DRUGS AND MUCUS
UNDERSTANDING THE WAY OF INTERACTION

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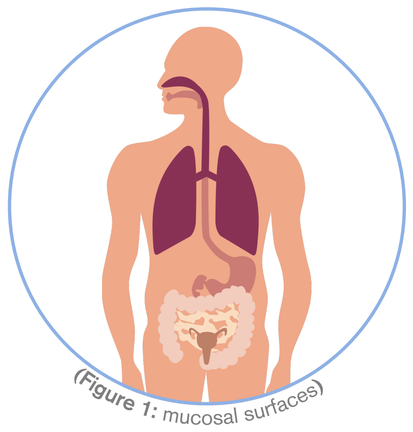
INTRODUCTION

All mucosal surfaces of the human body are covered with a thin **mucus** layer (Figure 1). From a structural and compositional point of view, mucus is a semi-permeable hydrogel with heterogeneous composition, and its primary function is to protect the underlying epithelium from environmental noxious agents such as air pollutants or bacteria. The barrier properties of mucus are principally governed by mucins, which are the mainly expressed glycoproteins within mucus [1].
However, mucus constitutes an important barrier also to drug absorption especially in those diseases characterized by mucus hypersecretion with altered chemical and structural features such as bronchial asthma, chronic obstructive pulmonary disease (COPD) and **cystic fibrosis** (CF).



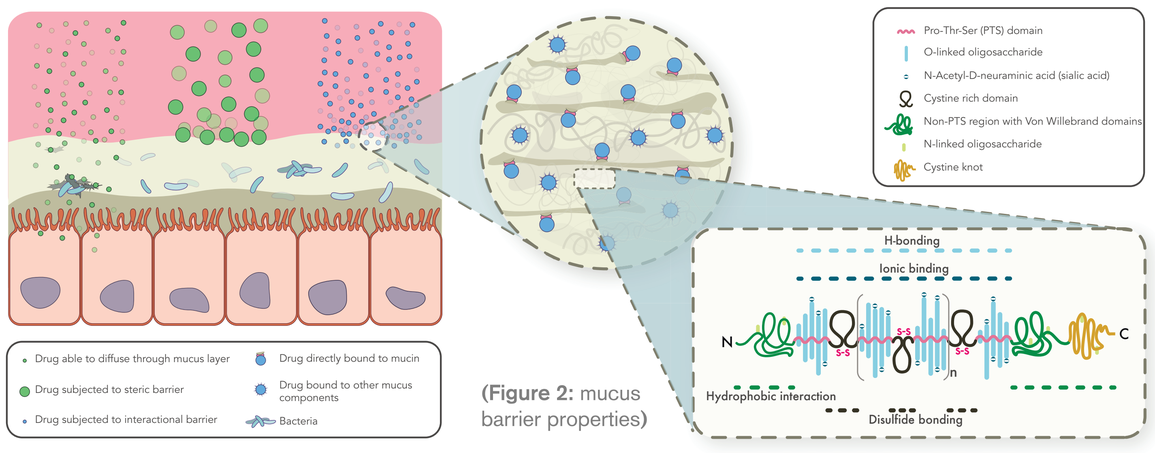
(Figure 1: mucosal surfaces)

CF is a chronical life-limiting autosomal recessive pathological condition caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene which encode for a cAMP-regulated epithelial chloride channel [2-3]. Most of the health problems (pulmonary and gastrointestinal) experienced by CF patients arise from the overproduction of the thick mucus. Even if there is no cure for CF, in the last 4 decades life expectancy of cystic fibrosis patients has improved significantly. The principal treatments involve mucolytics, bronchodilators, anti-inflammatory agents and **inhaled antibiotics**. Actually, only few mutation-specific drugs (ivacaftor, lumacaftor, tezacaftor) can treat the disease by acting on the underlying defect.

AIMS

The orally taken systemic drugs must pass through the gastrointestinal mucus barrier, whereas inhaled drugs must pass through airway mucus and their pulmonary deposition to reach their targets. Due to the wide variety of functional groups present into the mucin structure (Figure 2), many interactions can be established with molecules of hydrophilic as well as hydrophobic nature [4] and association (K_A) and dissociation (K_D) can be obtained.

The design of effective cystic fibrosis drugs must take into account the interaction of the potential candidates with mucin, and the diffusion across the mucus layer. The need to characterize drug behavior in a rapid, simple and reproducible manner has urged the development of airway **mucus models**. In this work, we investigate the affinity of some drugs to mucin and an airway mucus model composed by alginate and mucin, which aim to model both composition and rheological properties of the pathologic CF-mucus, is developed.



