




# Nanoparticles and rod-like particles for inhalation drug delivery

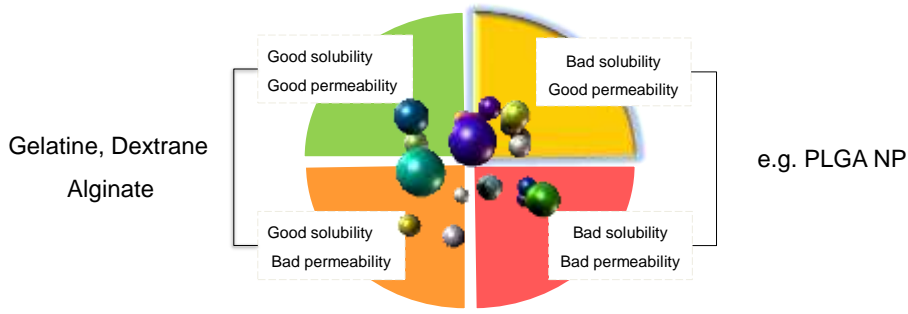
Prof. Dr. Marc Schneider

Department of Pharmacy  
Biopharmaceutics and Pharmaceutical Technology  
Saarland University

November 7<sup>th</sup> 2019

Chair of Biopharmaceutics and Pharm. Technology 



Gelatine, Dextrane  
Alginate

e.g. PLGA NP

Particulate carrier systems

Nano (nm)  
Micro ( $\mu\text{m}$ )

**Chair of Biopharmaceutics and Pharm. Technology**

Gelatine, Dextrane  
Alginatee.g. PLGA NP

**Particulate carrier systems**

Biological interaction

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**Cystic fibrosis**

**Healthy**

**Cystic Fibrosis**

- Autosomal recessive disorder
- Mutations in CFTR gene → defect chloride channel
- Abnormal thick mucus  
⇒ bacterial infections  
biofilm formation

**Current treatment** drawbacks:

- Change of phenotype, alginate production, slow division
- Hindered therapy due to biofilm formation
- Antibiotic resistance
- No effective local therapy

Cooper et al., Nature. 2011, Davies. Nature Rev Drug Discov. 2003

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## Treatment of CF

**Drugs:**

- Antibiotics
- e.g Azithromycin

Mucolytics

DNase, N-Acetylcystein,...

Antiphlogistics

Curcumin,...

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## Deposition pattern of particles

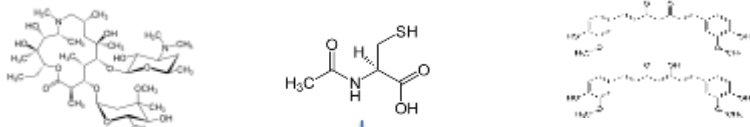
- Nasal, pharyngeal, laryngeal
- Tracheobronchial
- Alveolar

Oberdörster, G, Oberdörster, E & Oberdörster, J (2005). Environmental health perspectives. 113. 823-39.
24.11.2019

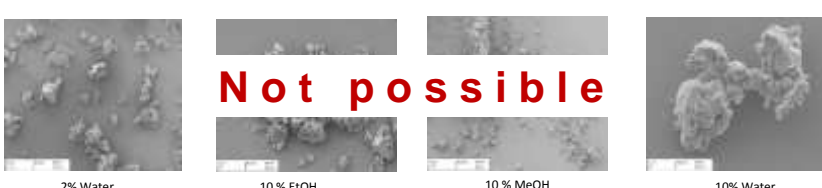
**Treatment of CF with inhalable microparticles**

Drugs: • Antibiotics      Mucolytics      Antiphlogistics

e.g. Azithromycin      e.g. N-Acetylcystein,...      Curcumin,...



Spray drying



2% Water      10% EtOH      10% MeOH      10% Water

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**Matrix formation for spray drying**

Drugs: • Antibiotics      Mucolytics      Antiphlogistics

e.g. Azithromycin      N-Acetylcystein,...      Curcumin,...

