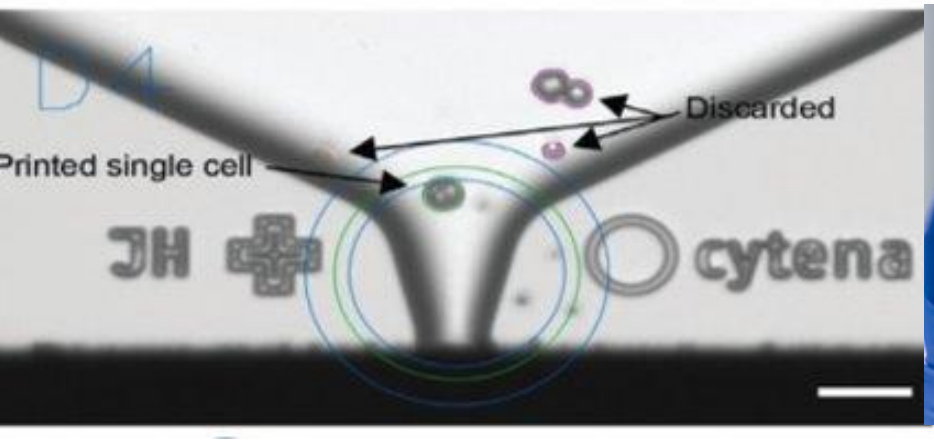


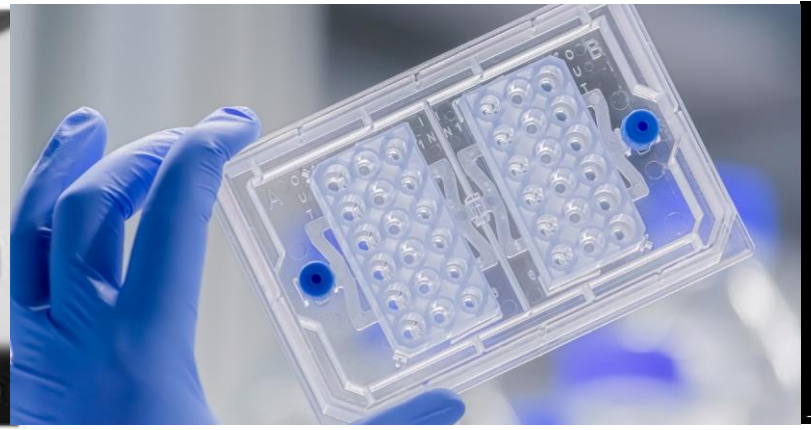
Biofilm

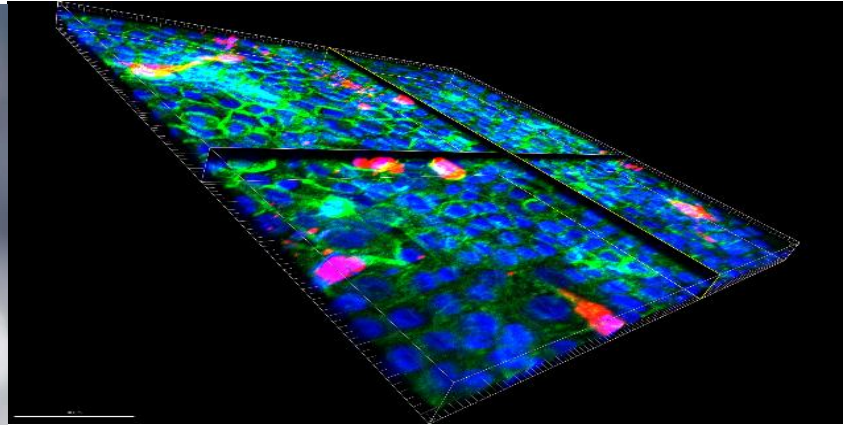
Bacterial cell envelope

CARRIERS for Delivering Antiinfectives and Vaccines

Understanding the BARRIERS in Health and Disease



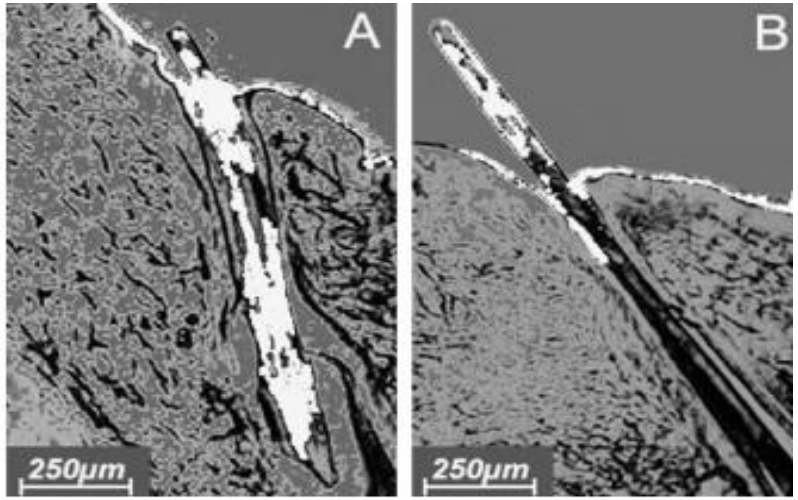




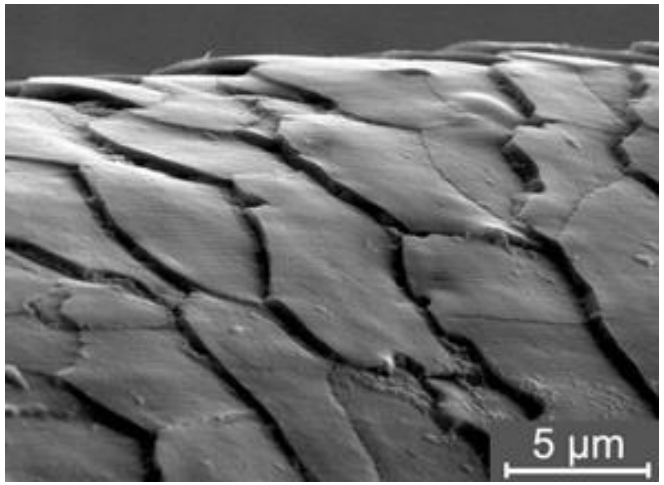
BARRIER #1:

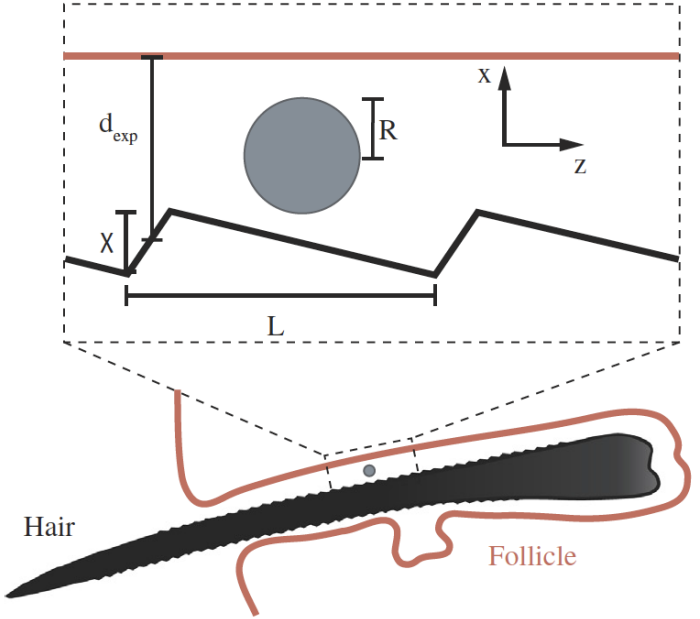
THE SKIN

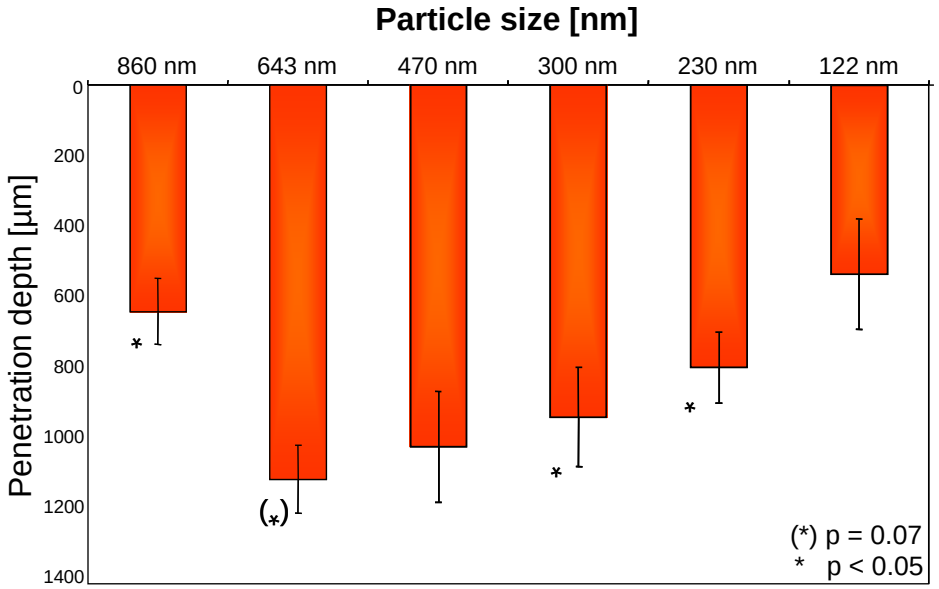
An interesting phenomenon, discovered in nano-safety research: Nanoparticles do penetrate into hair follicles!

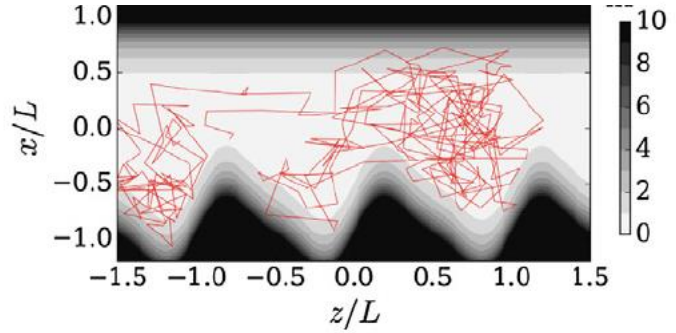


FITC-labeled PLGA-NPs Na⁺-Fluorescein in solution









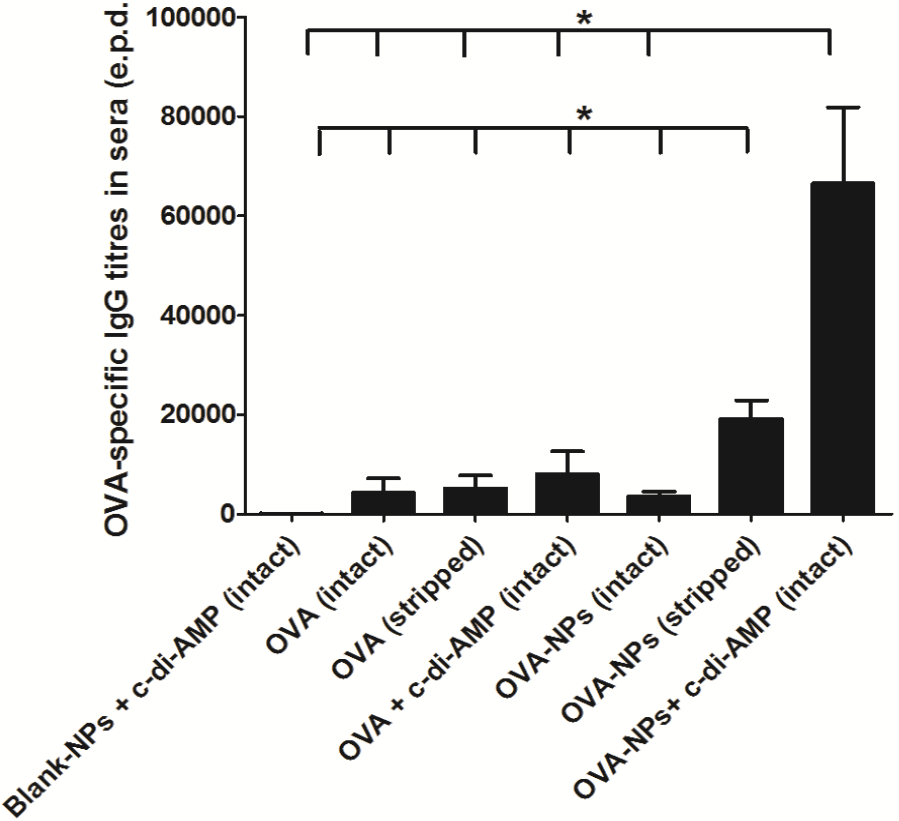
Lademann et al. (2007) **Eur J Pharm Biopharm**
Patzelt et al. (2011) **J Control Rel**
Radtke et al. (2017) **Eur J Pharm Biopharm**

Transfollicular delivery of antigens

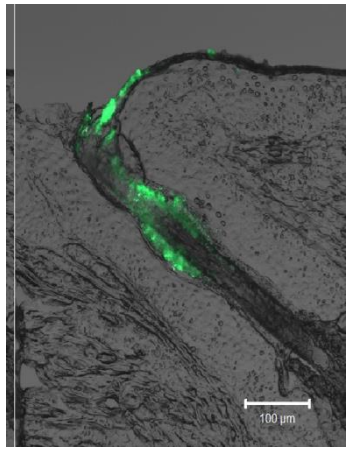
A perspective for needle-free vaccination?

Kai Schulze

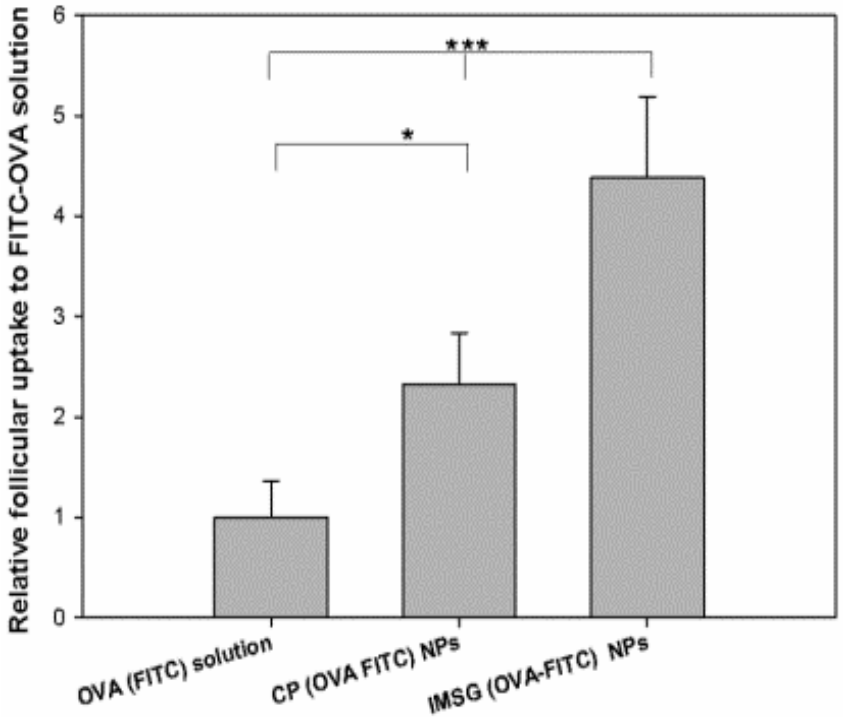
Carlos C Guzmán



C57BL/6 mice



Pig Ear Model



Mittal et al. (2015) **J Control Rel**

Mittal et al. (2013) **Vaccine**
Mittal et al. (2015) **Nanomedicine NBM**
Raber et al (2014) **J Control Rel**
Mittal et al, (2915) **J Control Rel**

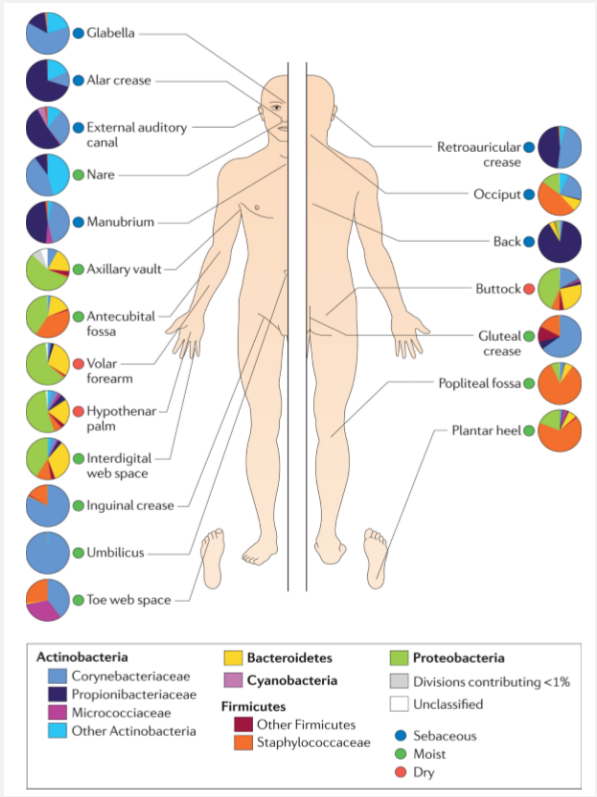
Targeted Delivery of Nano-Antiinfectives to Destructive Infections of Skin Hair follicles

