Giovanni Ferrarese
S288665

Thesis project:

Mucosal rheology in the airways of patients with severe lung disease

Thesis advisors:
Seyssiecq Isabelle
Marchisio Daniele

BIBLIOGRAPHICAL RESEARCH AND COMPARISON ON THE CHEMICAL COMPOSITION, INTERACTIONS AND THE RHEOLOGICAL CHARACTERISTICS OF COMPONENTS BETWEEN THE REAL AND RECONSTRUCTED PULMONARY MUCUS (BOTH OF HEALTHY SUBJECTS AND AFFECTED BY RESPIRATORY PATHOLOGIES):

This annex is a summary of the literature search set forth in Chapter n. 3 of the thesis and aims to:

- Classify all the components used in reconstructed mucosa
- Associate each component with the chemical bond that binds it to other components in the reconstructed mucosa
- Highlight the corresponding one in the real mucosa
- Highlight the rheological effect that such component produces in both real and reconstructed mucosa

Art.	Year	Reconstructed mucus Component	Real mucus Component	Kind of interaction	Rheological Influence (η, G', G'')
All evaluated	1997-2022	H_2O (deionized)	H_2O	The medium in which all the mucus components are dispersed. It should be as deionised as possible, given the crucial importance of ions in the interaction with the other components.	The increase (↑) in water leads to greater dilution, affecting the rheological characteristics of the reconstructed mucus as the real mucus in a decrease in density, viscosity and elasticity (η↓, G'↓, G''↓).
46 27 40 29 38 36 35	2021 2020 2002 2020 2021 2021 2015	Mucin from porcine stomach	MUC5AC	They are bound together by disulphide bridges and hydrophobic bonds due to cysteine domains, as well as Oligosaccharide side chains and hydrogen bonds, Van der Waals bonds, Ionic bonds . They repel each other through anionic interactions between the S/T/P domains.	The decrease (↓) of MUCINS affects negatively the density, viscosity and elasticity (η↓, G'↓, G''↓). Comparing the 2 types of mucins it appears that, since MUC5AC is shorter and with more concentrated site of cysteine the increase of MUC5AC (↑) induces a more important increase of the connections and also of the rheological behaviours.
39 42 45 44 37 21	2018 2019 2004 2017 2019 2020	Mucin from bovine submaxillary glands	MUC5B		
53 50 49 52 29 47 10 51	1995 2010 2021 2022 2020 2010 2010 2007	Deoxyribonucleic Acid from fish sperm	DNA Actin filaments	Both are anionic polyelectrolytes covered with a positive layer of counterions. Actin is associated with the globularisation of DNA. For this reason they bind electrostatically to the S/T/P domains of mucins. Two different binding mechanisms have been evaluated, consisting of a reduction in the mesh size of the mucin network through entanglements or disulphide bonds. The presence of DNA in mucus can also be associated with the presence of bacteria and the binding they cause between cells (infectious or not).	The increase (↑) of DNA is linked to the increase of density, viscosity and elasticity of mucus (η↑, G'↑, G''↑).

60 59 57 20 43	2019 2018 2015 2020 2017	NaCl	Na^+, Cl^-	Involved in the regulation of the intermembrane transducer. They are responsible for the dehydration of mucus due to an abnormal ion concentration between the epithelial cells and the mucus. Furthermore, Na^+ is involved in the neutralisation of S/T/P domains that allow mucin filaments to stand closer together with a kind of relative attraction, as opposed to normal repulsion. Finally, NaCl's ability to form so-called salt bridges increases the folded configuration of the globular, non-carboxylate part of mucins, decreasing binding in those mucin sections.	The increase (↑) of NaCl is linked to the increase (at quite neutral pH) and decrease (at low LV. of pH) of visco-elasticity (G* ↑↓). It is related to salt bridges and is progressively more relevant at lower and lower pH, due to the lability of these bridges under acidic conditions.
58	1995	KCl	K^+, Cl^-	Involved in the regulation of the intermembrane transducer. They are responsible for the dehydration of mucus due to abnormal ionic concentration between epithelial cells and mucus. It is known that bacterial infection can cause the mis regulation of ion between cells and mucus trading to an decrease of pH (↓) in the mucus.	The net physiological effect of the increase (↑) of KCl in the epithelial cells is the same induced by the decrease of pH (G* ↑) and dehydration (η↑), while in the reconstructed Mucus the effect is the same as NaCl.

