A mucus model to evaluate the diffusion of drugs for more efficient cystic fibrosis therapies

A MUCUS MODEL TO EVALUATE THE DIFFUSION OF DRUGS FOR MORE EFFICIENT CYSTIC FIBROSIS THERAPIES

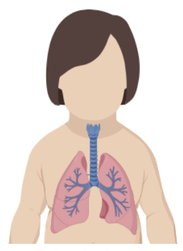
Pacheco DP[1], Butnarasu C[5], Bertoglio F[2,3,4], Suarez Vargas N[1], Briatico-Vangosa F[1], van Uden S[1], Visai L[3], Petrini P[1], Vallaro M[5], Caron G[5], **Visentin S[5]**

1 Department of Chemistry, Materials and Chemical Engineering "Giulio Natta" at Politecnico di Milano, Italy; 2 Molecular Medicine Department (DMM), Center for Health Technologies (CHT), UdR INSTM, University of Pavia, Italy; 3 Department of Occupational Medicine, Toxicology and Environmental Risks, Istituti Clinici Scientifici Maugeri S.p.A., IRCCS, Italy; 4 School of Advanced Studies IUSS Pavia, Italy; 5 Molecular Biotechnology and Health Sciences Department, University of Torino, Italy

18th PhysChem FORUM , Frankfurt , May 2019

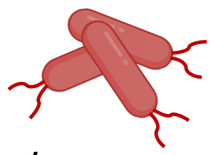
Introduction

Cystic fibrosis (CF) mucus exhibits altered chemical and viscoelastic features, limiting its clearance and leading to chronic bacterial infections. Current bacterial culture fails to recreate bacteria communities and microenvironments of lung microbiota. Additionally, it is difficult to induce representative human multi-bacterial infections in animals.



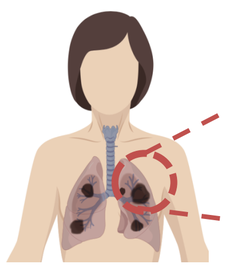
CF Children
Functional Lungs

Staphylococcus aureus

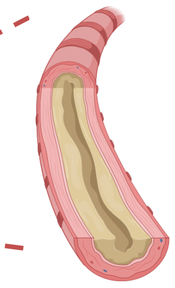


Pseudomonas aeruginosa

Consecutive infections exacerbate disease progression



CF Adults
Lung Failure leads to patient death

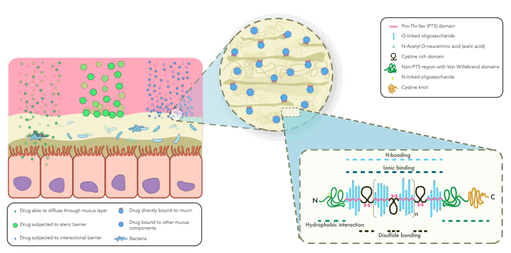


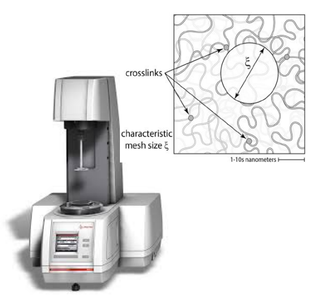
Mucus presents **altered chemical and viscoelastic properties**

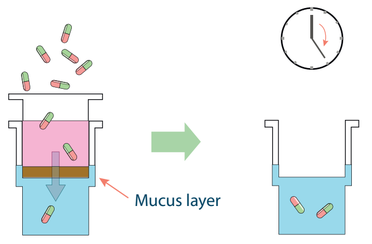
Important
Intestine
Eyes
Vagina

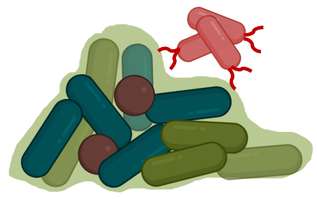
AIM Engineering a **mucus model of pathological CF mucus (Bac³Gel)** able to **model chemical, structural gradient** and **viscoelastic properties, while supporting CF lung microbiota** to be applied as a screening **platform for antimicrobial studies in the pharmaceutical field**