UdS-HIPS-Tandem
Interdisciplinary Graduate School for Drug Research

Inititative to characterize, modulate and model human microbiota

Human Microbiome

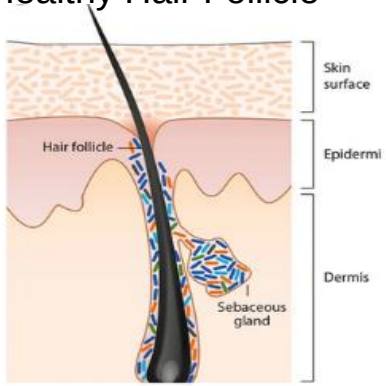
Aggregate of all microbiota on or within humans



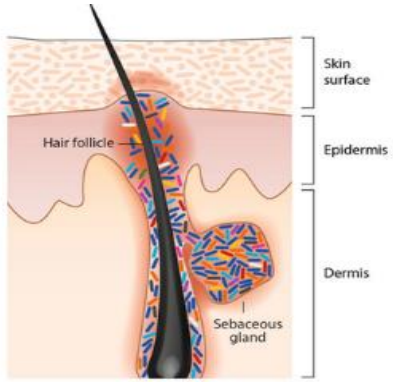
Grice & Segre *Nat Rev Microbiol* **2011**

Hair/Skin Microbiome

Healthy Hair Follicle



Inflamed Hair Follicle



Brandwein et al., 2016 npj biofilms and microbioms

Shift of skin microbiome towards „bad bacteria“

Acne inversa

Folliculitis decalvans

Severe infections lead to livelong scars

Fellows: Samy Aliyazdi, Sarah Frisch

Co-PI: Prof. Dr Thomas Vogt, UKS Dermatology

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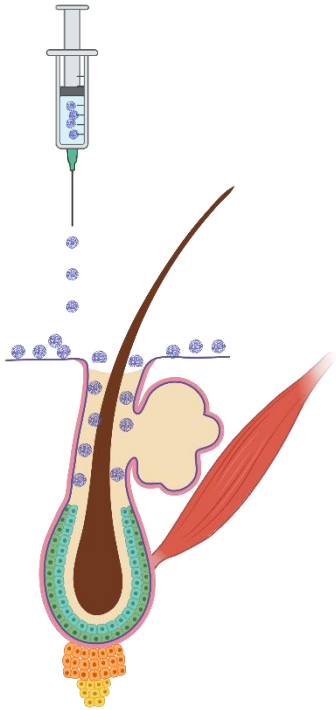
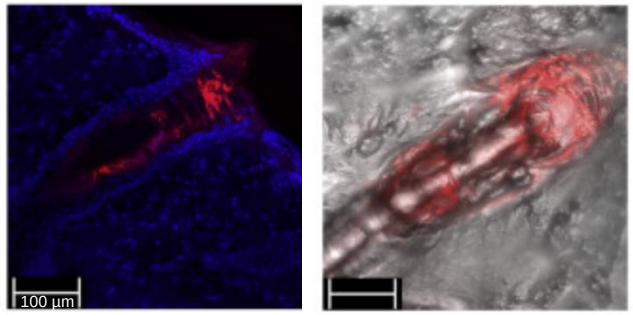
Can we model human hair follicles in vitro?

We know that nanocarriers can penetrate hair follicles.





Christmann et al., 2020 JID

Developing targeted nanocarriers for novel ani-infectives needs adequate testing platforms!

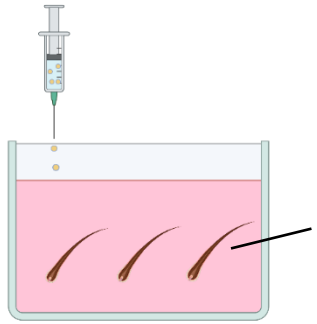
The mouse is not a good model,

Especially as regads skin and hair,

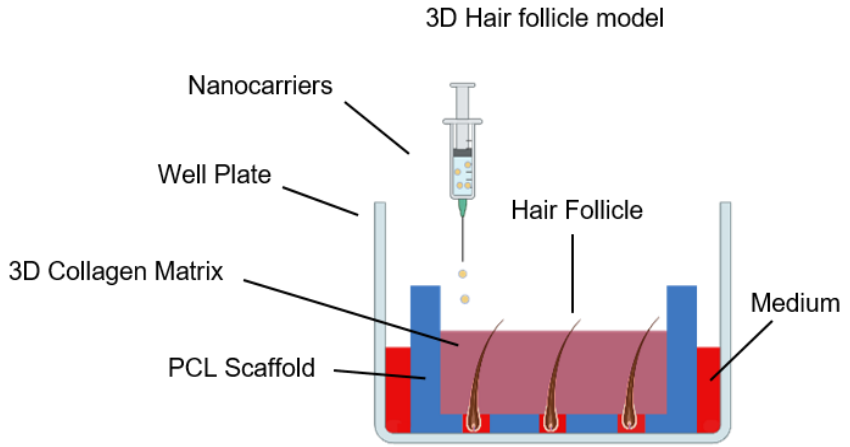
due to major histological differences

(besides genetics and immunology

However, current human hair models are not suitable to study follicular penetration.



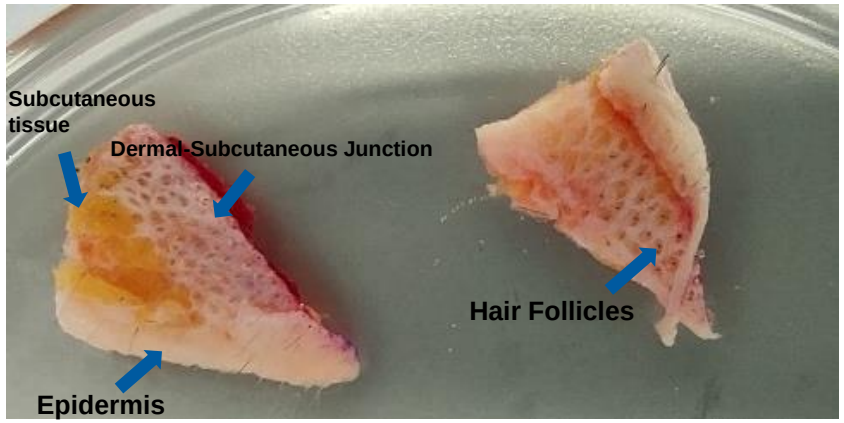
Project goal: 3D organ culture of hair follicles



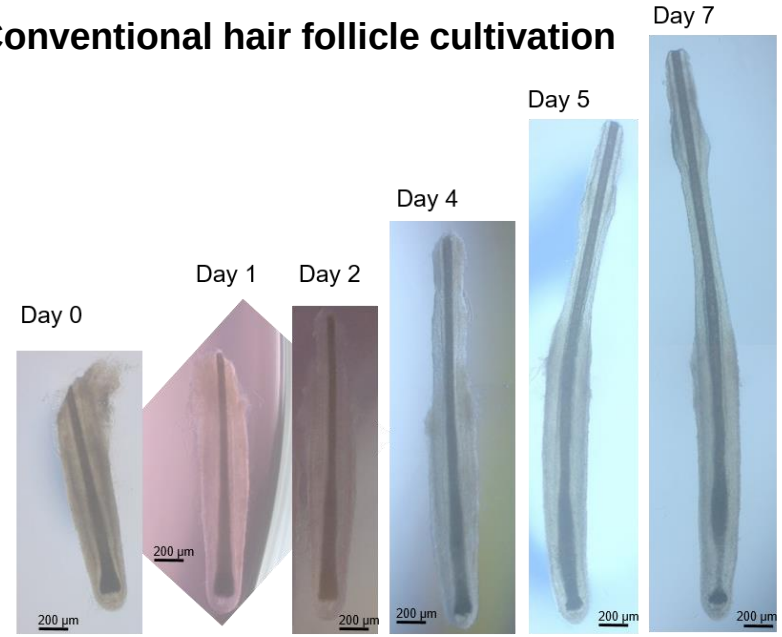
2D Cultivation of human hair follicles

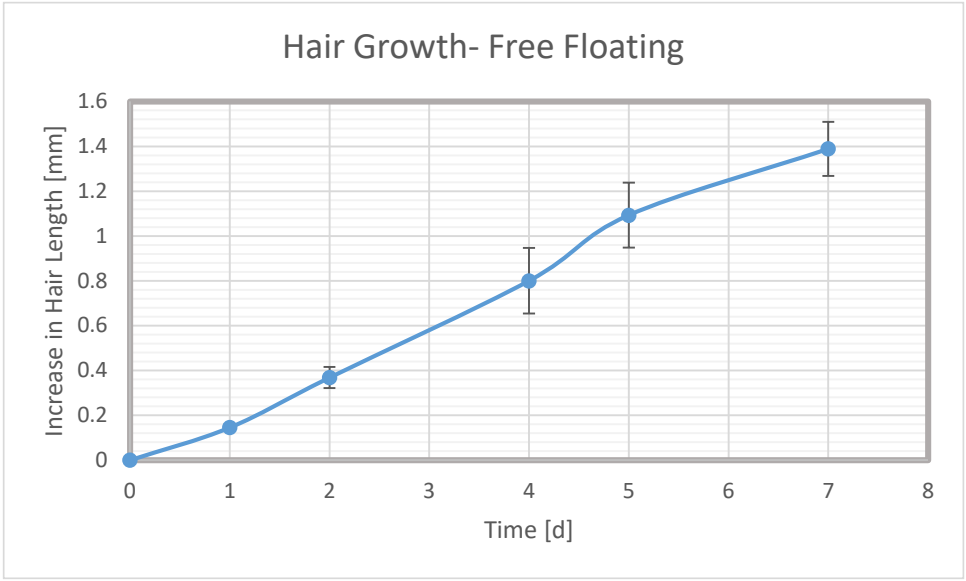
Collaboration Partner:
Dr. Barbara Veldung, Cosmetic Surgeon, Saarbrücken
Approval by Ethics Committe , **Reg.-Nr. 117/20 30.06.2020**

Hair follicles were isolated from skin tissue and cultivated free floating in media



Conventional hair follicle cultivation

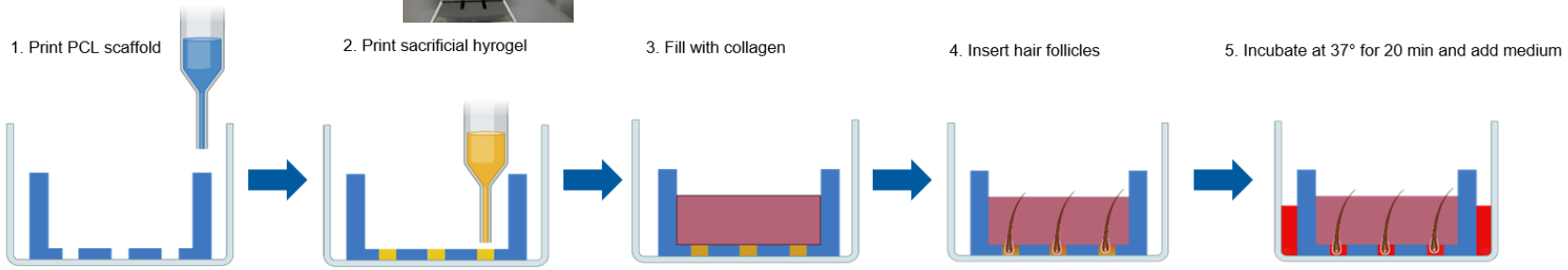


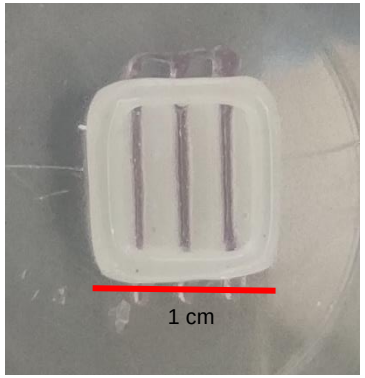


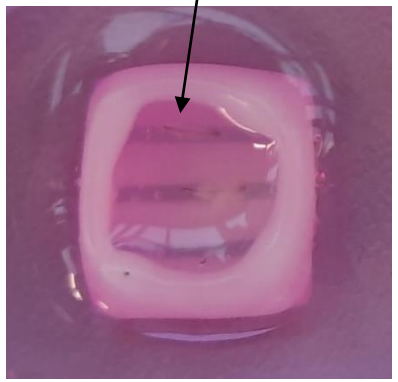
Hair follicles grow over 1 mm within a week

3D Hair Follicle Organ Culture

Procedere via 3D Printing



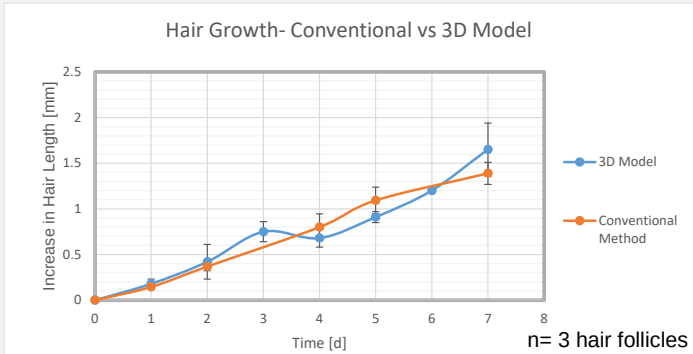




Hair Follicle Growth

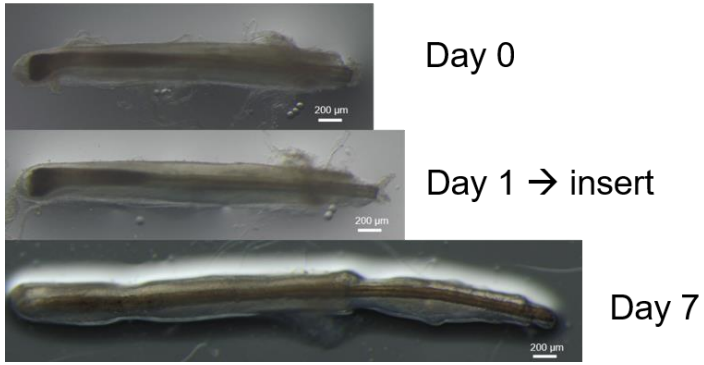
Conventional vs 3D conditions

- Seperate models for each day
- Healthy HF‘s in model grow similar to free floating follicles
- Same observation made on day 7 → hair shaft detaches from papilla



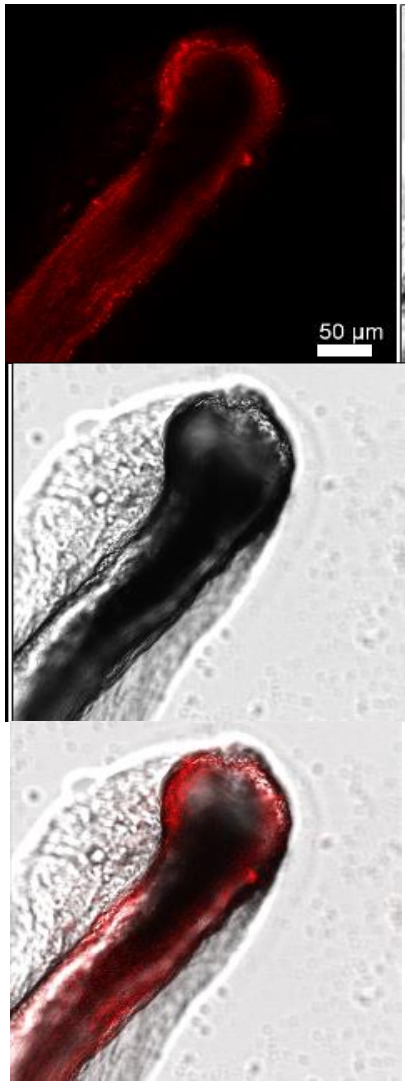
Healthy HF‘s in model grow similar to free floating follicles

3D Model

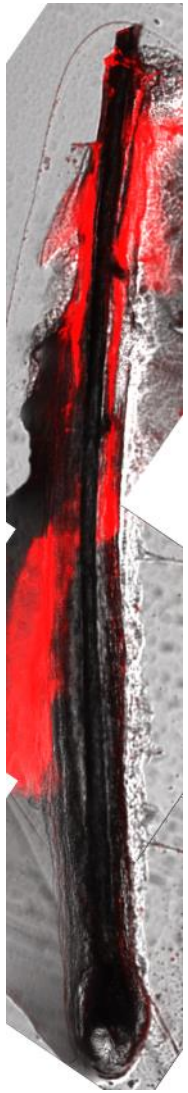


Samy Aliyazdi, Sarah Frisch et al., ACS Biomat Sci. Eng. 2024

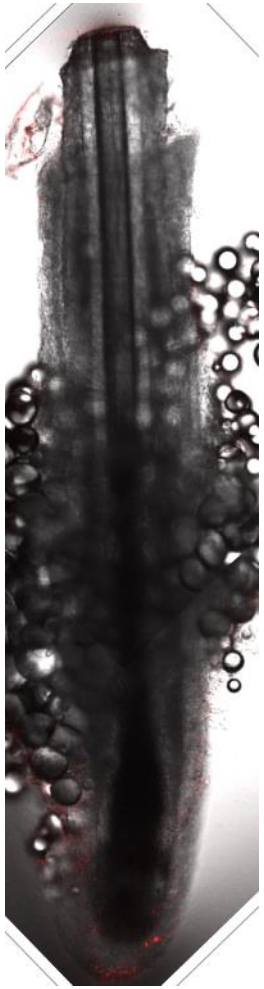
Follicular Penetration of Nanocarriers and S. aureus infection



