# Hydrogel-based platforms to mimic *in vivo* drug diffusion: A multicenter research

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## Abstract

An airway mucus model is proposed thus serving as an *in vitro* screening tool with the aim to reduce the number of non-effective drugs reaching the preclinical trials. The engineered mucus model is an easy-to-use and easy-to-produce tool that can be easily coupled to state-of-art diffusion models and it is compatible with high throughput analysis. This platform will serve as the basis to implement the complexity of the model in terms of components, also including the effect of bacteria.

## Introduction

Mucus is a natural barrier of all organs exhibiting an air-liquid interface, which selectively filters the passage of gases, pathogens, pollutants, and nutrients, but also a steric and interactive barrier for drug permeability.[1,2] Mucus also hosts particular microbial niches that co-exist in symbiosis or dysbiosis within the human body, yet the consequences of the latter are only now being significantly addressed mainly in animal testing and from the clinical point of view. In diseases like cystic fibrosis (CF), mucus exhibits altered physicochemical properties, which hamper clearance mechanisms and limits drug diffusion. The inefficient mucus secretion enables the establishment of bacterial colonies that trigger chronic infection, and lately lead to lung failure. New strategies are developed to overcome mucus barrier.[3,4] The need to characterize drug permeability in a rapid, simple and reproducible manner has urged the development of mucus models.[5] As the full reproduction of the complexity of the mucus barrier is an ambitious task, an airway mucus model is herein proposed to disassemble the complexity of the mucus barrier following a modular approach.

## Materials 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 behaviour 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 analyse changes in 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

The steric retention of CF mucus was reproduced by targeting its mesh size (approximately 50 nm) and rheological properties, while the interactive barrier was reproduced by adopting a composition inspired by the CF mucus. The mucus models, mainly composed of mucin in an alginate (Alg) hydrogels, were developed aiming at specifically modelling the chemical-physical properties of CF mucus. A combined set of experimental and mathematical techniques was developed and applied to target similar viscoelastic properties as those reported for CF sputum at both breathing and mucociliary beating frequencies. Optimized mucus models, composed of 3 mg/mL Alg and 25 mg/mL mucin, exhibited a G' increasing from ~ 21.2 to 55.2 Pa and G" ranging from ~ 5.26 to 28.8 Pa in the frequency range of 0.1 to 20 Hz. Stability analysis in water, PBS, and DMSO, at 25°C, revealed an increased weight and thickness mainly in the early hours. Diffusion studies of three model drugs through the mucus models unveil the ability of the mucus model to discriminate their mucin-drug interaction and the steric barrier of a mucus layer with respect to PAMPA that models the phospholipidic cell membrane, the state-of-art screening tool for passive drug diffusion.

## Conclusions

The mucus model is proposed as an *in vitro* tool for early drug discovery, representing a step forward on modelling the mucus layer that is often not considered when assessing drug permeability. The engineered mucus model is an easy-to-use and easy-to-produce tool that can be easily coupled to state-of-art diffusion models (*e.g.* PAMPA membranes) and it is compatible with high throughput analysis. Finally, the production method allows to easily incorporating, in a modular approach, other mucus components (*e.g.* albumin, phospholipids, among others) to further recreate the chemical composition of mucus, while allowing to distinguish the contribution of each component over drug permeability. This platform will serve as the basis to implement the complexity of the model in terms of components, also including the effect of bacteria.

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Key words: *In vitro* models; diffusion profiles; hydrogels; mucins; permeability.

Conference presentation: this paper was presented at the Second Centro 3R Annual Meeting - 3Rs in Italian Universities, 2019, June 20-21, University of Genoa, Italy.

Received for publication: 28 October 2019.
Accepted for publication: 6 November 2019.


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*Biomedical Science and Engineering 2019; 3(s2):101*
*doi:10.4081/bse.2019.101*

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