Materials & Methods

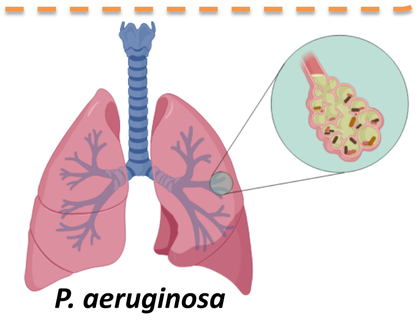








P. aeruginosa colonization



P. aeruginosa Infections

1 — Interaction of CF drugs with mucin

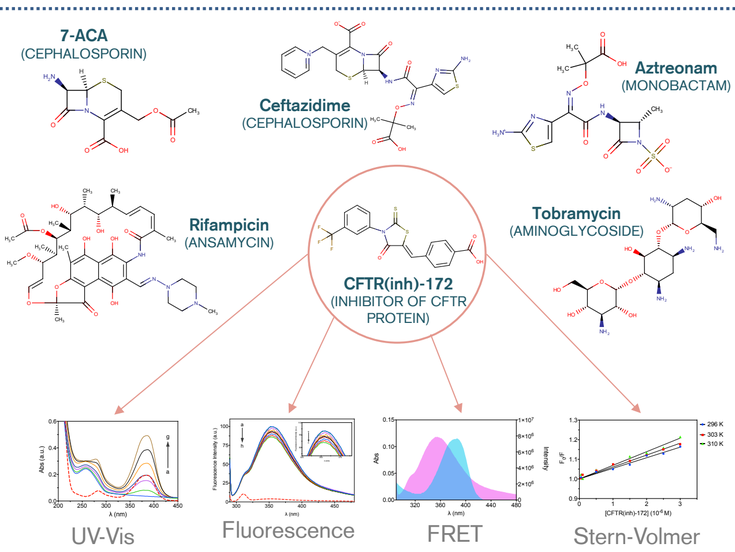
2 — Preparation and rheological analysis of **the 3D mucus model**

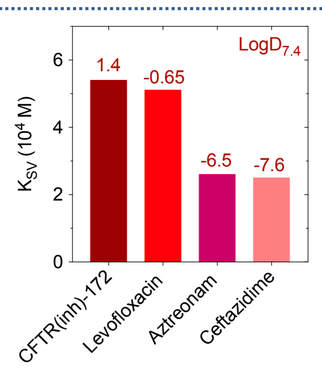
3 — PAMPA/cells & mucus model **Drug diffusion**

4 — Confocal microscopy to determine **bacterial migration**

5 — **Antimicrobial Treatment**

1. Interaction with mucin





However, the study of the only mucin-drug interaction is incomplete.

3D model for HTS is needed

Fig1. Selected drugs and mucin interaction

3. PAMPA & mucus model

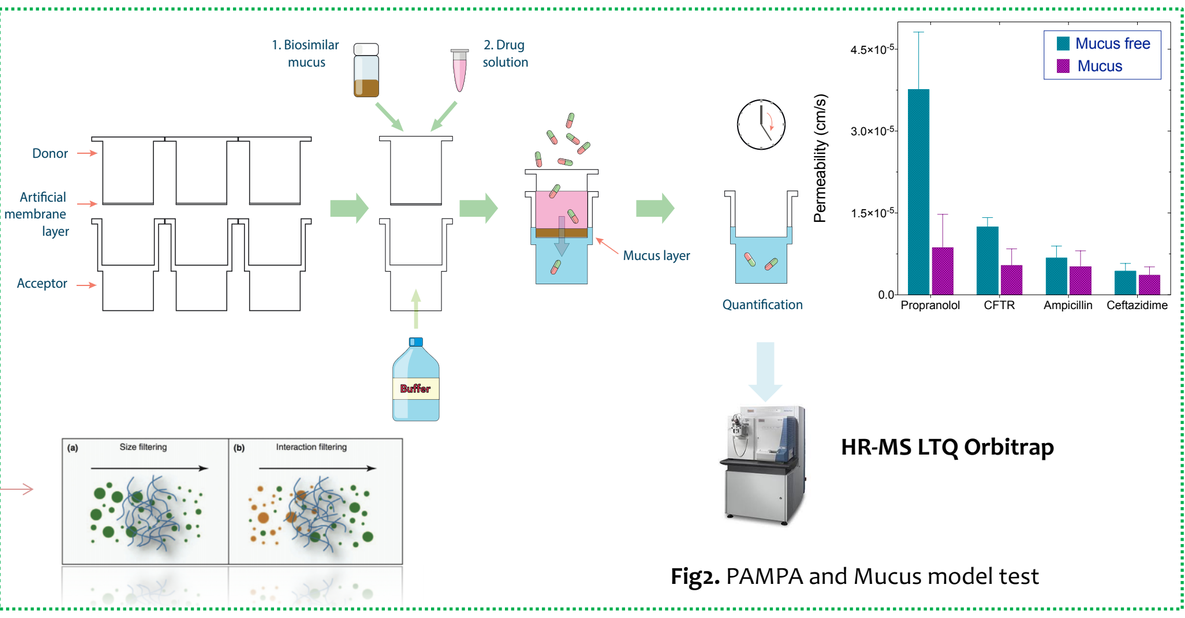


Fig2. PAMPA and Mucus model test

2. A 3D Mucus model: Bac³Gel

Alginate-based mucus model (Patent Pending)

