Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supplementary Information

Table S1 – Available *in vitro* cell-free models found in the literature.

Mucus Model	Model Chemical Composition	Model Rheological Properties	Limitations
Sputum of Patients	+	+	Poor reproducibility due to subject-to-subject variability.
Mucin-based solutions			
20, 30, or 50 mg/mL pig gastric mucins with sodium phosphate and sodium carbonate buffer [20]	+	-	This model does not consider the steric barrier.
5 mg/ml pig gastric mucin, 4 mg/ml DNA, 5.9 µL/ml diethylene triamine pentaacetic acid (DTPA), 5 mg/ml NaCl, 2.2 mg/ml KCl, 5 µL/ml egg yolk emulsion *	+/-	-	These models include exogenous compounds, as DTPA or Tween 80 that may interact with drugs. Do not model the steric barrier.
4 mg/ml pig gastric mucin, 30 mg/ml lipid mixture, 31 mg/ml pig serum albumin, 5 mg/ml DNA, 0.75 %(v/v) Tween 80 [22]	+/-	-	
3D Mucin-based structures			
Purified pig gastric mucus, 0.92 mg/ml calf thymus DNA, ~80 mg/ mL bovine serum albumin (BSA) [9]	+/-	+/-	The viscoelastic properties and composition were different from those of CF sputum. The model requires that the end-user performs mucus purification of pig gastric mucus, which entails complex steps and may lead to poor reproducibility.
3 – 9 mg/ml PAA, 50 mg/ml pig gastric mucin, 31 mg/ml BSA, 6 – 30 mg/ml lipid mixture, polysorbate 80, 1.3 mM $CaCl_2$, 1.0 mM $MgSO_4$ and 137 mM NaCl [23]	-	+	These models include exogenous compounds, as PAA, polysorbate 80, locust bean gum, sodium tetraborate or HEMA that may interact with drugs.
5 – 17 mg/ml locust bean gum and 0.1 M sodium tetraborate [25]	-	+	
Gels made of N-acryloyl-D-glucosamine (AGA) to mimic the neutral sugars of mucins copolymerised with 2-hydroxyethylmethacrylate (HEMA) in a proportion of either 20:80 or 30:70 %(v/v)	-	Not known	
Proposed Mucus Model	**Model Chemical Composition**	**Model Rheological Properties**	**Advantages**
25 mg/ml pig gastric mucin, 3 mg/ml alginate, 7.07 mg/ml, 1 mg/ml Ca^{2+} ions	+	+	The proposed model only includes components present in CF mucus, while modelling its rheological properties.

			The model relies on commercially available products offering interlaboratory reproducibility. Does not require specific or complicated experimental set-ups.

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Supplementary Information

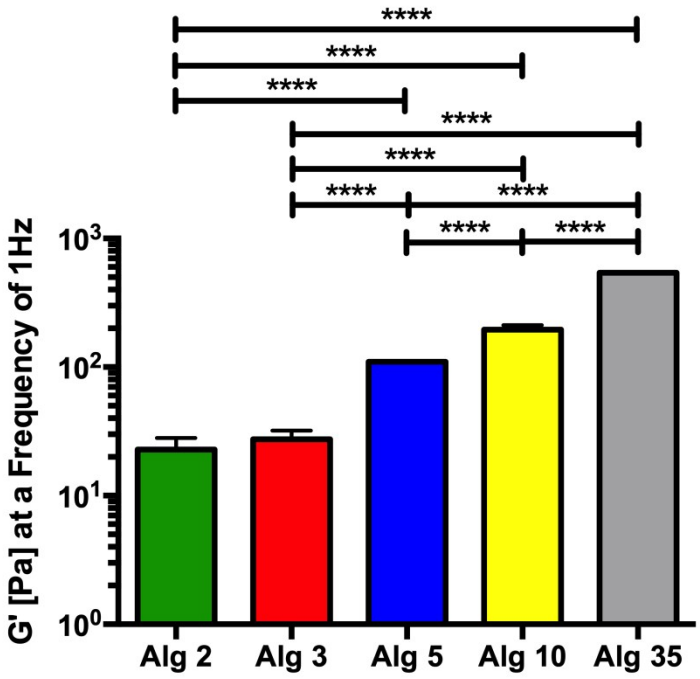

Fig. S2 Storage modulus hydrogels made of different concentrations of Alg, including 2, 3, 5, 10 and 35 mg/ml at a frequency of 1 Hz. Significant differences were set for *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001.