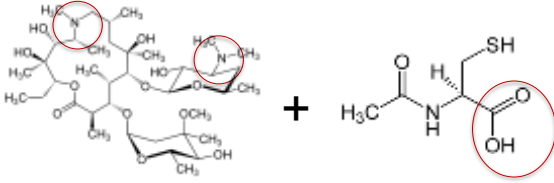
hydrophobic      hydrophilic      hydrophobic

How to combine them ?

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**Matrix formation for spray drying**

<p>Drugs: • Antibiotics</p> <p>e.g Azithromycin</p> <p>hydrophobic</p>	<p>Mucolytics</p> <p>N-Acetylcystein,...</p> <p>hydrophilic</p>	<p>Antiphlogistics</p> <p>Curcumin,...</p> <p>hydrophobic</p>
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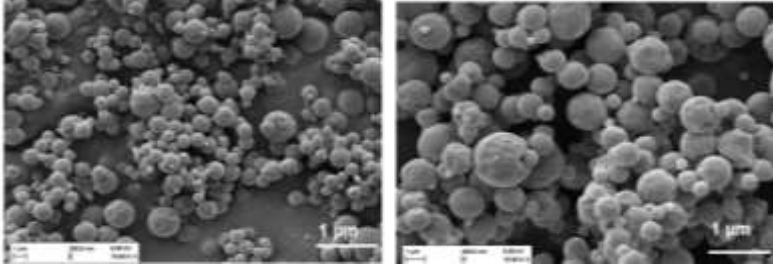


Complex formation by charges

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Lababidi et al, (2019) JCR
24.11.2019

**Spray-drying of compounds**

<p>Drugs: • Antibiotics</p> <p>e.g Azithromycin</p> <p>hydrophobic</p>	<p>Mucolytics</p> <p>N-Acetylcystein,...</p> <p>hydrophilic</p>	<p>Antiphlogistics</p> <p>Curcumin,...</p> <p>hydrophobic</p>
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Lababidi et al, (2019) JCR
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## Particle characterization

Glass transition temperature

Sample Name	Experimental $T_g$	Predicted $T_g$ based
Azi/NAC	$99.0 \pm 0.9$	66
Azithromycin	$108.34 \pm 0.5$	-
N-acetyl-cysteine (NAC)	$6.9 \pm 1.9$	

XRD

DSC

**MPs with an amorphous structure**

Page 11 Lababidi et al, (2019) JCR

## Characterization of SD Microparticles

### Aerodynamic properties

Formulation	AziNAC
MMAD [ $\mu\text{m}$ ]	$2.51 (\pm 0.06)$
GSD	$1.58 (\pm 0.04)$
FPF [%]	$68.83 (\pm 6.11)$

### Viscosity

Plate – cone geometry

### Biological activity

High fine particle fraction

reduced mucus viscosity

reduced biofilm formation

Lababidi et al, (2019) JCR

**Treatment of CF**

Drugs: • Antibiotics  
e.g Ciprofloxacin

Mucolytics  
DNase, N-Acetylcystein,...

Antiphlogistics  
Curcumin,...

hydrophobic

Embed in  
PLGA nanoparticles

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**Treatment of CF**

Drugs: • Antibiotics  
e.g Ciprofloxacin

Mucolytics  
DNase, N-Acetylcystein,...

Antiphlogistics  
Curcumin,...