200 nm Fluorospheres

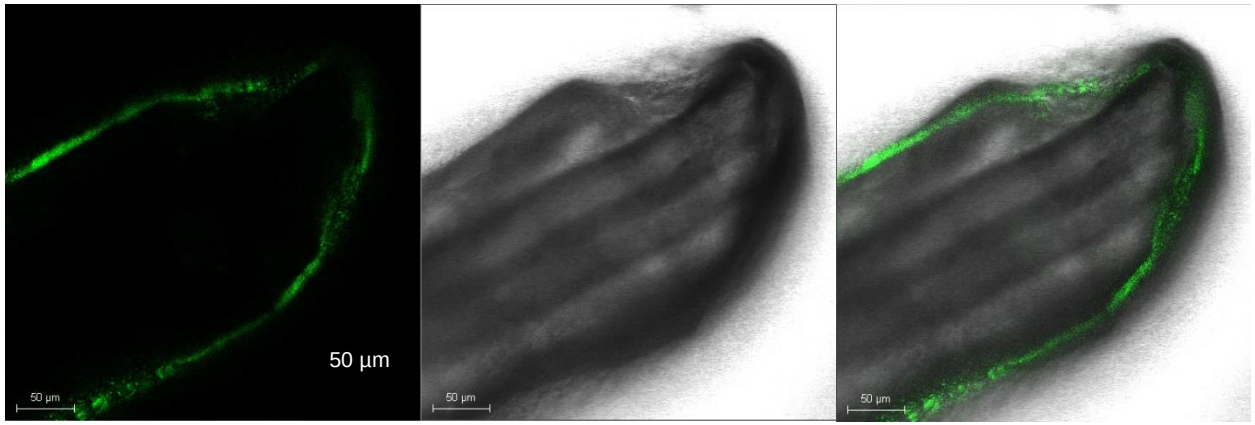


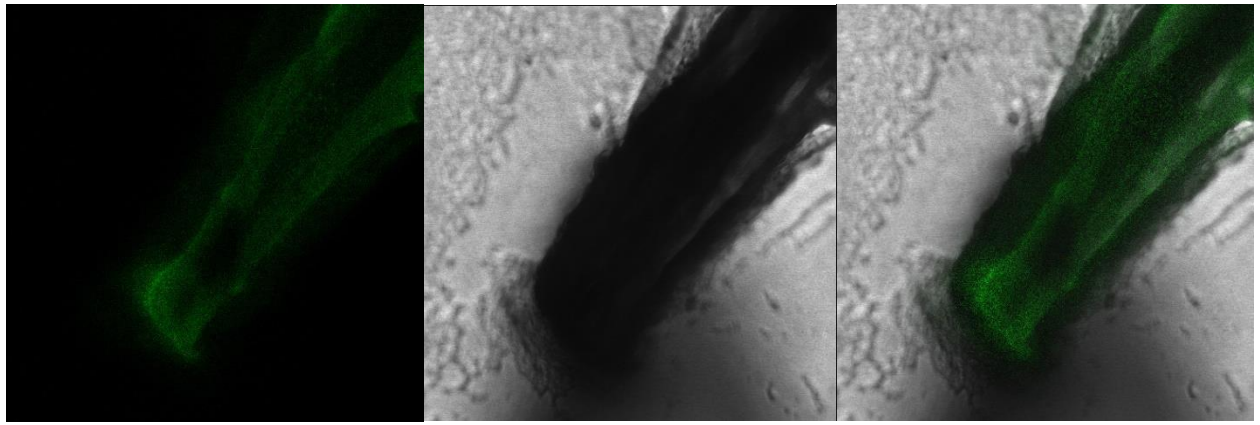
500 nm Fluorospheres



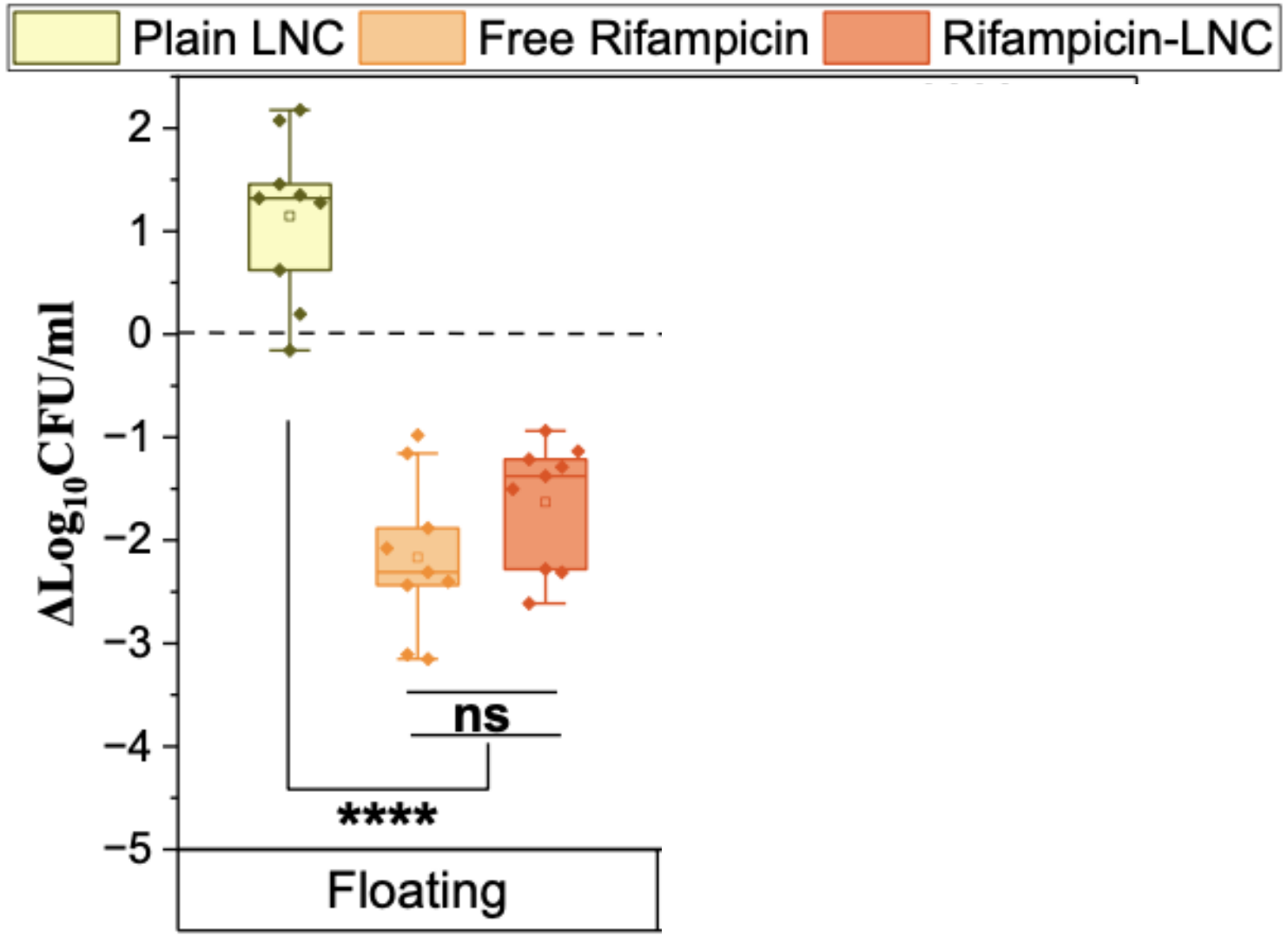
GFP *Staph. Aureus colonization of hair follicles*

Fluorescence **Bright-Field** **Merged**

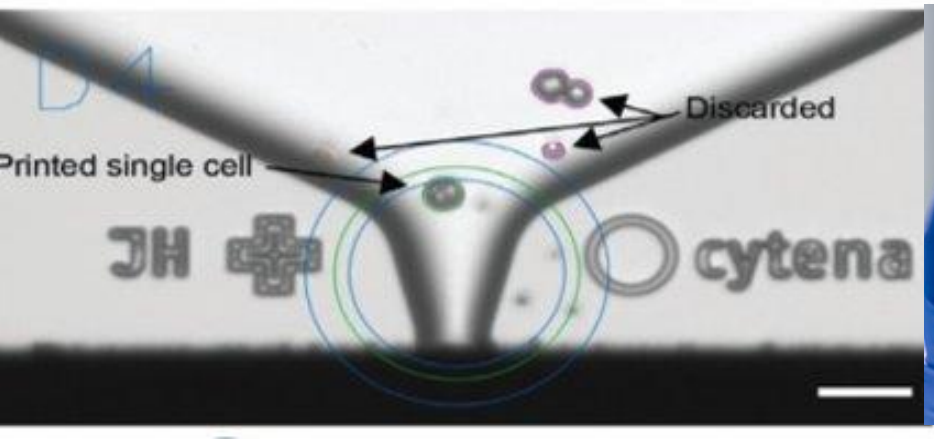


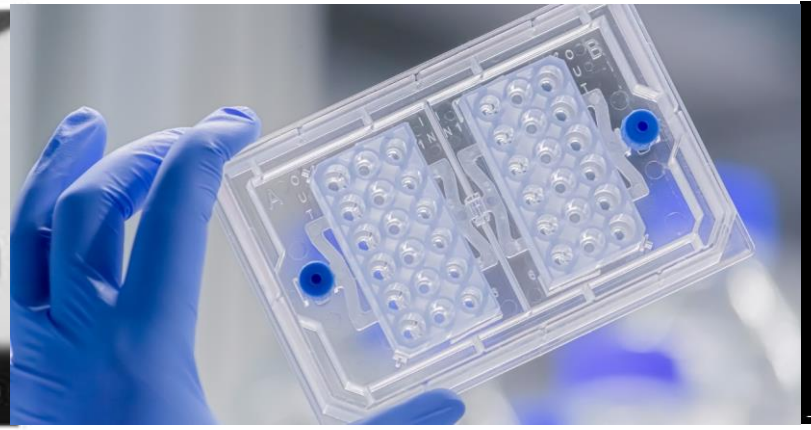


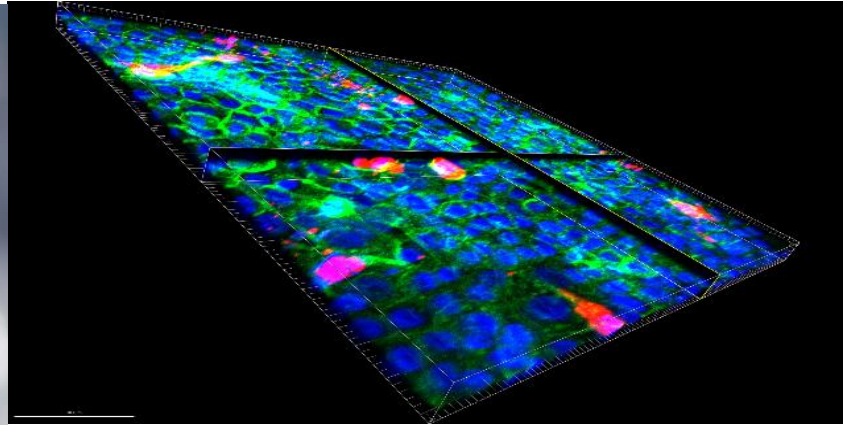
Rifampicin-LNC treatment of S.Aureus-infected hair follicles



Samy Aliyazdi, Sarah Frisch et al., ACS Biomat Sci. Eng. 2024



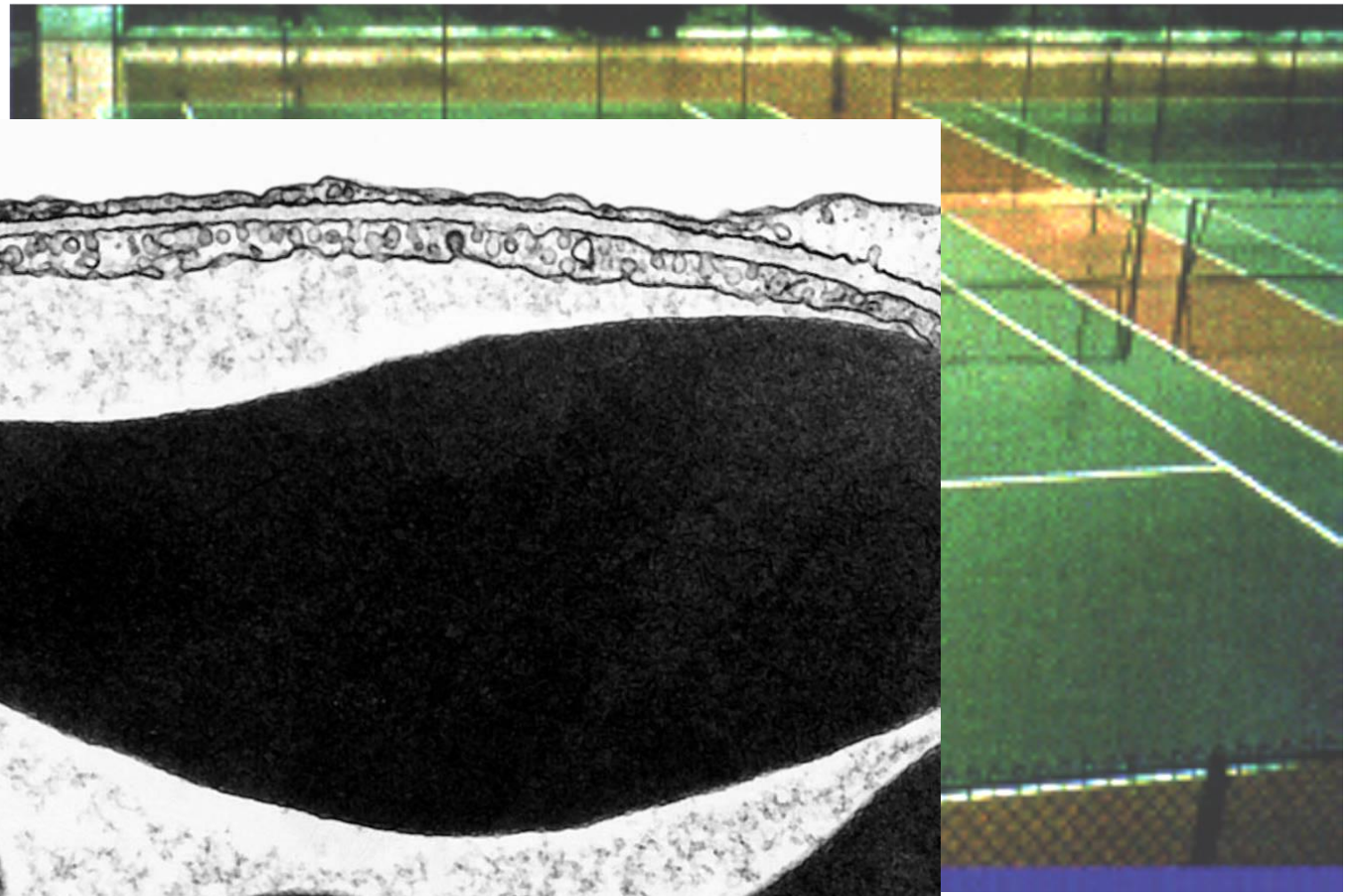




BARRIER #2:

THE LUNGS

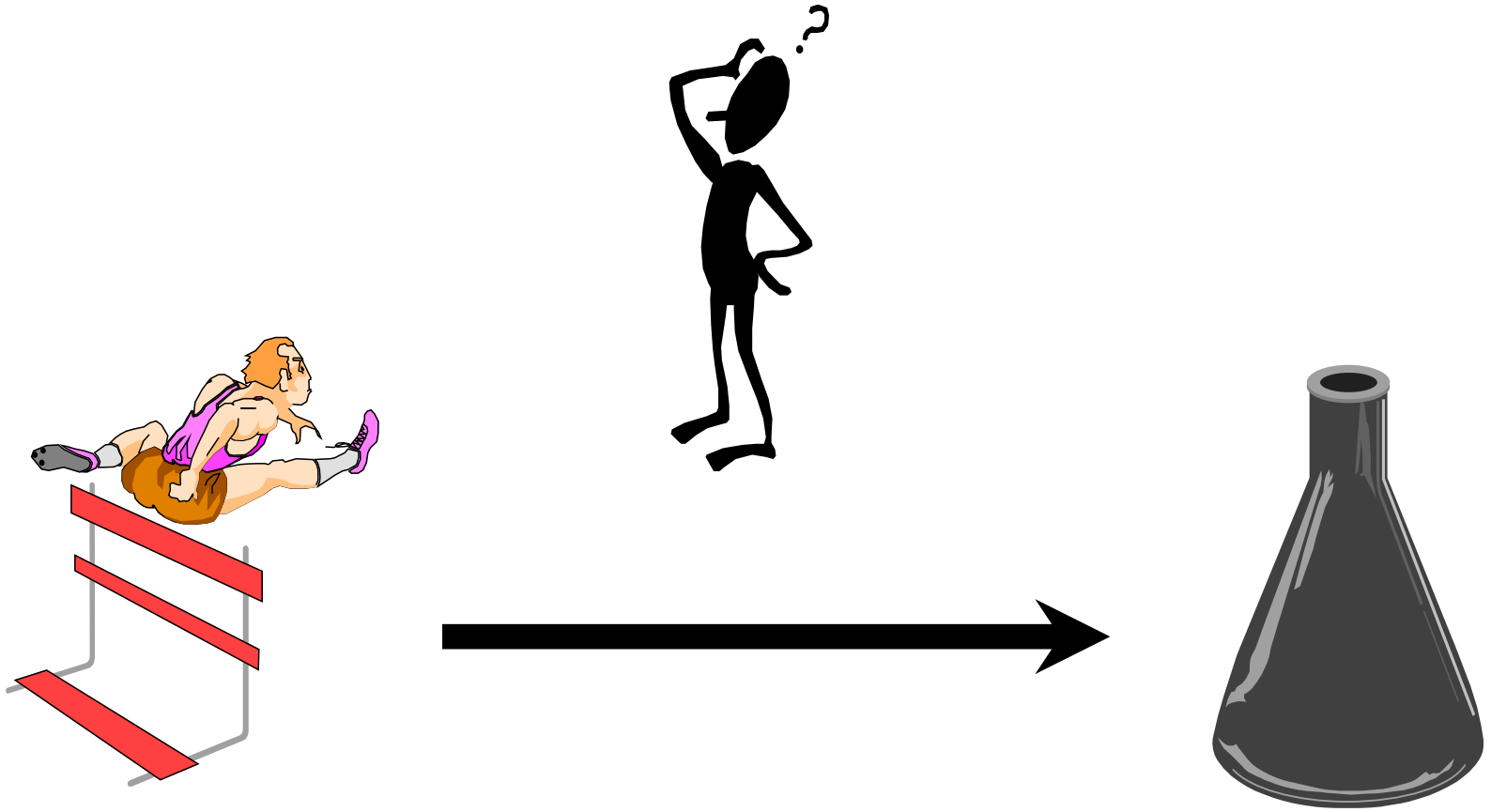
What makes the lung attractive for drug delivery ?