(Figure 2: mucus barrier properties)

MUCIN-ANTIMICROBIC DRUGS INTERACTION

Retention in the mucus layer may be strongly influenced by the interaction with mucus components, especially with mucin. Mucin-drug interaction may play an important role on the drug pharmacokinetics as a strong bind with the protein may results in a reduced drug absorption. The extent of interaction between the dataset drugs with a mucin solution was measured using **UV-VIS** and **fluorescence** spectroscopy. Spectra of mucin upon increasing concentration of drugs were recorded. Quenching mechanism, binding constants, number of binding sites, thermodynamic parameters and binding distance of the interaction were obtained (Figure 3).

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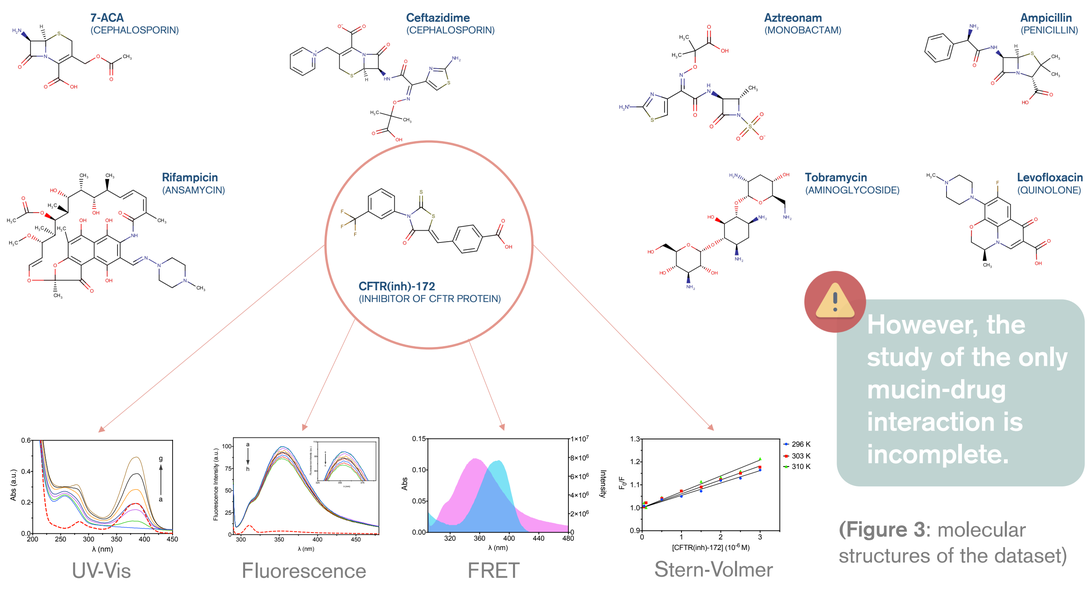


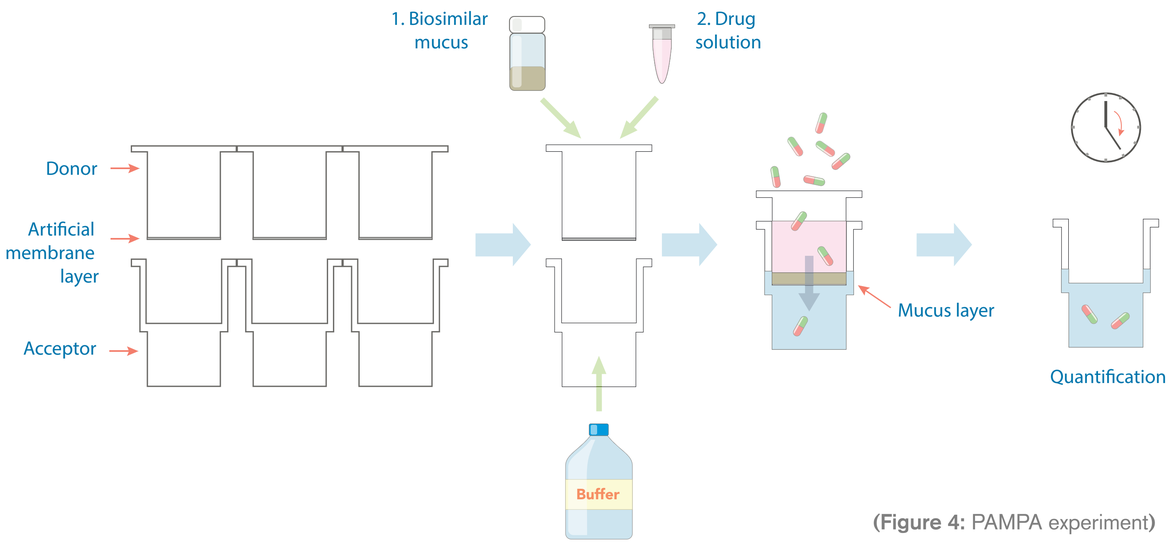
(Figure 3: molecular structures of the dataset)

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

MUCUS MODEL AND PAMPA TEST

Alginate/mucin hydrogels were developed taking advantage of the internal crosslinking mechanism of alginate in the presence of Ca^{2+}. Rheological parameters such as the elastic or *storage modulus* (G') and the viscous or *loss modulus* (G") of the biosimilar mucus were studied in order to obtain values as similar as possible to the values of the pathological mucus.