Nanoparticle formation (µfluidics)

Production parameters to Size

Z-Ave [nm] Encapsulation [%]

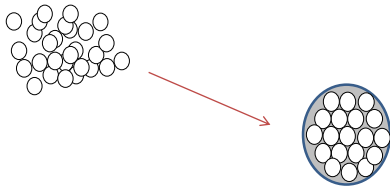
Flow rate (ratio)

Flow rate (ratio)	Z-Ave [nm]	Encapsulation [%]
0.1	~145	~90
Bulk	~135	~25
MF 0,1	~105	~100


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Lababidi et al, Beilstein J Nanotech, accepted

Combining Nano and Micro



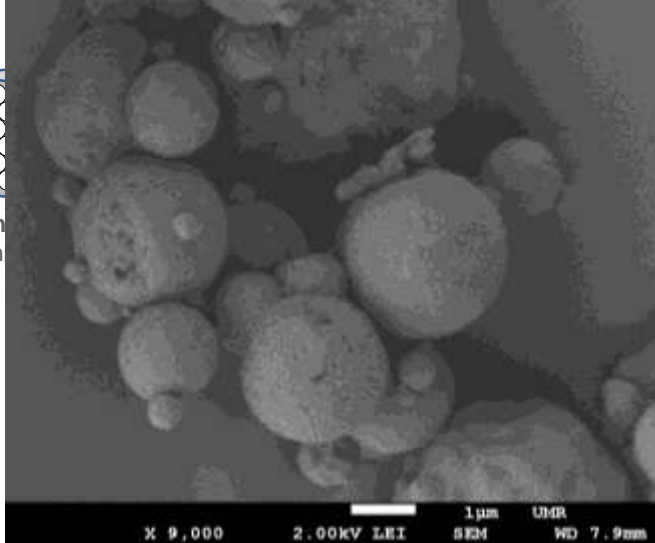
**Spherical particles**  
(Spray drying)




20% NP fraction

Page 15 Torge et al. Europ J Pharm Sci, 2017 104:171, Baghdan et al. Europ J Pharm Sci, 2019, 132:63 24.11.2019

Spray-dried Particles



Cylind  
(Tem



X 9,000 2.00kV LEI SEM WD 7.9mm 1µm UMR

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**What do we need after inhalation?**

Deposition of the inhaled particle

What happens after landing?

Kirch et al. PNAS 2012

**Redispersibility in lung conditions**

Microparticle

Membrane

100 % rel. humidity

Gel

## Treatment of CF

Drugs: • Antibiotics

e.g Azithromycin

hydrophobic

Mucolytics

N-Acetylcystein,...

hydrophilic

Antiphlogistics

Curcumin,...

hydrophobic

Nanoparticle formation (µfluidics)

Formulation	MMAD [µm]	GSD	FPF [%]
Azi/NAC	2.63 (±0.03)	1.53 (±0.06)	67.40 (±9.41)
Azi/NAC/NP	2.51 (± 0.06)	1.58 (±0.04)	68.83 (± 6.11)

Production parameters to Size

Flow rate (ratio)

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Lababidi et al, Beilstein J Nanotech, accepted
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## Functionality of drug-loaded nanoparticles

**A**

Without NAC alone

■ No Curcumin ■ +PLGA-Curcumin-NP 55 µg/ml

**B**

With formulation

■ No Curcumin ■ +PLGA-Curcumin-NP 55 µg/ml


THP1 + PMA 10ng/mL 48 hours → Infection 6 hours

Legend:


- Macrophage-like cells
- Bacteria (PAO1 *P. aeruginosa*) Biofilm
- PLGA-NP
- Drug Curcumin
- Smart Matrix Microparticles

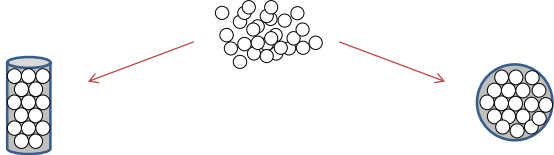
C. Montefusco

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In collaboration with HIPS – Prof. CM Lehr
24.11.2019



## Microparticles for inhalation





**Cylindrical particles**  
(Template technique)

**Spherical particles**  
(Spray drying)

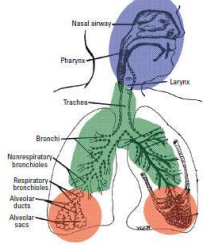
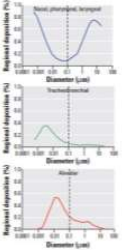
Size influences deposition of drug carrier