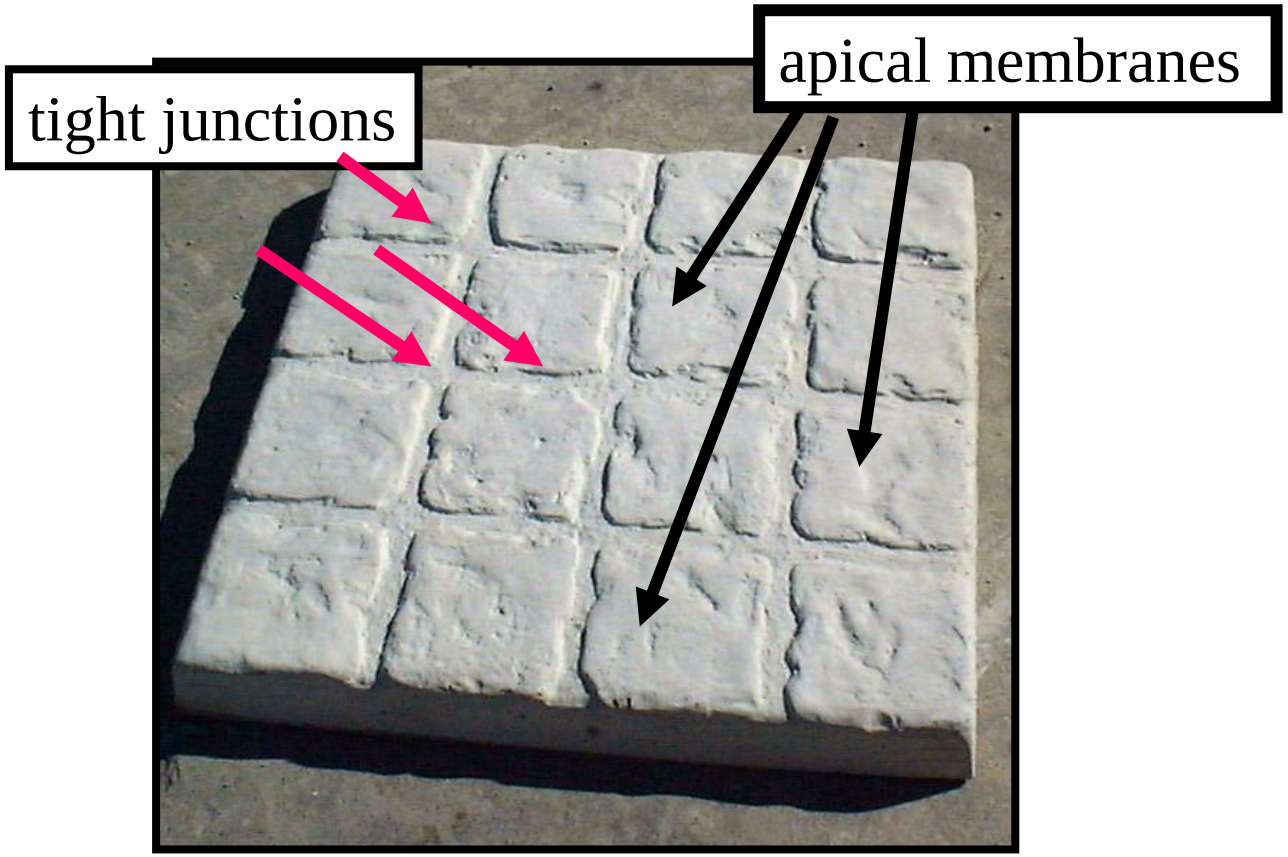
courtesy J. Patton, USA

courtesy P. Gehr, Bern

How to put the air-blood barrier in a test tube?



NEEDED: Barrier Properties!

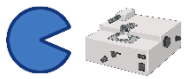


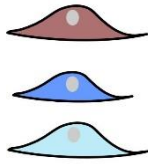
A functional epithelium is more than just an assembly of cells!

Modelling the air-blood-barrier of the deep lung: the human alveolar epithelial cell line „Arlo“

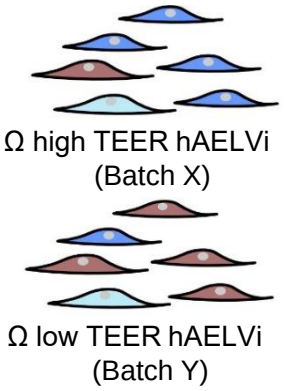
Patrick Carius

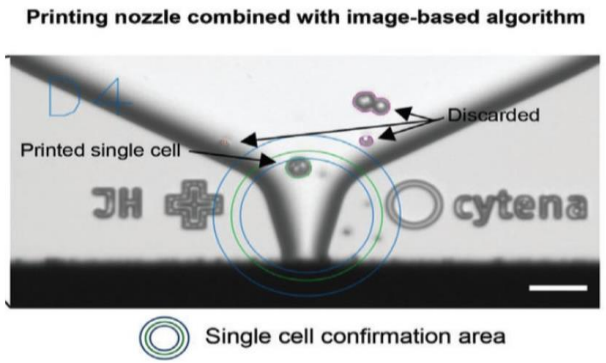
Dr. Nicole Schneider-Daum

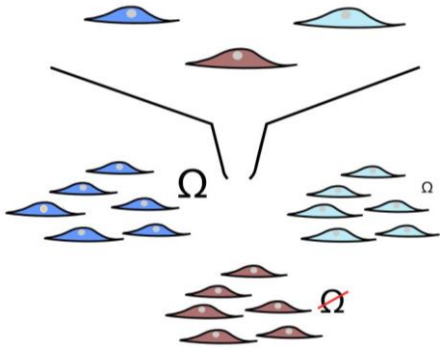




human **A**lveolar **E**pithelial **p**rimary **C**ells (**hAEpC**)





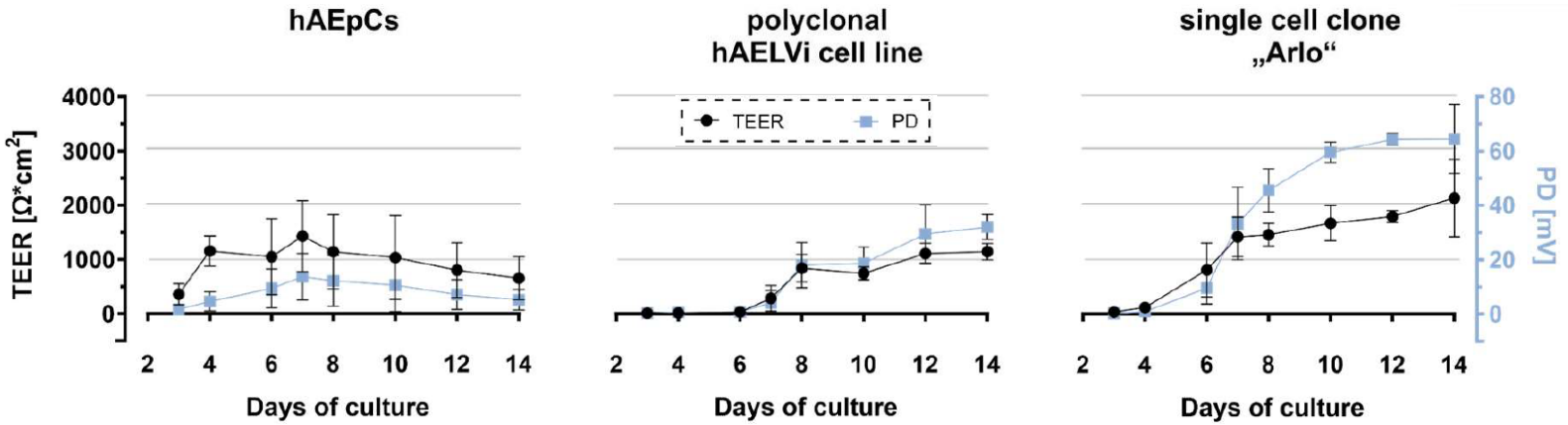


Elbert et al., **Pharm. Res.** (1999)
Daum et al., **M Mol. Biol.** (2012)

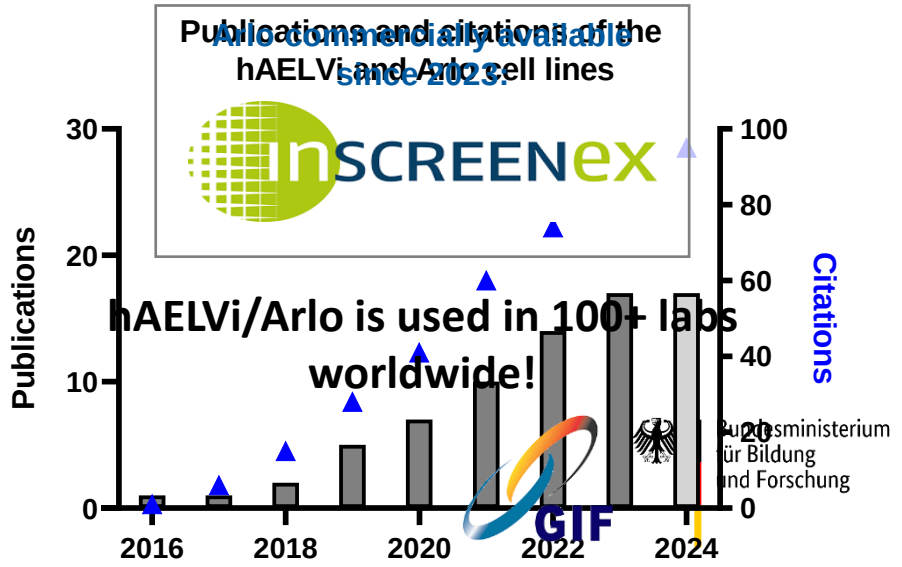
Kuehn et al.,**ALTEX (2016).**

Carius et al., **Adv. Sciences** (2023)

Characterization of the Arlo cell line

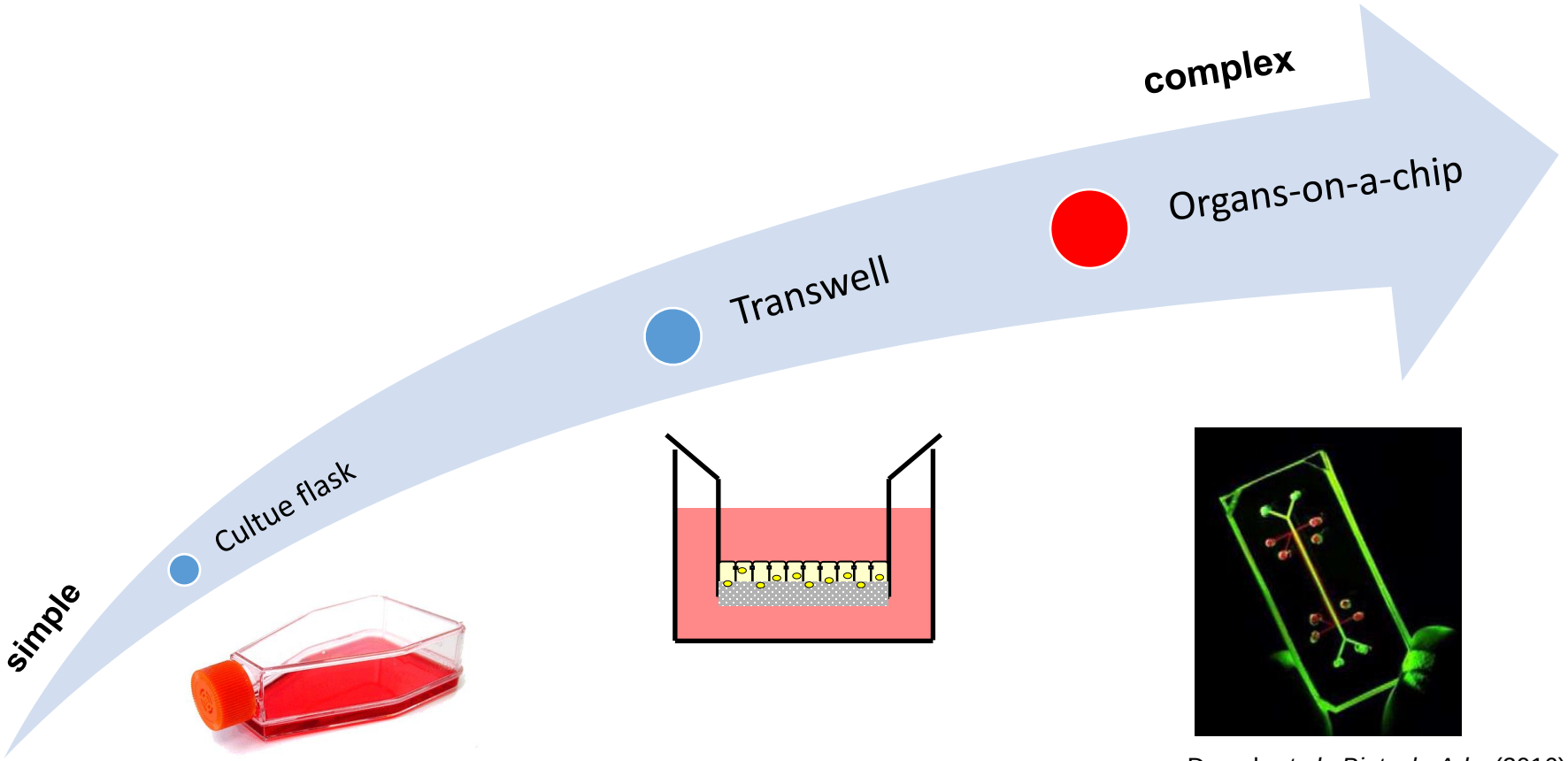


Carius et al., **Adv Sciences** (2023).