The biosimilar mucus model is a fast and economic tool suitable for high throughput screening purposes indeed, it can be employed on **parallel artificial membrane permeability assays** (PAMPA) in order to evaluate the diffusion of drugs (Figure 4).



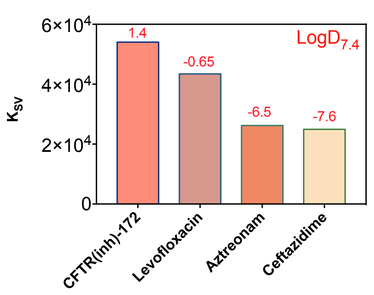
(Figure 4: PAMPA experiment)

RESULTS

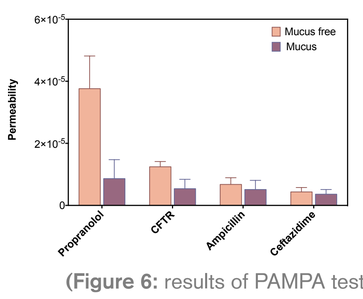
Fluorescence quenching data indicate that ceftazidime, aztreonam, CFTR(inh)-172 and levofloxacin binds mucin, whereas no interaction is observed for 7-ACA, ampicillin, tobramycin and rifampicin. The increasing quenching upon increasing temperature for ceftazidime and CFTR(inh)-172 indicate a dynamic quenching process. The K_A and n values indicate that a weak mucin-drug bind is established and there is only one principal site of binding. Contrary to what was expected, the charge of the molecule seems to play not such a fundamental role upon interaction with mucin, as positively charged molecules at pH 7.4, such as tobramycin, have no interaction, whereas negatively charged drugs such as CFTR(inh)-172 or aztreonam can interact. It seems that an undefined relation exists between drug lipophilicity and interaction with mucin, particularly, hydrophobic drugs tend to interact more with mucin (Figure 5: the greater the $logD_{7.4}$ is, the greater the K_{SV}). The permeability of some drugs was measured in presence and in absence of mucus. Compared to a highly permeable compound (propranolol) the drugs we tested are low permeable already in absence of mucus and consequently the permeability is less influenced by mucus if compared to propranolol (Figure 6).

	n	K_A (M^{-1}) 10^3	K_D (M) 10^{-4}
Ceftazidime			
296 K	0.74 (± 0.040)	1.1 (± 0.081)	9.14
303 K	0.73 (±0.064)	1.3 (± 0.15)	7.62
310 K	0.78 (± 0.039)	4.6 (± 0.29)	2.16
Aztreonam			
296 K	0.64 (± 0.035)	0.32 (± 0.026)	32
303 K	0.63 (± 0.064)	0.26 (± 0.038)	39
310 K	0.64 (± 0.11)	0.30 (± 0.076)	34
CFTR(inh)-172			
296 K	0.72 (± 0.049)	1.3 (± 0.12)	7.8
303 K	0.81 (± 0.032)	5.0 (± 0.25)	2.0
310 K	0.93 (± 0.045)	24 (± 1.5)	0.41
Levofloxacin			
296 K	0.75 (± 0.049)	1.4 (± 0.14)	6.9
303 K	0.90 (± 0.098)	9.4 (± 1.4)	1.1
310 K	0.72 (± 0,0094)	0.96 (± 0.019)	10

(Table 1: number of binding sites *(n)*, association *(K_A)* and dissociation *(K_D)* constants**)**



(Figure 5: values of K_{SV} and $logD_{7.4}$**)**



(Figure 6: results of PAMPA test)

CONCLUSIONS

Even though some of the antibiotics herein investigated (ceftazidime, aztreonam, levofloxacin) can interact with mucin, the order of magnitude of K_A is quite low (Table 1) while tobramycin showed no affinity to mucin. All these data could in part explain why these antimicrobial drugs are the most employed antibiotics in CF. The low affinity of the tested drugs could be in part explained by their high hydrophilicity. However, in order to obtain a relation between the molecular structure and the retention in the mucus blanket a broader database should be investigated.

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20-22 February 2019

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