Density


**Shape**

$$r_{ae} = r_g \sqrt{\frac{\rho}{\chi}}$$


- Nasal, pharyngeal, laryngeal
- Tracheobronchial
- Alveolar





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## Shape & size influence biodistribution/ uptake





**Why to prepare particles?**

- Can be inhaled (powder inhalator)
- MΦ clearance can be modified (aspect ratio)
- For same diameter higher volume to be loaded

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Production Method

Tem  
Bloc

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Stabilization by LbL technique and harvest

Membrane dissolution


Filtration

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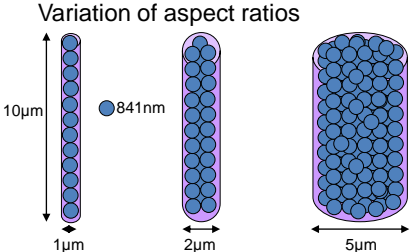
24.11.2019

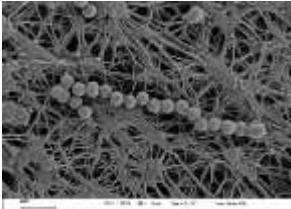


## Size variations of cylinder particles

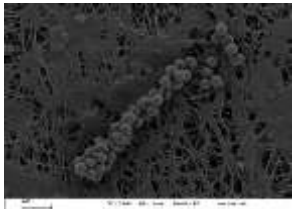


Variation of aspect ratios






SiO<sub>2</sub> (841nm) +3bilayers PAH/PSS  
in 1µm PC-Membrane



SiO<sub>2</sub> (841nm) + 5 and +3bilayers PAH/PSS  
in 2µm PC-Membrane




SiO<sub>2</sub> (841nm) +5 and +3bilayers  
PAH/PSS in 5µm PC-Membrane


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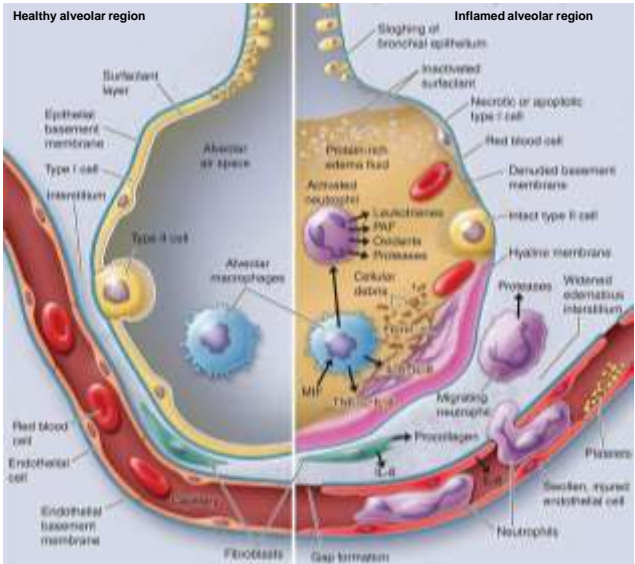
Kohler et al. (2011) Adv Mater

24.11.2019



## Impact of Macrophages









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## Delivery System Requirements





Therapeutic Approach

*Genetic Modulation of Macrophages*





Delivery system – rods with

- PE(+)
  - Polyethylenimine, Chitosan
  - DEAE-Dextran
- PE(-)
  - Dextran sulfate
  - pDNA