Created with BioRender.com

Moving from Transwells® to Organs-on-a-Chip



Doryab *et al.*, *Biotech. Adv.* (2016)

Advanced *in vitro* models of the human lung: Project AIM4Doc

AIM4DoC = Advanced Inhalation Model for Drug Discovery on Chip

Founded by:

eurostars™

AlveoliX
In-vitro models inspired by nature

Lung-on-chip development

© AlveoliX AG

b
UNIVERSITÄT
BERN

Biological implementation

VITROCELL® SYSTEMS

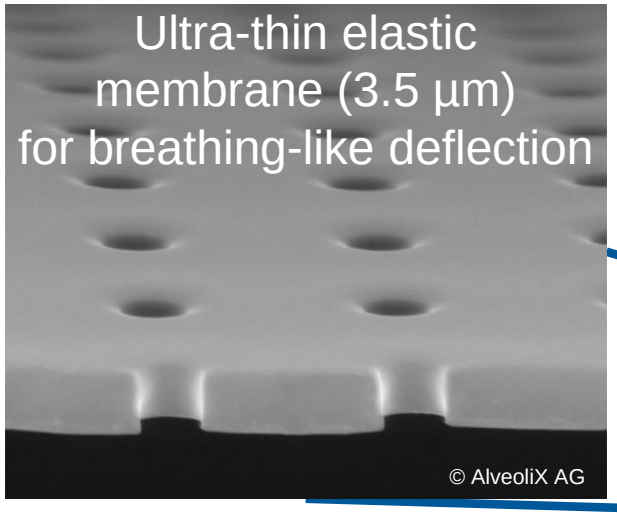
Exposure system development

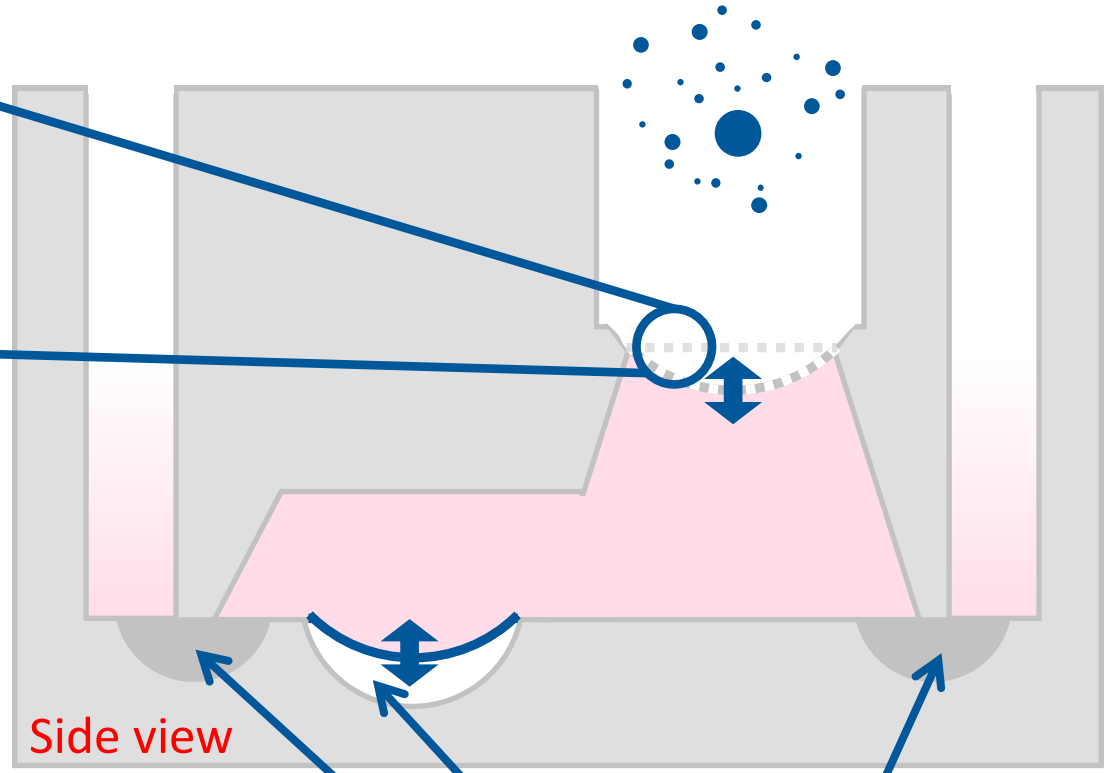
HZI HELMHOLTZ Centre for Infection Research

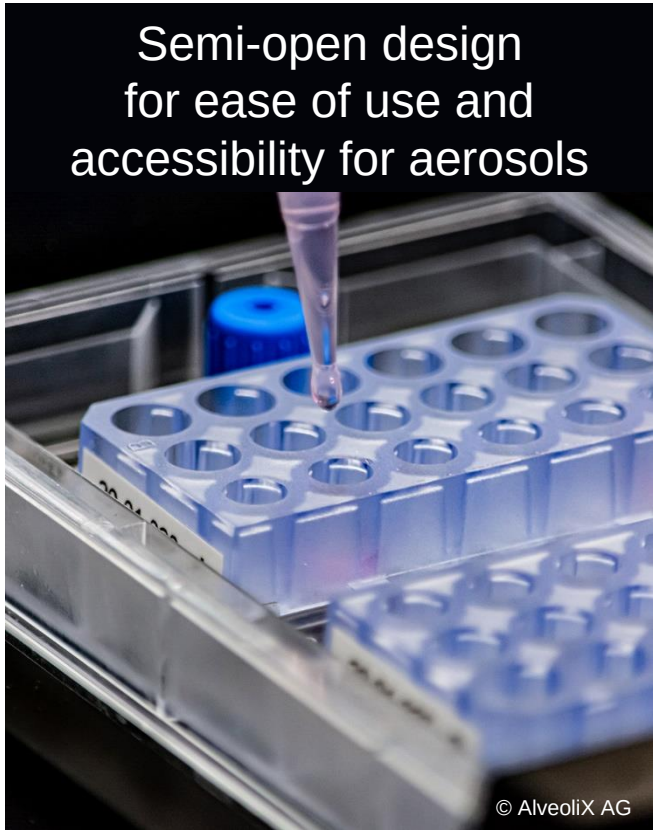
Biological implementation

The breathing lung-on-chip *AX12* – A microfluidic system

Founded by:
eurostars™



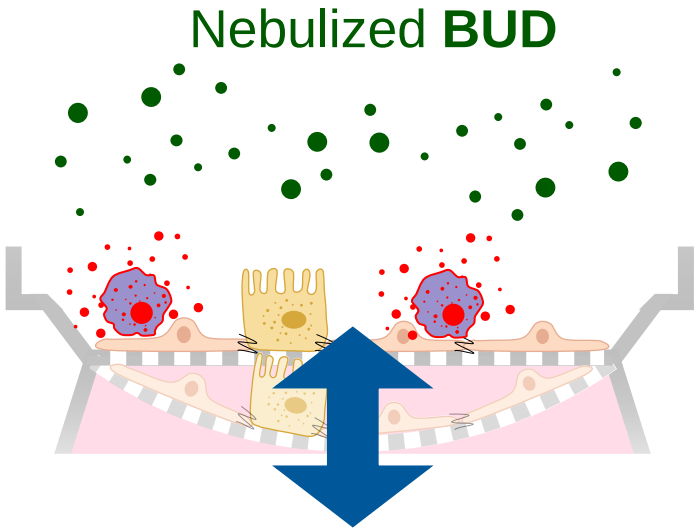


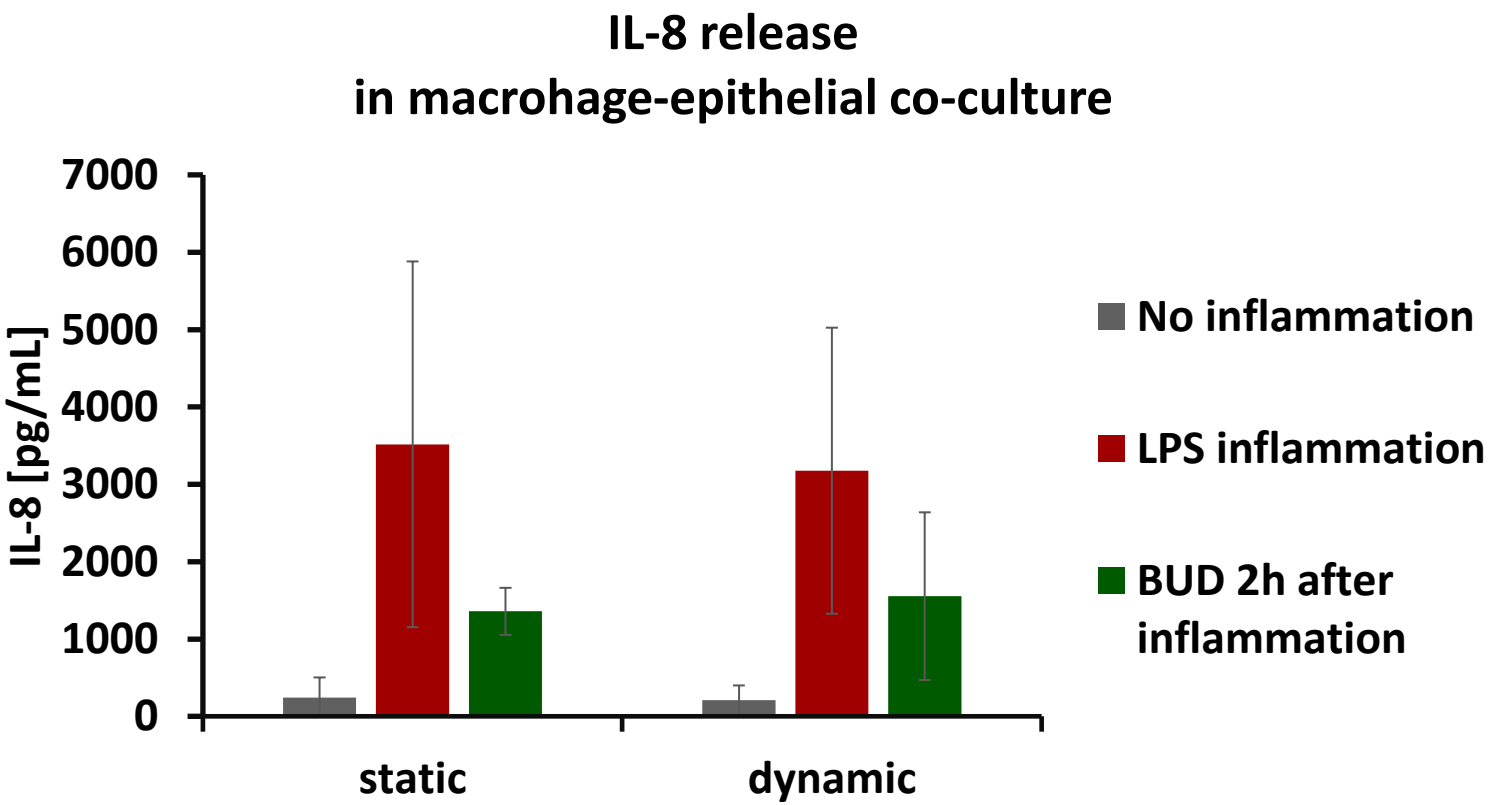


Adapted from: **Stucki et al.**, *Lab on a chip* 2018

EU Project "AIM4DoC“:
Advanced Inhalation Models for Drug Discovery on Chip

BIOENGINEERING & TRANSLATIONAL MEDICINE





Breathing dynamics seem to have **little effect** on the strength of **LPS-induced inflammation** nor on the efficacy of anti-inflammatory treatment

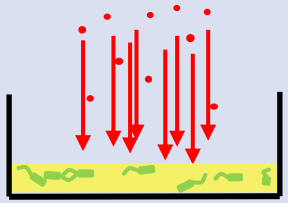
Static: n = 7-9 out of 3 independent experiments, mean ± SD
Dynamic: n = 12-13 out of 4 independent experiments, , mean ± SD

Modelling a CF-patient in vitro

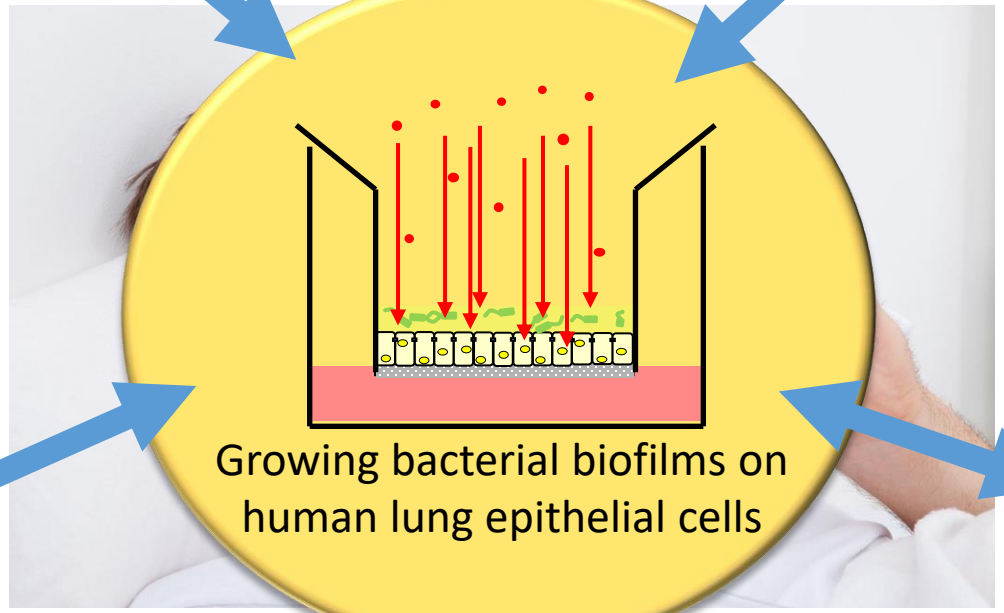
Planktonic bacteria on epithelial cells

Anderson et al., Infect Immun, 2008

Aerosolized treatment of Biofilm



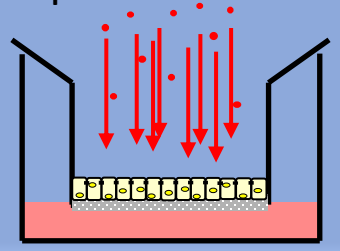
Loo et al., Pharm Res, 2018



P. aeruginosa Biofilm

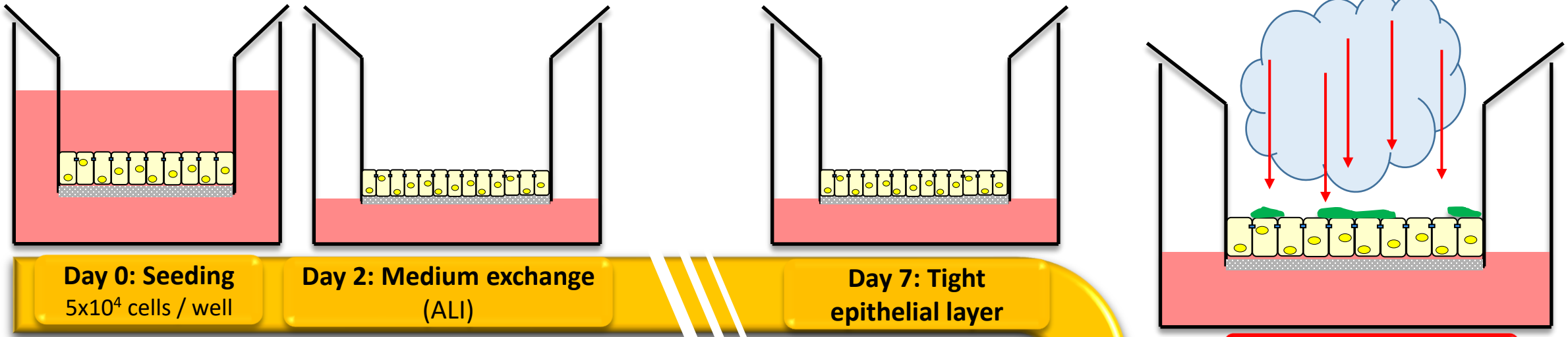
Macia et al., Clin Microbiol Infect, 2014

Nebulization on epithelial cells



Röhm et al., Int. J. Pharm., 2017

The “trick”: Host cell monolayers and bacterial biofilms must be grown separately!



Day 0: Seeding
5x10^4 cells / well

Day 2: Medium exchange
(ALI)

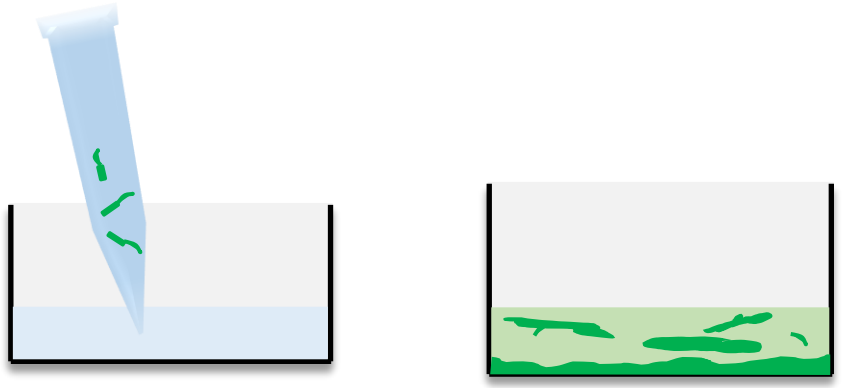
Day 7: Tight epithelial layer

Bronchial epithelial cells

Treatment after x h

1 | 24 | 48

Biofilm



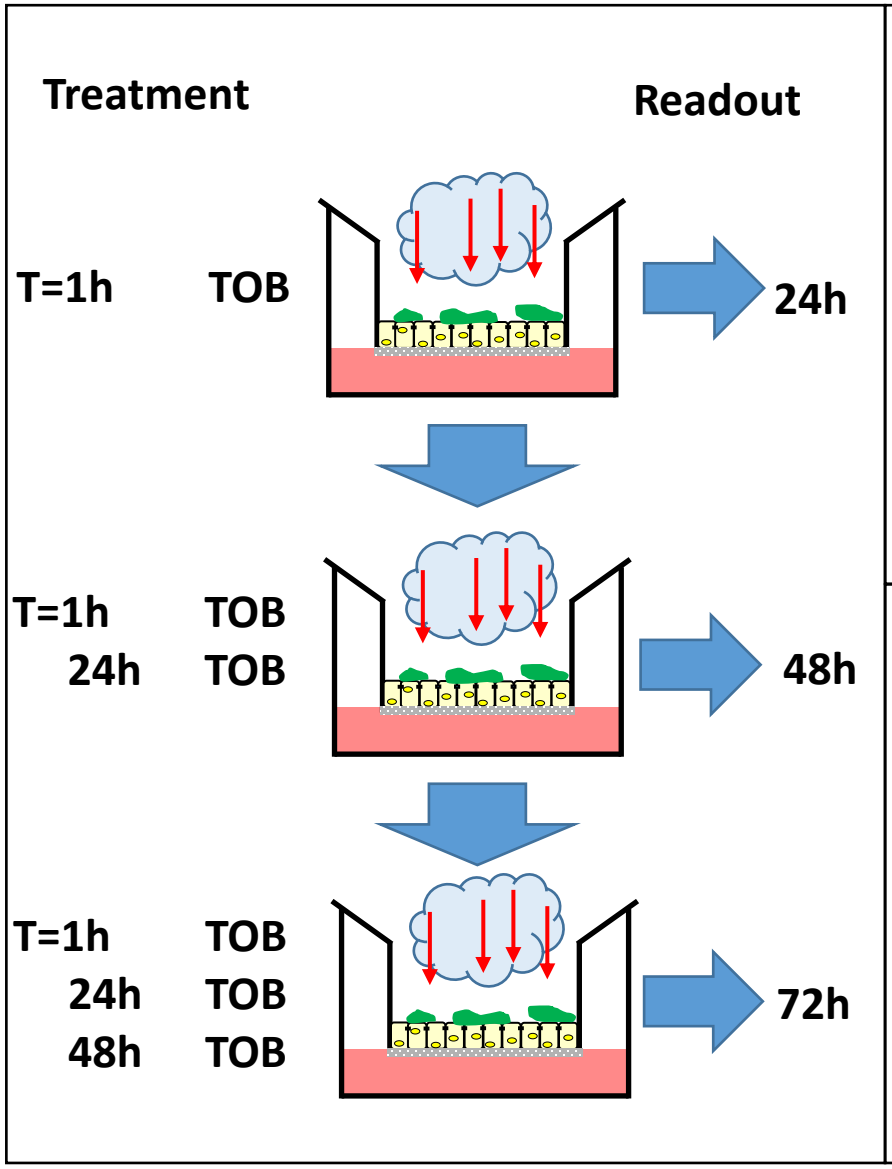
Day 4: Start biofilm growth
1 x 10^6 bacteria

