Airway mucus microenvironment modelling to be applied on cystic fibrosis drug discovery

Petrini P[a], Pacheco DP[a], Pontremoli C. 3[b], Caron G.[c], Visentin S.[c]

[a] Dipartimento di Chimica, Materiali e Ingegneria Chimica, Politecnico di MilanoMilano, 20133, Italy
[b] Dipartimento di Scienza Applicata e Tecnologia (DISAT), Politecnico di Torino, Torino, 10129, Italy
[c] Department of Molecular Biotechnology and Health Science, Torino, 10125, Italy
e-mail: sonja.visentin@unito.it

Keywords: (mucus , drugs , cystic fibrosis, mucin)

Introduction

Cystic fibrosis (CF) is the most common life-threatening autosomal recessive genetic disease in pediatric age. CF is caused by mutations in the transmembrane conductance regulator (CFTR) gene encoding for an ion channel located on the apical membrane of lung epithelial cells.

Most of the health problems experienced by CF patients arise from the overproduction of the thick mucus (mucus is composed of water, ions, lipids, and approximately 2% of proteins: the protein mainly expressed is mucin, a high molecular O-glycosylated protein). These thick, sticky secretions plug up the ducts (small tubes) that should carry the secretions either outside of the body or into a hollow organ such as the lungs or the intestines. This can affect vital body functions such as breathing or digestion. There is no cure for CF, but pharmaceutical developments envisioned to tackle mucus accumulation/obstruction and CFTR-targeted therapies have improved average life expectancy of CF patients. Over the past decade, advanced technologies have enabled high throughput screening (HTS) approaches to drug discovery that yielded orally bioavailable small molecule compounds capable of targeting the underlying defect. CFTR modulators are designed to treat the underlying cause of cystic fibrosis by targeting the CFTR protein defect. Small molecule pharmacologic agents that target defects in CFTR gating, processing, and synthesis have undergone rigorous preclinical and clinical evaluation over the past decade and include CFTR potentiators (e.g. VX-770 also known as ivacaftor), correctors (e.g.VX-809 also known as lumacaftor), and translational read-through agents (aminoglycoside antibiotics e.g. gentamicin, tobramycin).

Although the encouraging results of pharmaceutical companies, mucus overproduction remains the major factor determining drug efficacy in CF patients. In fact, 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.

The need to characterize drug behavior in a rapid, simple and reproducible manner has urged the development of airway mucus models. In this work, an airway mucus model composed by alginate and mucin is herein proposed aiming to model both composition and rheological properties of the pathologic CF-mucus.

Material and Methods

Alginate (alginic acid sodium salt, from brown algae)/mucin (from porcine stomach, type III) hydrogels were developed taking advantage of the internal crosslinking mechanism of alginate, in the presence of NaCl (final concentration 7 mM). Rheological measurements were carried out to access the viscoelastic and shear thinning behavior of the developed gels and further compared to the pathological CF-mucus. Stability analysis was also conducted to acquire using both water and PBS, at 25 ºC, to analyze changes on weight percentage and volumetric increase. Finally, both drug diffusion and interaction through alginate and alginate/mucin gels were carried out using aspirin, cephalexin and epirubicin, as well as gold nanoparticles (GNP) as model drugs.

Results and Discussion

Hydrogels composed by alginate and mucin were developed. As observed for CF mucus (1-3), the viscosity of the mucus model decreases with the increasing of shear stress, with no differences observed between both mucus model and CF mucus at both breathing and ciliary beating frequencies. Additionally, no differences on the dissipative modulus were detected between the CF and model mucus, although small differences were detected over storage modulus (1-3). Stability analysis in both water and PBS, at 25°C, revealed an increased weight and size mainly in the early hours. Diffusion studies of drugs and gold nanoparticles through the gels exhibited compositional and structural dependency, thus effecting the interaction with mucin (4,5). The diffusion of drugs was also related to both alginate-drug interactions or steric barrier effect of the gel. Likewise, the diffusion of GNP was hindered by alginate-mucin gels compared to alginate gel, probably related to mesh size of the model.

Conclusion

A mucus model was proposed to study drug permeability in presence of mucus secretion. This platform will serve as the basis to implement the complexity of the model in terms of components, also including the effect of bacteria.

References

1. M. Robinson et al. J. Aerosol Med Pulm Drug Deliv. (2016), 337-345.
2. Y. Shaopeng et al. Sci Transl Med. (2015),1–19.
3. S.K. Lai et al. J.Adv Drug Deliv Rev. (2009), 86-100.
4. G. Caron et al. Int J Pharm. (2015), 67-69.
5. C. Pontremoli et al. Bioorg Med Chem. (2015),6581-6586.

.

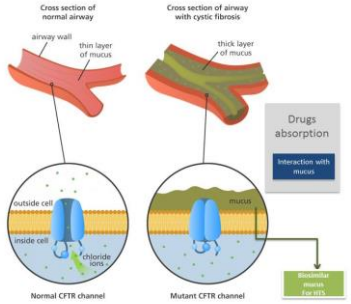

Figure 1 The overproduction of thick, sticky mucus is responsible for the efficacy of drugs used in the treatment of patients affected by CF