Boorsma et al. 2013

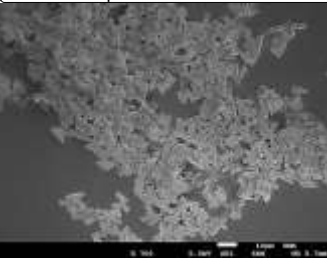
## Microrods as Gene Delivery Platform


pDNA  $\mu$ R Production



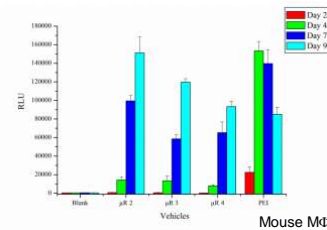
Optimization Yield



Optimization *in vitro* Performance

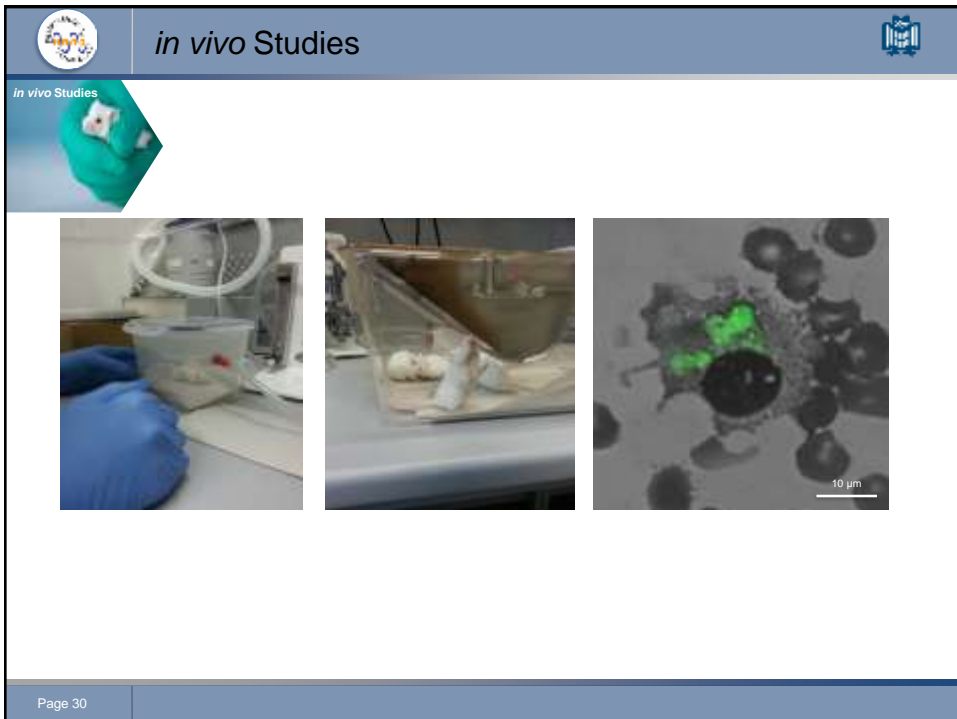
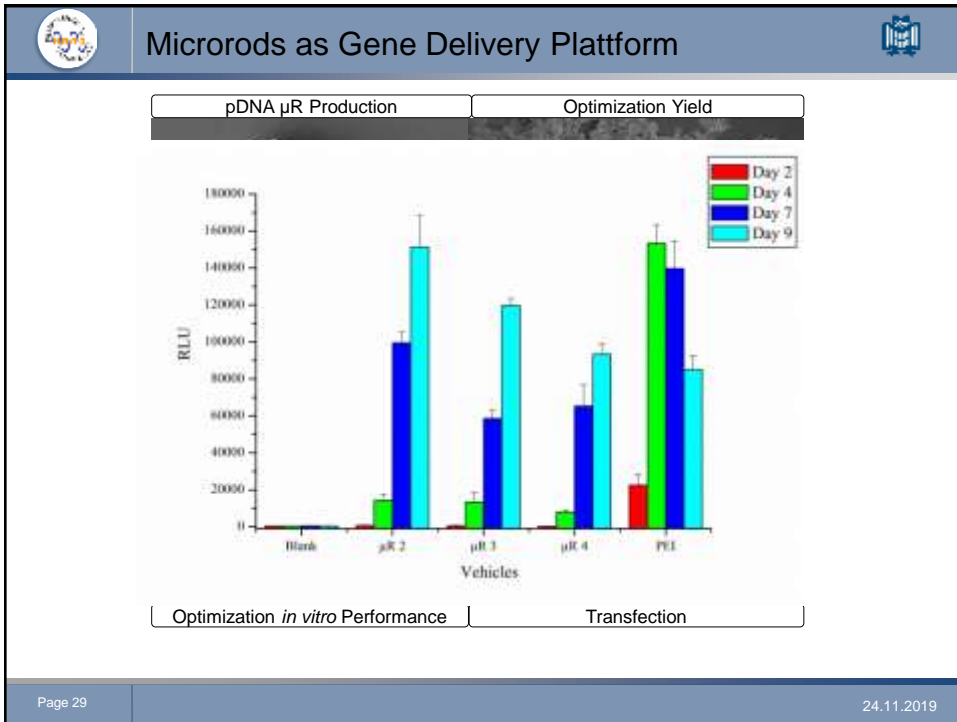