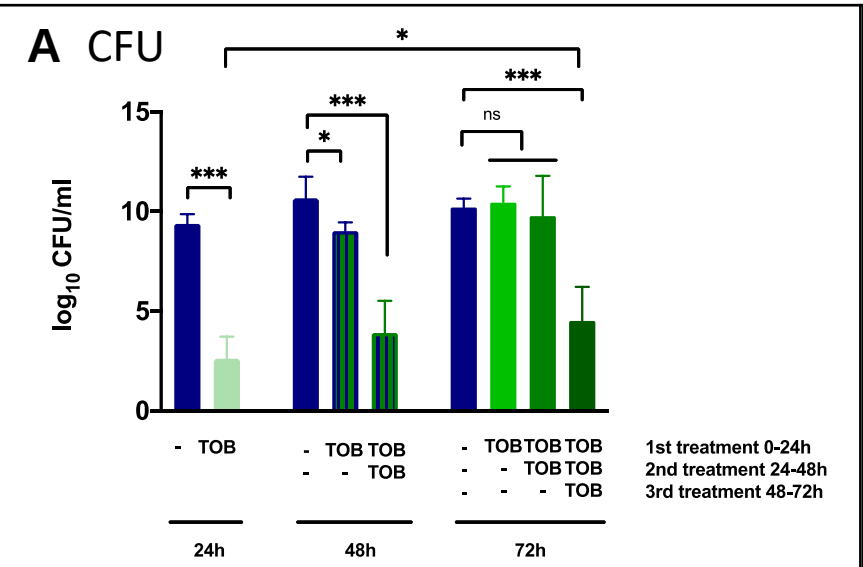
Day 7: Mature biofilm

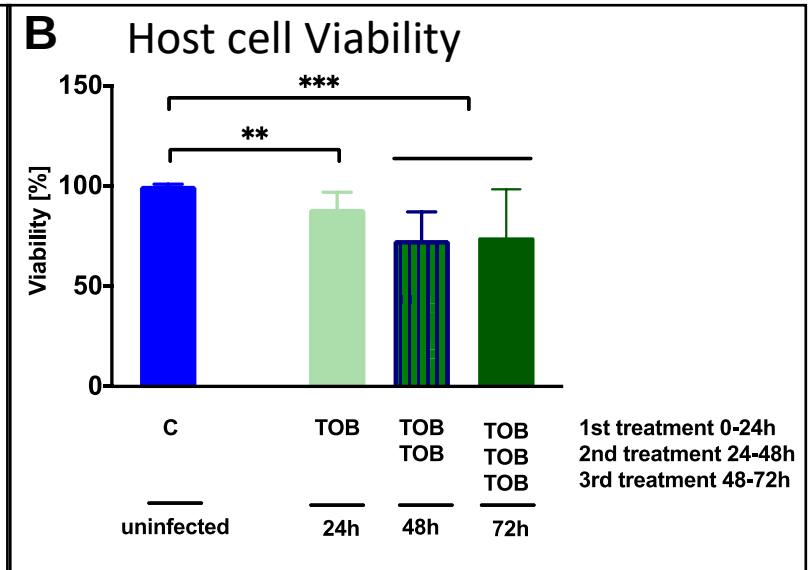
4/24h Readout | 48h Readout | 72h Readout

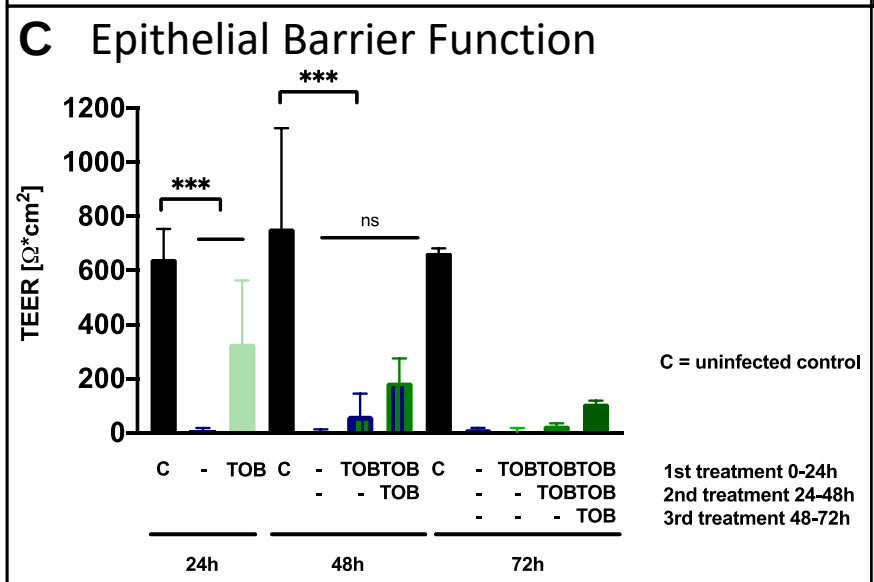
Chronic infected co-culture

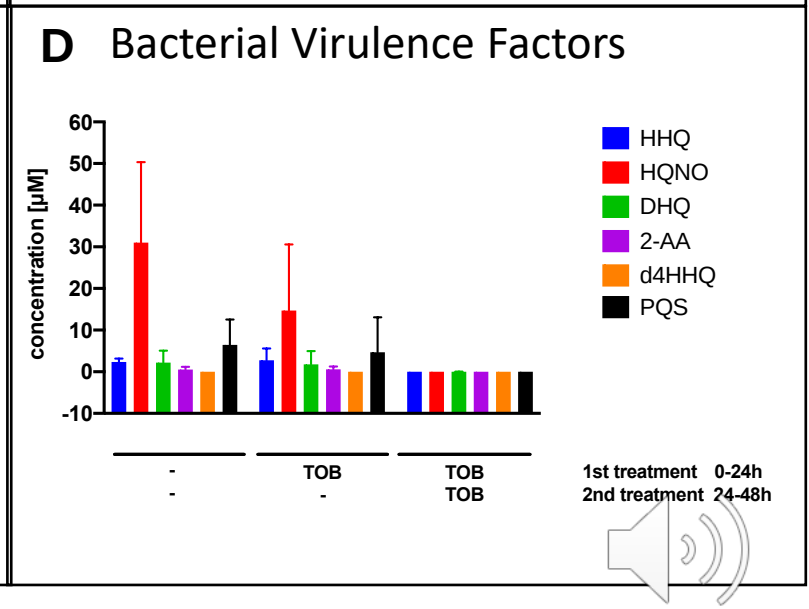
Repeated antibiotic aerosol administration allows to control bacterial biofilm and monitor epithelial barrier function up to 72 hours post-infection











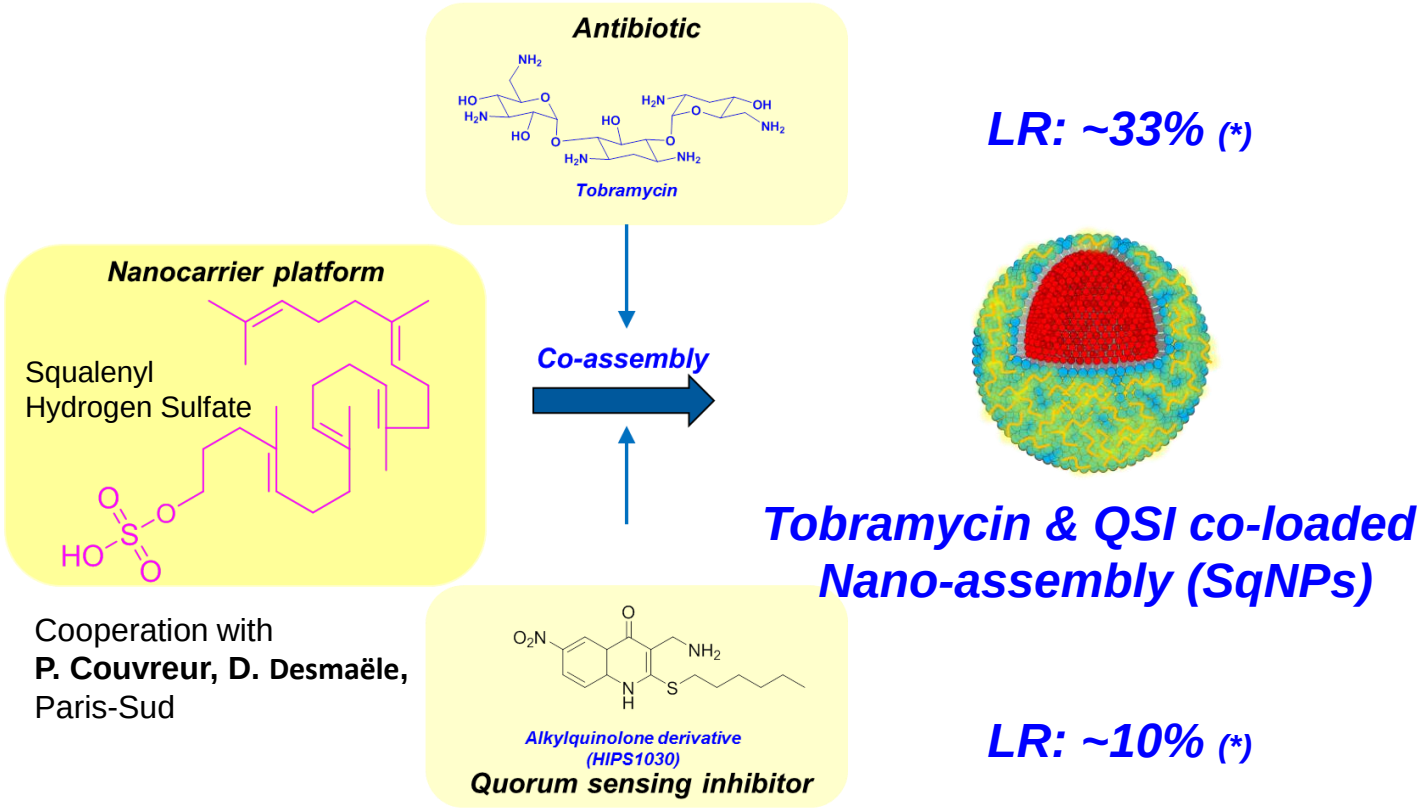
Horstmann et al., *ACS Infect. Dis.* **2022**, 8, 1, 137–149

Co-delivery of Antibiotic (Tobramycin) + Biofilm-Inhibitor (PQSI) by self-assembling Nanocarriers

Duy-Khiet Ho, Brigitta Loretz

Ho et al. Eur. J Pharm, 144 (2019) 110–124

Squalene Nanoassemblies for dual drug delivery



Cooperation with
P. Couvreur, D. Desmaële,
Paris-Sud

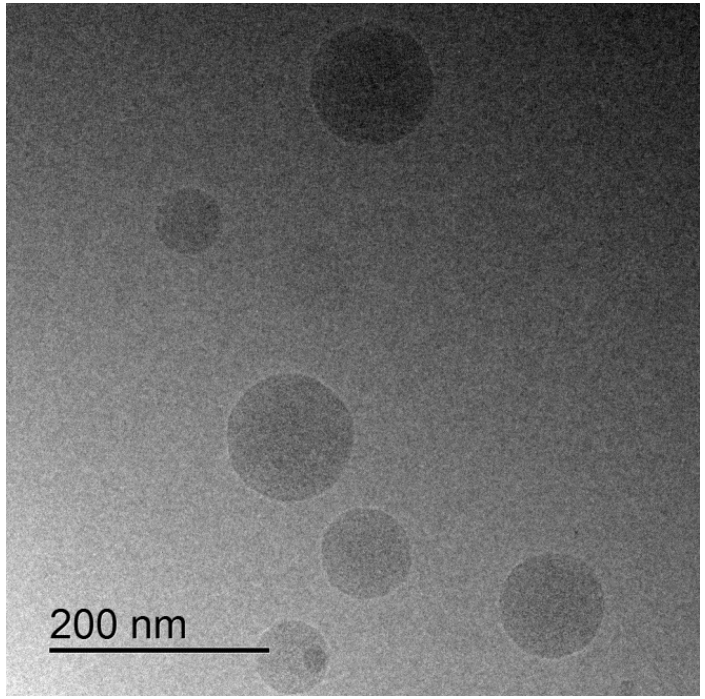
Cooperation with
R. Hartmann, M. Empting, HIPS

LR: ~33% (*)

LR: ~10% (*)

Tobramycin & QSI co-loaded Nano-assembly (SqNPs)