Art.	Year	Reconstructed mucus Component	Real mucus Component	Kind of interaction	Rheological Influence (η, G', G'')
62 60 59 58 61 20	2018 2019 2018 1995 2003 2020	Trizma base	pH regulator	Involved in the neutralization of pH. This regulator is necessary not only to mimic the variation of H^+ as a function of the disease state, but also to reduce the pH susceptibility of reconstructed mucus, essential to linkage of certain components such as PAA . In addition acidic pH is related to a decrease in number and strength of the so called saline bridges, decreasing the folded configuration of the globular, non carboxylate part of the mucins, increasing the linkages in those sections.	The decrease (↓) of Trizma base is linked to a decrease of pH so an increase of H^+ in the solution and trade to the increase of viscoelasticity (G* ↑) while the viscosity not seems to be affected from the pH swing. For the great affinity with triethanolamine, Trizma base for the PAA stabilization is a key element of the reconstruction. In fact, without Trizma base PAA can not assume the necessary stability to link other molecules resulting ineffective.
62 63 86 94 31 33	2018 2003 2017 2018 2005 2005	Diethylenetriaminepentaacetic acid gadolinium(III) dihydrogen salt hydrate	Ionic sequester and chelate	Involved in the linkages of the cysteine's domains, mediate the reaction between them and PAA macromolecules, avoiding the proteolysis of mucins in the reconstructed mucus. The use of a chelator mimic the condition of a culture substrate rich in Fe^{3+}, essential for the growth of certain bacteria such as Pseudomonas Aeruginosa (involved in the CF bronchial sputum infection).	The increase (↑) of DEPA alone is not sufficient to explain a real effect on the rheological characteristics of reconstructed mucus. However, it is important to note that this substance has been associated with other chelators such as DTPA (Penteticacid) and EDTA (Ethylenediaminetetraacetic), which are involved in the stabilization of the binding of other monomers used to mimic the effect of bacterial presence (such as Alginate, PAA and Xantan gum). Therefore, the effect of this component is the same as that found for such substances (G* ↑, η↑).

62 46 63 65 66 31 64	2018 2021 2003 1997 2022 2005 2003	PAA Poli(acrylic-acid)	Cysteine domains Component of the interbacterial layer	Added to restore the natural number of bonds between the CYS domains lost in the mucin extraction process, the conformation of PAA is very susceptible to pH, which is why it is combined with components such as Trizma base. With the help of chelators such as EDTA DPA and Diethyl... it thus increases the hydro-cross-links between mucins. As it is also a component of the interbacterial layer, its presence in reconstructed mucus mimics the presence of non-pathogenic bacteria that also inhabit healthy bronchial mucus.	The increase (↑) of PAA is associated to an increase of all the rheological properties (η↑, G'↑, G''↑).
55 56 33	1990 2016 2005	Alginic acid	Alginate	It is part of the outer capsid of bacteria that develop in a weakened oxide condition and is a sign of chronic infection. Increased expression of alginate is linked to enhanced biofilm production (Alginate was also found in the intra-bacterial layer) and so to the resistance to killing of bacteria by leucocyte.	An Alginate increase (↑) is associated to an enhance of linkages between the bacteria and so to an improvement of the mucins entanglement and so the rise of the mucus viscoelasticity (η↑, G'↑, G''↑).

Art.	Year	Reconstructed mucus Component	Real mucus Component	Kind of interaction	Rheological Influence (η, G', G'')
72 70 69 71 77 68 76	2002 2013 2005 2004 2020 2007 2012	Egg yolk	Lipidic and protea surfactant component of the mucus	It is mainly found in the mucus surface and it role is to decrease surface tension to improve the mucus's ability to capture substances. It is composed of lipids, proteins, phospholipids and lipoproteins in a globular and plasma structure. It structure is continuously perturbed by two mechanisms that enable the transport of macromolecules in the mucus and adaptation to its modifications (adsorption of low-density lipoprotein (LDL) and adsorption of phosphitin). The interaction with NaCl is important for the dispersion of the granular component. This substance is considered essential to replace the surface purulence of mucus, especially infected mucus.	The addition of egg yolk generates a simultaneous increase (↑) of the surfactant lipidic and proteinic components by a 2 to 3 factor inducing a small increase of the rheological properties due to the Ionic or hydrophobic interactions (h↑, G'↑, G''↑).
59 75 33 67 28 76	2018 2020 2005 2015 2011 2012	CHF5633	Lipidic and protea surfactant component of the mucus	It has the same similar interactions as egg yolk, but the composition is quite different, particularly, for the less substantial protein component. The difference is that this engineered component may be closer to the actual structure of the bronchial mucus since its constituting elements are those present in the lipidic fraction of the mucosa. (see in the articles the importance of DPCC, PC, SP-C, SP-A, SP-D).	The increase (↑) of the artificial surfactant in the mucus not only induces the reduction of the superficial tension but also of the rheological properties (η↓, G'↓, G''↓).
32 78 85 79 80 82 84	2022 2022 1996 2016 2004 2007 2010	Casein Hydrolysed (Casamino acids) (CH)	Additional presence of NH_2 group in CF mucus, natural contribution of free amino acids and proteins in bronchial mucus.	CH can be added to replace a pool of generic amino acids. It also contains several small peptides (such as casein) present in the actual mucus. These are known to be largely related to the body's immune response to bacterial infections and to be characterised by a cationic structure that allows linkage between S/T/P domains. In addition, cysteine residues have also been found in hydrolysed casein that may enhance binding across the cysteine domains of mucins, mimicking the contribution of several immune proteins rich in cysteine groups (HNP1-3).	Casein Hydrolysed simulates the presence of free AA in the pulmonary mucus and gives the possibility to mimic the presence of proteins too SP-A and SP-C. The increase (↑) of CH is supposed to induce an increase of S/T/P and Cysteine linkages that involve an increase of -all the rheological properties (η↑, G'↑, G''↑).