



**Thesis project:**

Mucosal rheology in the airways  
of patients with severe lung disease

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**MAIN RECIPES FOR PULMONARY RECONSTRUCTED MUCOSA WITHOUT BACTERIAL CULTURE DEVELOPMENT:**

This appendix, which is referred to in Chapter n. 3, aims to identify based on different publications, the main elements and their concentrations for reconstructing the lung mucosa of healthy and/or respiratory ill subjects (without bacterial culture development).

Articles	Year	Sample	Adopted procedure	Composition	Anal.	Rheological behaviours
28	2011	Mucin Alginate Gels	Mucins were purified from a pool of pig gastric mucus (containing both surface epithelium and gland gene products, which are homologous to the human MUC5AC and MUC6 gene products) using a purification procedure that avoids the use of denaturing agents and preserves rheological properties. Alginate (MW 350 000, F(G) 0.45) was donated by FMC Biopolymer AS, Drammen, Norway. High molecular weight DNA was isolated from salmon spermatozoa according to the method of Johansen and Valla. Mixed biopolymer systems were prepared in 50 mM NaCl by gently mixing the solutions of the individual biopolymers to avoid the formation of bubbles.	H <sub>2</sub> O: 1 mg Mucins: 18 mg/mL Alginate: 0-1 mg/mL	θ	G': 0,94 Pa G'': 0.5 Pa tan δ: 0.47 G <sub>c</sub> *: 1 Pa
		Mucin DNA Gels		H <sub>2</sub> O: 1 mg Mucins: 18 mg/mL DNA: 0.6 mg/mL		G': 2 Pa G'': 0.53 Pa tan δ: 0.27 G*: 4 Pa
		Mucins		H <sub>2</sub> O: 1 mg Mucins: 18 mg/mL		G': 2.82 Pa G'': 0.5 Pa tan δ: 0.18 G*: 8 Pa
30	2019	Pulmonary mucus Surrogate	Briefly, porcine stomach mucin (type II mucin from porcine stomach, Sigma), deoxyribonucleic acid (low-molecular-weight from salmon sperm, Sigma), diethylenetriaminepentaacetic acid (Sigma-Aldrich) stock solution (DIETHYsol <sub>0.15M</sub> ), NaCl (Sigma), KCl (Sigma), and of Trizma base (Sigma) were added to a volume of of ultrapure water under constant stirring. The solution was stirred for several hours at 800 rpm to dissolve the mucins completely. To increase the viscoelastic properties of mucus, it has added poly(acrylic)acid (PAA) (Carbopol 974P NF) and stirred for several hours until PAA was completely dissolved. The pH was adjusted to 7.0 and experiments performed immediately. After adding of casein hydrolysate (Sigma-Aldrich), the pH was adjusted to 7.0 by adding Trizma base. Finally, egg yolk emulsion (Oxoid) was added to the solution and the final volume made up to 10 mL. Experiments were performed immediately after preparation.	H <sub>2</sub> O: 8 mL Mucins: 50 mg DNA: 50 mg DIETHYsol <sub>0.15M</sub> : 100 µL NaCl: 50 mg KCl: 22mg Trizma base: 18.1 mg Casein Hydrolysed: 50 mg Trizma base: 1,82 mg Egg yolk emulsion: 50 µL PAA: 90mg	ζ	G': 19 Pa G'': 2.4 Pa tan δ: 0.12 G*: 19 Pa

29	2020	Artificial sputum medium (ASM)	It has been mixed mucin from pig stomach mucosa (NBS Biologicals), DNA (Fluka), diethylene triamine pentaacetic acid (DTPA) (Sigma), NaCl, KCl, egg yolk emulsion (phosphatidylcholine as source of lecithin) (Oxoid) and amino acids per L of water (pH 7.0). The same medium without amino acids was designated ASM. For a standard experiment, an inoculum equivalent to OD600 0.05 of an overnight culture of <i>P. aeruginosa</i> PAO1C grown in tryptone soy broth (TSB) was added to 1 ml of sputum medium in 24-well cell-culture plates and incubated for 16 h at 37 °C with gentle shaking. To determine the growth rate, viable counts were performed with cultures grown for 6 h. Cells were treated for 30 min with cellulase (1 mg cellulase ml, 400 g chloramphenicol ml in 0.05 M citrate buffer, pH 4.6) to dissolve the clumps and plated on agar medium. Xanthan gum (T622, CP Kelco) in powdered form was added into ASM with concentration 0.5% to make the solution of ASM+XG. These solutions were mixed thoroughly until the powder was dissolved.	H <sub>2</sub> O: 1 L Mucins: 5 g DNA: 4 g DTPA: 5.9 mg NaCl: 5 g KCl: 2.2 g Amino acids: 5 g Egg yolk emulsion: 50 mL	ε	G': 6.5 10 <sup>-4</sup> Pa G'': 1.5 10 <sup>-2</sup> Pa tan δ: 2.3 G*: 1.6 10 <sup>-3</sup> Pa
		Artificial sputum medium (ASM+0.5% XG)		H <sub>2</sub> O: 1 L Mucins: 5 g DNA: 4 g DTPA: 5.9 mg NaCl: 5 g KCl: 2.2 g Amino acids: 5 g Egg yolk emulsion: 50 mL Xanthan gum: 0.5 w%		G': 5.1 Pa G'': 2.2 Pa tan δ: 0.43 G*: 5.6 Pa