Size [nm]	PDI	Zeta [mV]
192,0 ±1,1	0,104 ± 0,024	6,8 ± 1,3

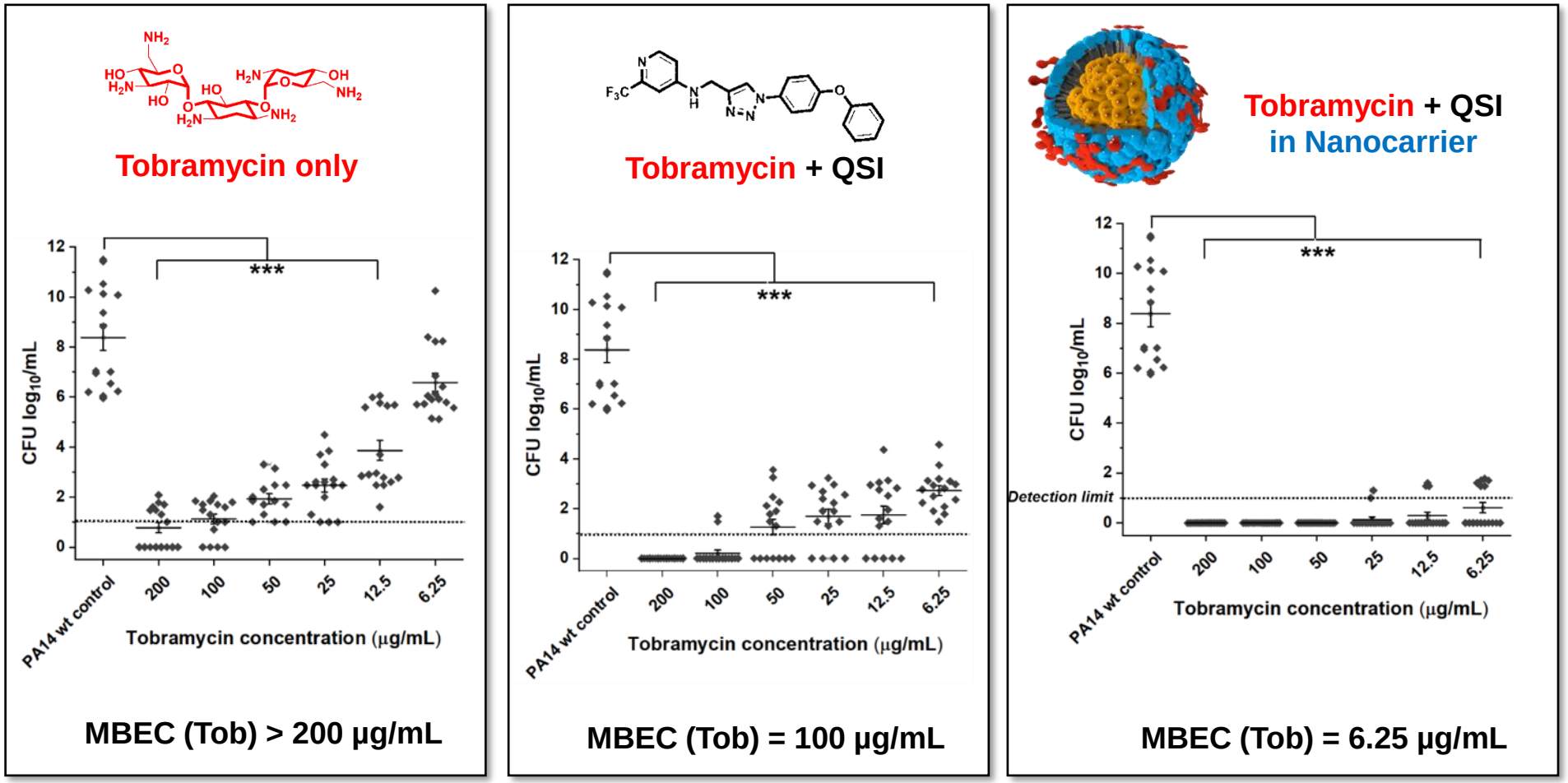


Cryo-TEM images of SqNPs
by M. Koch, INM

Ho D.K., Loretz B., Lehr C.-M., EP17179011

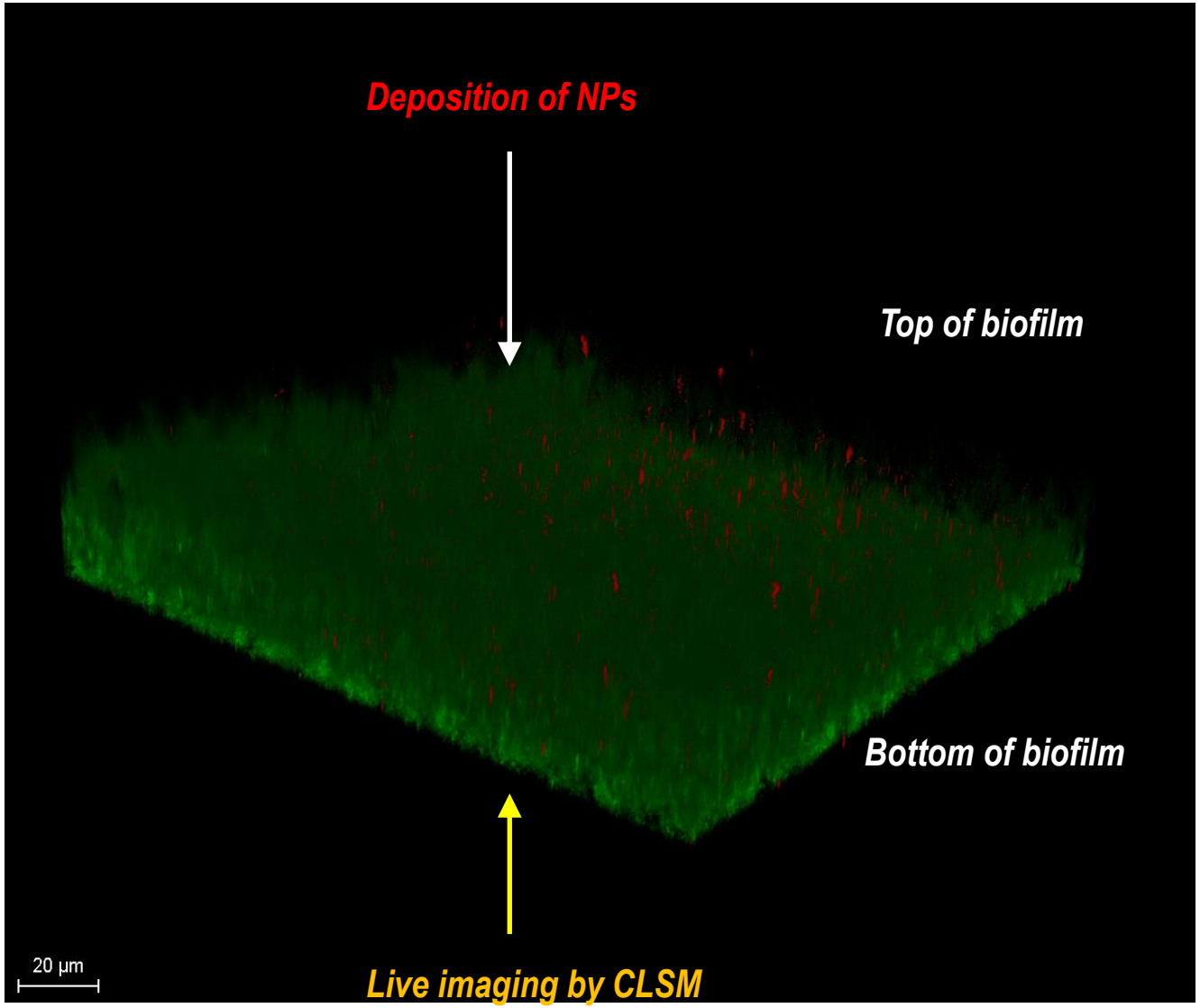
(*) Loading rate as quantified by LCMS

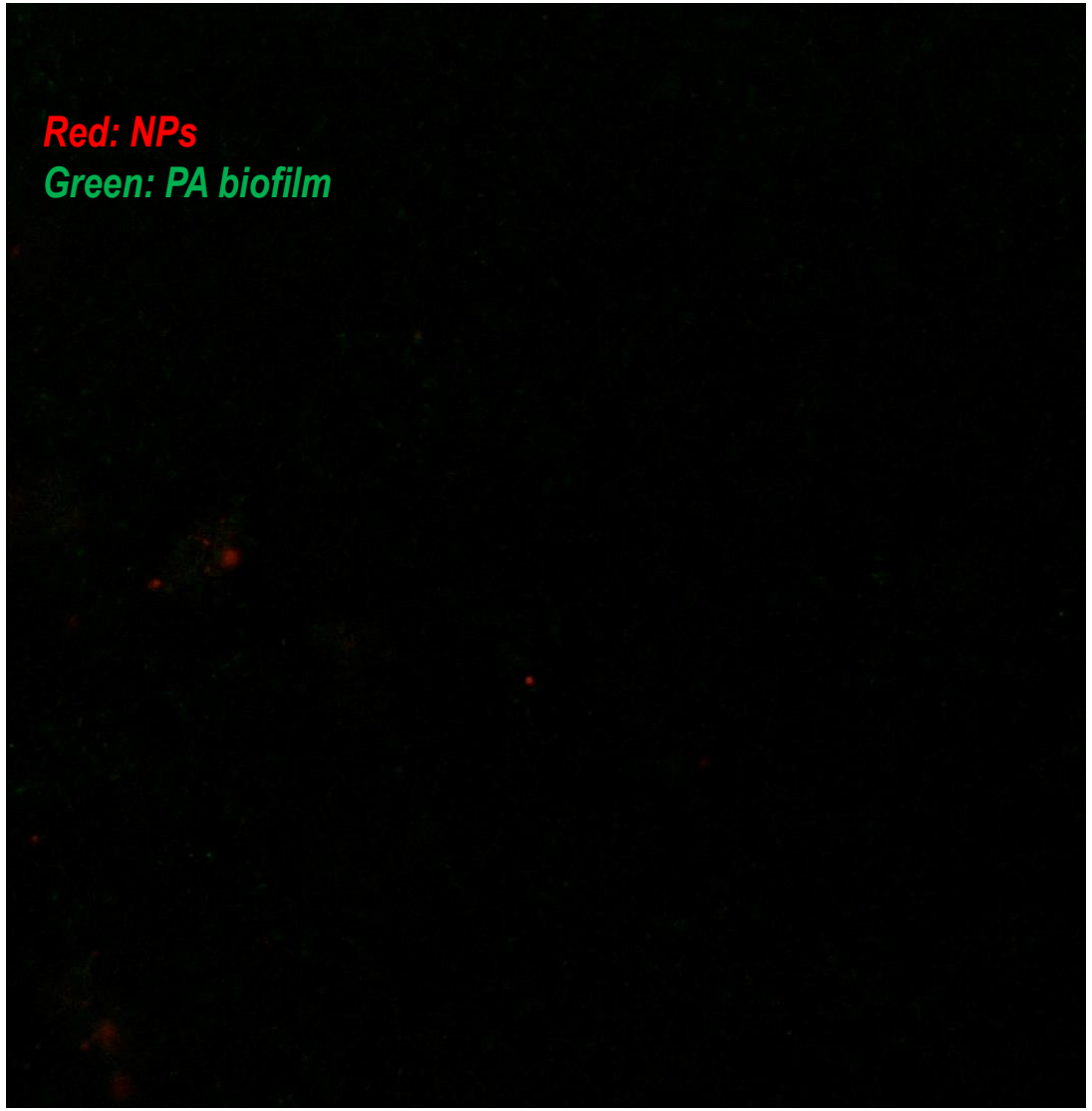
Nanocarriers Boost the Synergy of QSI with Tobramycin



Ho et al., ***Angew. Chem.*** (2020)
Schütz et al., ***Advanced Science*** (2021)

Getting through the biofilm is the key!





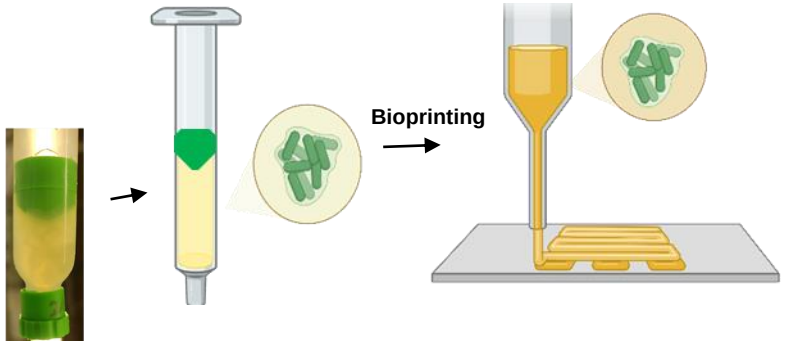
Ho et al., ***Angew. Chem.* (2020)**

3D Bioprinting of *Bacterial* Biofilms on Calu-3 cells

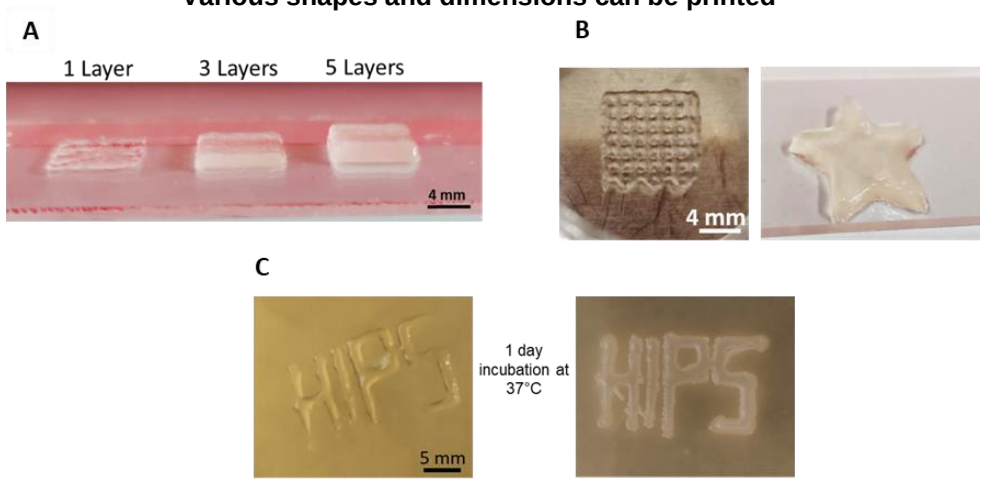
Samy Aliyazdi

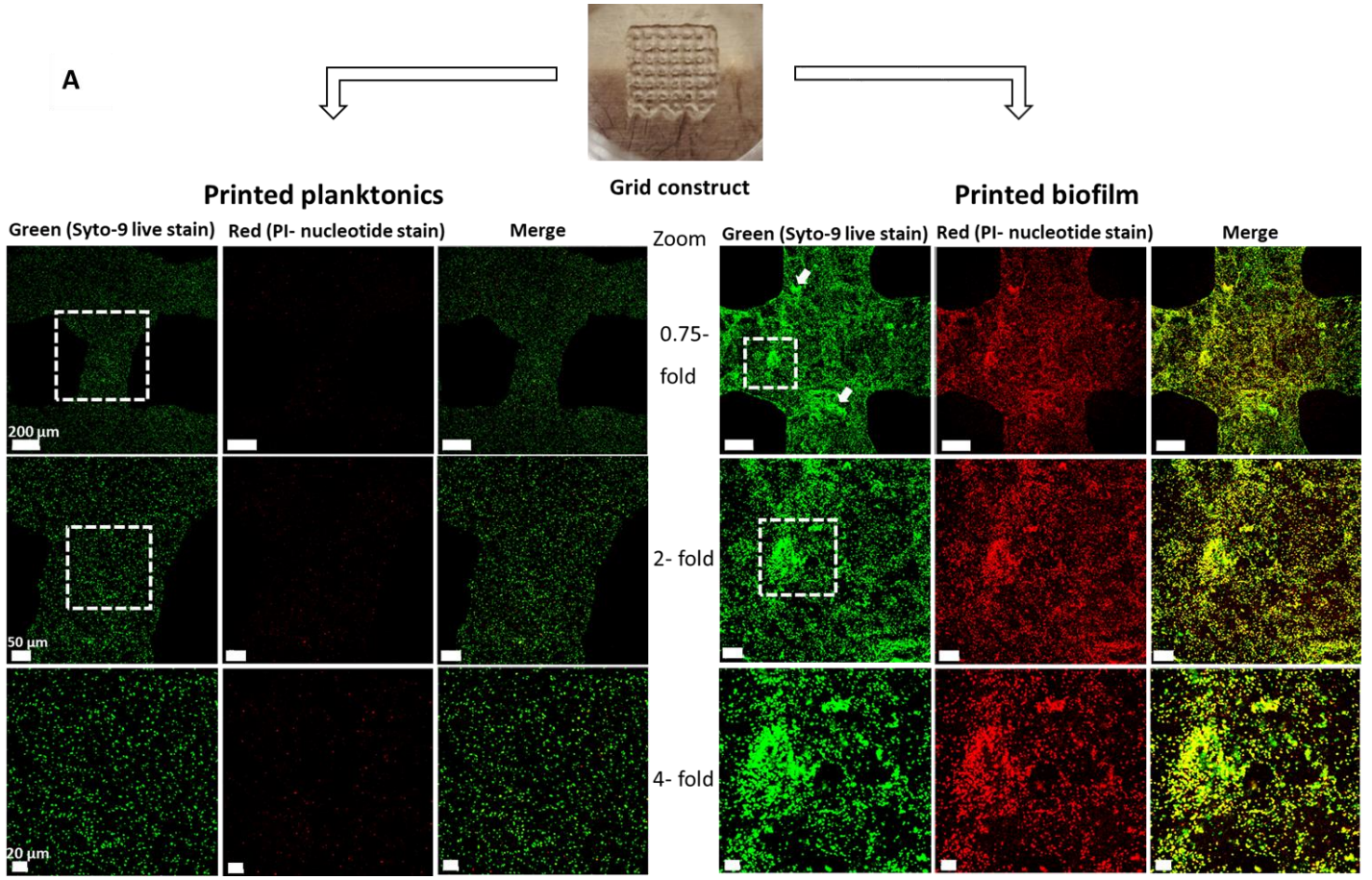
Bioprinting *E. coli* MG1655 biofilms

As model strain *E.coli* MG1655 biofilms were formed inside a gelatin-alginate bioink



Various shapes and dimensions can be printed





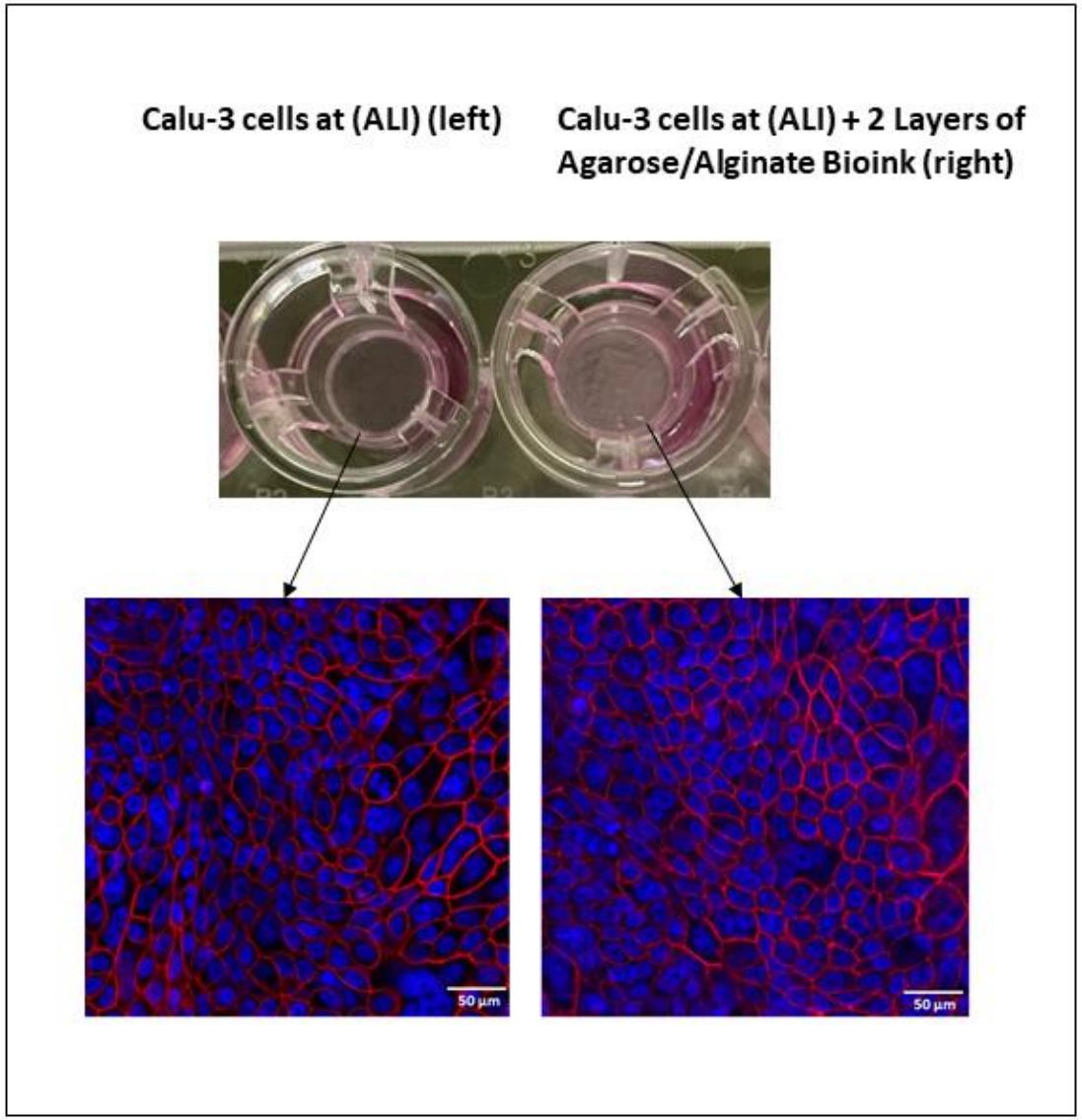
Biofilm morphology confirmed by bacterial aggregations and release of extracellular nucleic acids