Transfection



Condition	Day 2	Day 4	Day 7	Day 9
Blank	~0	~0	~0	~0
$\mu$ K.2	~10000	~100000	~100000	~150000
$\mu$ K.3	~10000	~100000	~100000	~120000
$\mu$ K.4	~10000	~100000	~100000	~100000
PEI	~10000	~100000	~100000	~100000

Mouse M $\phi$



**in vivo Studies – Histology**

Day 1 Day 9

pulmonary vessels  
 pulmonary arterioles  
 alveolus  
 capillary network

*Park et al. 2012*

Page 31 Möhwald et al. (2017) Adv Healthcare Mater

**in vivo Studies – Luciferase Assay**

**Luciferase Assay MH-S**


Group	Day 2	Day 4	Day 7	Day 9
Sham	~0	~0	~0	~0
pH 1	~10000	~20000	~30000	~40000
Vehicle	~10000	~20000	~30000	~40000
pH 4	~10000	~20000	~30000	~40000
pH 7	~10000	~20000	~30000	~40000

**Luciferase Assay BALB/c**


Day	Luciferase Expression (RLU/mg Protein)
Placebo	~0
4	~1000
7	~12000
9	~58000

Page 32 Möhwald et al. (2017) Adv Healthcare Mater

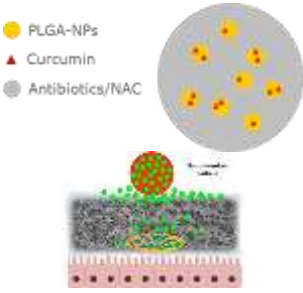






## Summary




- Preparation of multifunctional particles
  - Inhalable particle
  - Fully active MP matrix
  - Incorporation of drug loaded nanoparticles
  
- Preparation of rod-like particles
- Loading with genetic material
- Transfection of macrophages *in vitro* & *in vivo*





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## Acknowledgements





**Saarland University**

Dr. D. Kohler  
Dr. A. Torge  
Dr. M. Möhwald  
M. Pourasghar  
T. Fischer  
Dr. N. Lababidi



**HIPS**

Prof. Dr. C.-M. Lehr  
Dr. N. Schneider-Daum  
Dr. C. Wodarz-Carvalho  
C. Montefusco




**UKS**

Prof. Dr. Thomas Tschernig  
B Wonneberg




**Philipps-Universität**

Prof. Dr. U Bakowsky  
Dr. S. Pinapireddy  
Dr. C. Tscheka




**Boehringer Ingelheim**


Dr. M. Krüger †  
Dr. M. Wolkenhauer



**UNIVERSITÄT DES SAARLANDES**  
**BERGISCHE UNIVERSITÄT WUPPERTAL**

Prof. Dr. Martin Simon  
F. Drews








Thank you  
for your attention