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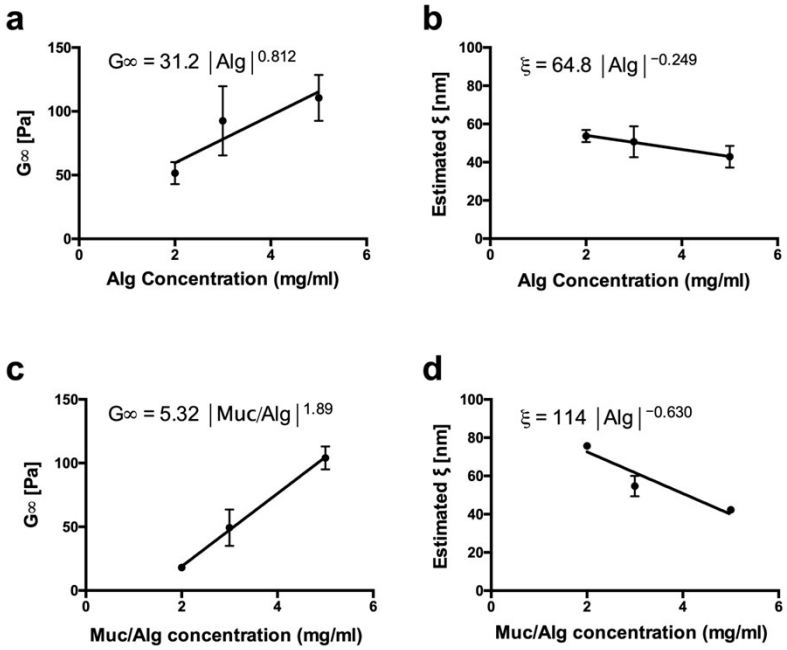

Fig. S3 Mesh size (ξ) and Shear Modulus (G) after fitting the GMM on the obtained rheological data of the developed mucus models: (A) and (B) Alg-based hydrogels; (C) and (D) Mucin/Alg-based hydrogels.

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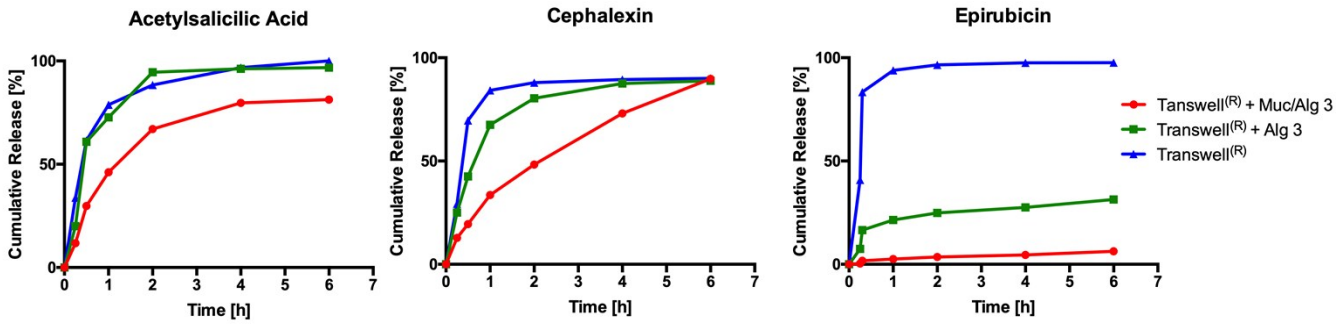

Fig. S4 Drug diffusion tests through the developed airway mucus model. Cumulative release of acetylsalicylic acid, cephalexin and epirubicin through the empty Transwell® supports (blue line) and in the presence of either Alg 3 (green line) or Muc/Alg 3 hydrogels (red line). Acetylsalicylic acid diffusion through the Transwell® supports was performed using 50:50 %(v/v) ethanol/dH_2O to avoid interactions with the Transwell® supports.