Aliyazdi et al., Biofabrication (2023)

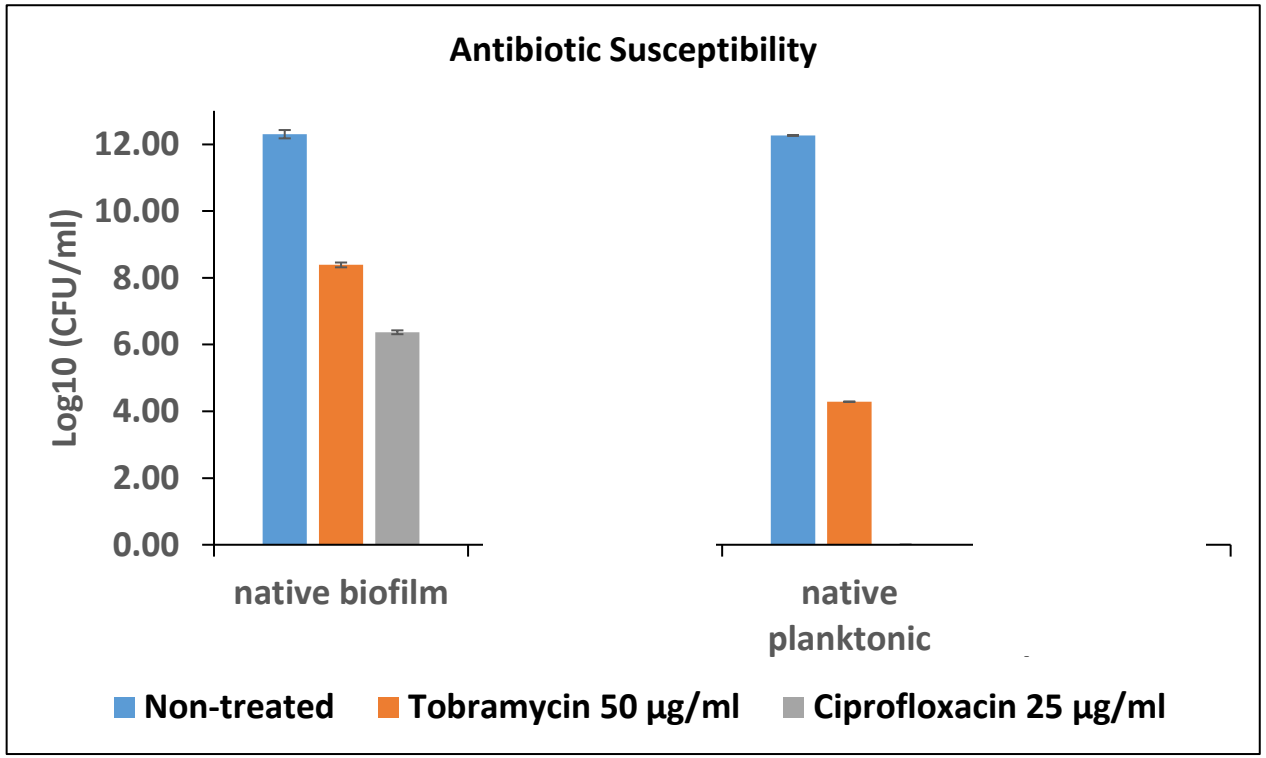
The next step: Transfer from *E. coli* to *P. aeruginosa*

Aghiad Bali

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

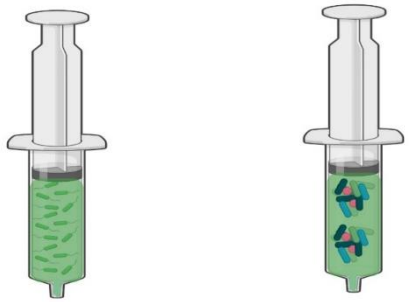


CLSM images of Calu-3 cells grown 12 days at ALI stained with DAPI (blue) and Phalloidin (red)



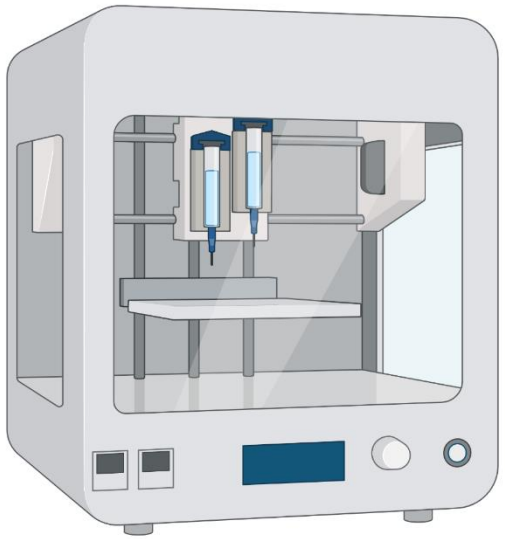
Antibiotic resistance of native/3D-printed biofilm vs. native/3D-printed planktonic bacteria. n=9, N=3. Error bars represent standard deviation.

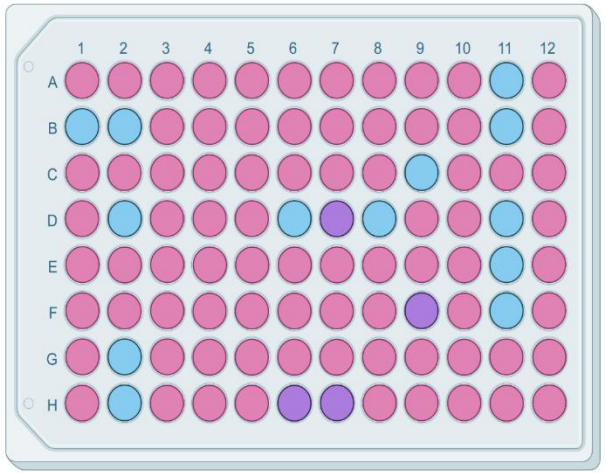
Platform for high-throughput drug testing in 96-well plate



Planktonic bacteria embedded in Bioink

Biofilm bacteria embedded in Bioink





Metabolic activity (viability) assessment

Add test compounds (overnight incubation)

Add reagent e.g. Presto blue

PAO1 Biofilm

PAO1 Planktonic

PA14 Biofilm

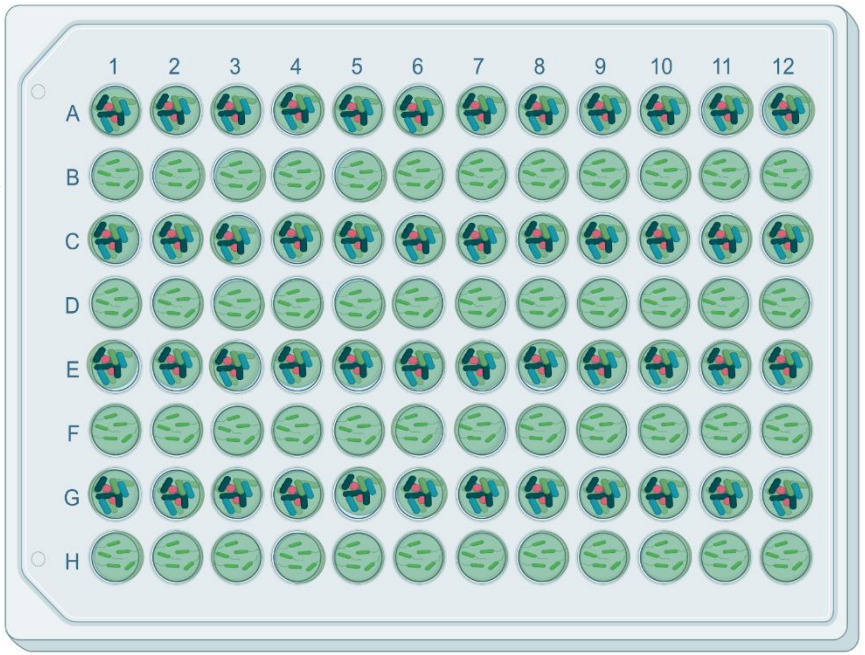
PA14 Planktonic

PAN Biofilm

PAN Planktonic

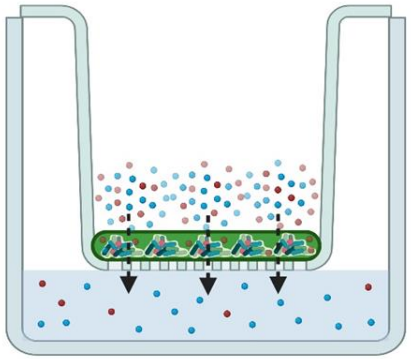
PAK Biofilm

PAK Planktonic



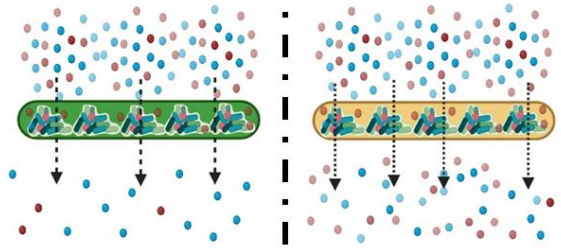
3D-printed biofilm on transwells for investigating drug permeation through biofilms

Concept



- Concept: Evaluate the permeation of molecules with different properties e.g. Fluorescein (hydrophilic), Rhodamine B (hydrophobic

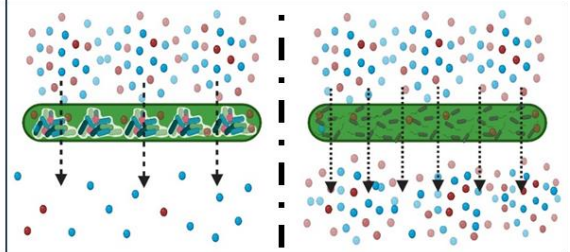
Different Bacteria produce different Biofilms



Transport through bacterial biofilms of Strain 1

Transport through bacterial biofilms of Strain 2

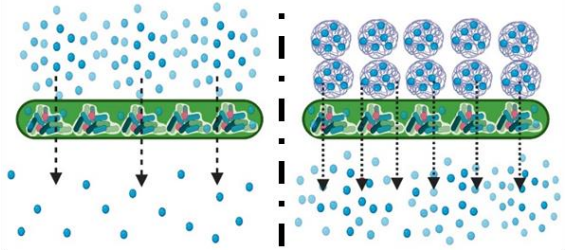
Drug permeation through weakened Biofilm



Transport through normal bacterial biofilms

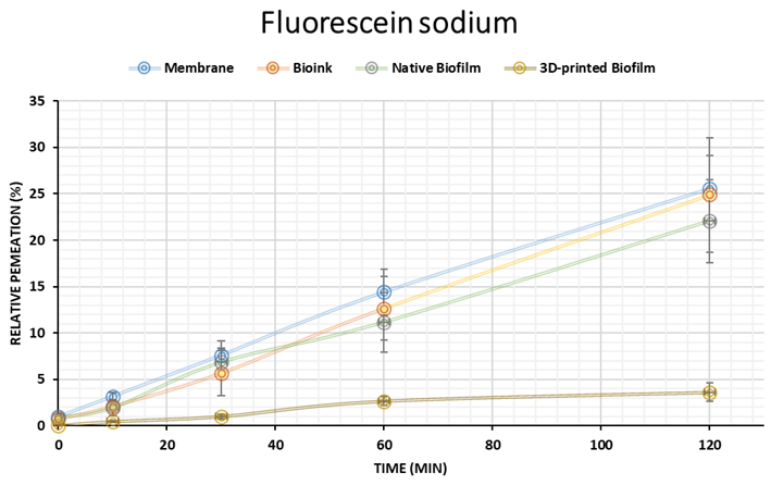
Transport through weakened bacterial biofilms

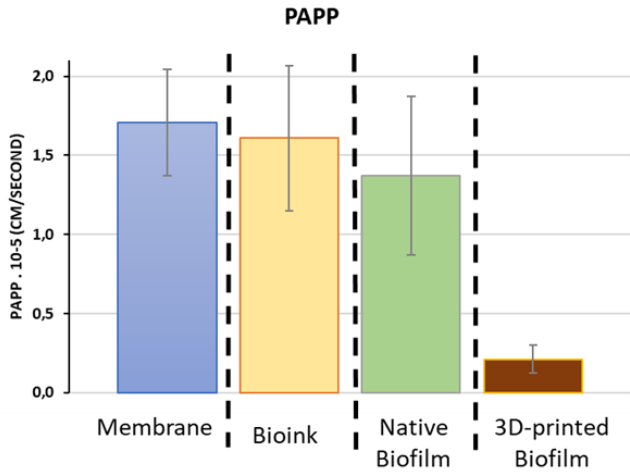
Nanoparticles vs. free drug permeation



Transport of free drug molecules through bacterial biofilms

Transport of drug-loaded nanoparticles through bacterial biofilms





Transport study through bacterial biofilms (7 days PAO1 biofilm). n=6, N=2. Error bars represent standard deviation

Thanks for your attention!

Check out our posters:

Achim Biesel

Poster #158 Lipid Polymer Hybrid Nanoparticles for Pulmonary mRNA Delivery

Max Koumou-Okandze

Poster #215 Acid-cleavable Tobramycin cross-linked nanogels for antibiotic delivery to *P. aeruginosa biofilms*

Jun.-Prof. Sangeun Lee

Poster #321 Biodynamers, dynamic protein nanorods, potentiate antibiotics via membrane interactions

We hope to welcome you in March 2026 in Saarbrücken, Germany!

30th CRS DeChAt Local Chapter Meeting meets

14th International Conference on

BIOLOGICAL BARRIERS

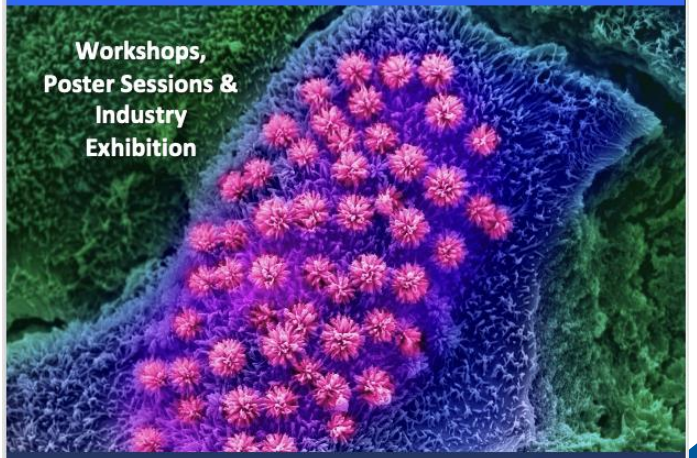
3 - 6 March 2026
Saarbruecken, Germany

Scientific Agenda

- Advanced Drug Delivery Technologies
- Microphysiological *in vitro* Models ("on the Chip")
- Infectious Diseases and Microbiota
- Career Development & Networking

Target Audience

- Young and Experienced Researchers
- Industry Professionals
- Start-Ups and Entrepreneurs



HIPS HELMHOLTZ Institut für Pharmazeutische Forschung Saarland

UNIVERSITÄT DES SAARLANDES

Confirmed Invited Speakers Include

Cameron Alexander (Nottingham, UK)
Ana Beloqui (Louvain, BE)
Elias Fattal (Paris, FR)
Hamid Ghandehari (Salt Lake City, US)
Rainer Haag (Berlin, DE)
Sarah Hedtrich (Vancouver, CA)
Anna Hirsch (Saarbruecken, DE)
Andreas Keller (Saarbruecken, DE)
Hagar Labouta (Toronto, CA)
Karsten Lindhardt (Copenhagen, DK)
Jens Puschhof (Heidelberg, DE)
Otmar Schmid (Munich, DE)
Avi Schröder (Haifa, IL)
Simone Schürle (Zurich, CH)
Maria Vicent (Valencia, ES)
Wilfried Weber (Saarbruecken, DE)
Maike Windbergs (Frankfurt, DE)
Elisabeth Zeisberg (Goettingen, DE)

Program Schedule

Tue/Wed	Wed/Thu	Thu/Fri
14th International Conference on Biological Barriers	GALENOS meets TALENTS: Workshops for Young Scientists	30th CRS DeChAt Local Chapter Meeting

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