2018 SWITCH 2 PRODUCT INNOVATION CHALLENGE

Bac³Gel

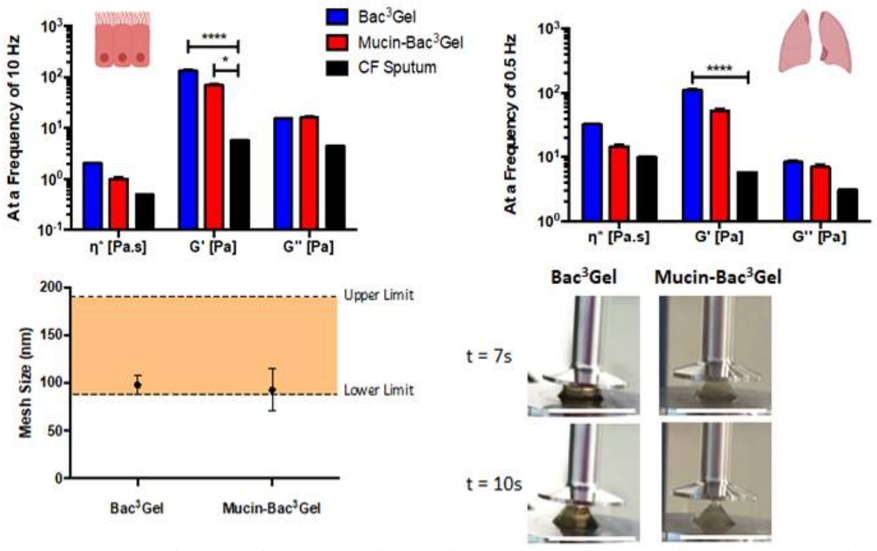


Fig. 3 Viscoelastic properties of both Bac³Gel and Mucin-Bac³Gel in comparison to those reported for pathological CF mucus[1]. **Fig. 4** Estimated mesh size of both Bac³Gel and Mucin- Bac³Gel in comparison to that reported for pathological CF mucus[2]. The developed mucus models appear to display mucus-*like* behaviour. The scale bar corresponds to 25 mm

4. Recreating Lung Microbiota of CF Patients

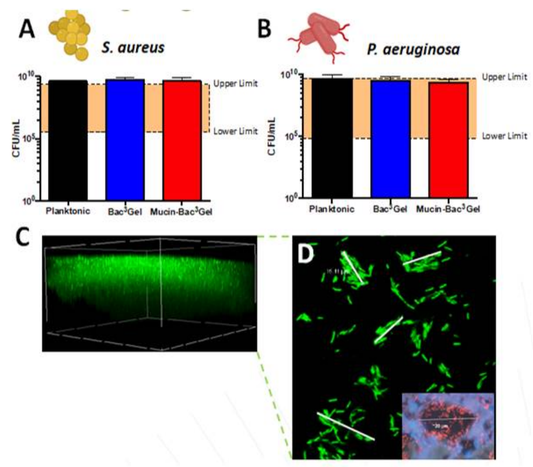


Fig. 5 CFU count after infecting both Bac³Gel and Mucin-Bac³Gel with 1000: (A) *S. aureus*; and (B) *P. aeruginosa* for 24 hours depicting physiological ranges[3]. (C) Confocal laser scanning microscope images of Bac³Gel colonized by *P. aeruginosa*, with a close-up view of bacterial aggregates resembling those (D) observed in CF sputum[4].

5. Antimicrobial Treatment of *P. aeruginosa* infections

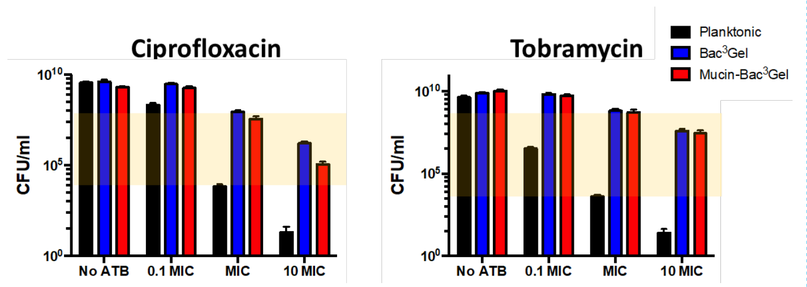


Fig. 6 Antimicrobial treatment of *P. aeruginosa infections:* (A) Ciprofloxacin and (B) Tobramycin. The Miniminal Inibitory Concentration (MIC) of Colony Forming bacteria was determined following the EUCAST guidelines. Afterwards, both infected Bac³Gel and Mucin-Bac³Gel were treated with three different concentrations: 0.1, 1 and 10 MIC.
The mismatch determined between planktonic cultures and both infected Bac³Gel and Mucin-Bac³Gel was three-orders of magnitude higher, confirming what is extensively stressed by clinics[5].

References

1.Butnarasu *et al.*doi.org/10.1016/j.ijpharm.2019.04.0322; 2. Yuan S, *et al.* DOI: 10.1126/scitranslmed.3010525; 2. Suk JS, *et al.* DOI: 10.1016/j.biomaterials.2008.12.076; 3. Wong K, *et al.* DOI: 10.1099/00222615-17-2-113; 4. Bjarnsholt T, *et al.* DOI: 10.1016/j.tim.2013.06.002; 5. Macià MD, et al. DOI: 10.1111/1469-0691.12651

sonja.visentin@unito.it