

Biophysical Journal, Volume 99

Supporting Material

**Combined and Independent Action of Proteins SP-B and SP-C
in the Surface Behavior and Mechanical Stability of Pulmonary
Surfactant Films**

David Schürch, Olga L. Ospina, Antonio Cruz, and Jesús Pérez-Gil

Supplementary Table 1:

Influence of SP-B and SP-C on surface activity

Sample	Protein (%)	Adsorption				Quasi static cycles			Dynamic cycles		
		γ_i (mN/m)		γ_{post} (mN/m)		γ_{min} QS4 (mN/m)	Area change QS4 (%)	γ_{max} QS4 (mN/m)	γ_{min} D20 (mN/m)	Area change D20 (%)	γ_{max} D20 (mN/m)
		20s	5min	20s	5min						
Lipid extract (protein depleted)	0	55 ± 2	50 ± 1	52 ± 2	45 ± 5	2.3 ± 0.8	52.3 ± 4.6	52.3 ± 1.6	2.6 ± 0.3	34.7 ± 5.5	56.6 ± 3.5
Lipid extract + SP-B	0,4	33 ± 4	24 ± 1	45 ± 2	25 ± 1	1.6 ± 0.1	18.8 ± 1.4	31.4 ± 1.4	2.1 ± 0.3	14.8 ± 2.8	32.7 ± 2.3
	1,0	25 ± 1	25 ± 1	24 ± 1	24 ± 1	1.6 ± 0.2	21.0 ± 3.8	30.4 ± 1.2	1.9 ± 0.3	18.5 ± 2.1	32.2 ± 2.4
	1,2	24 ± 1	24 ± 1	24 ± 1	24 ± 1	1.8 ± 0.1	18.5 ± 2.4	28.2 ± 0.1	1.8 ± 0.2	19.3 ± 1.8	31.7 ± 1.4
Lipid extract + SP-C	0,6	33 ± 1	31 ± 1	42 ± 5	30 ± 4	2.2 ± 0.3	32.5 ± 6.8	32.2 ± 1.7	2.4 ± 0.5	19.0 ± 2.9	36.6 ± 4.8
	1,0	34 ± 1	30 ± 1	31 ± 3	28 ± 2	2.1 ± 0.2	25.7 ± 3.3	31.5 ± 2.1	2.2 ± 0.6	20.1 ± 1.2	34.5 ± 2.1
	1,2	37 ± 1	30 ± 1	30 ± 2	27 ± 1	2.3 ± 0.3	27.7 ± 2.8	30.0 ± 0.4	2.3 ± 0.2	20.3 ± 0.7	33.5 ± 0.2
	1,6	35 ± 4	29 ± 2	29 ± 4	28 ± 1	2.4 ± 0.3	29.7 ± 1.4	29.4 ± 1.3	2.2 ± 0.1	18.1 ± 1.2	31.9 ± 0.5
Lipid extract + SP-B + SP-C	0,4, 0,6	32 ± 2	22 ± 1	24 ± 1	23 ± 1	1.6 ± 0.2	21.3 ± 3.4	29.9 ± 2.5	2.1 ± 0.5	18.7 ± 2.9	34.7 ± 0.5
	1,2, 1,2	34 ± 4	24 ± 1	24 ± 1	23 ± 1	2.0 ± 0.3	28.3 ± 2.4	27.1 ± 2.6	2.3 ± 0.2	18.5 ± 3.9	34.5 ± 1.0
	1,2, 1,6	28 ± 2	23 ± 1	24 ± 1	23 ± 2	2.1 ± 0.3	30.3 ± 3.4	29.6 ± 0.4	2.1 ± 0.3	22.1 ± 2.9	32.1 ± 1.0
Native surfactant	NA	23 ± 1	22 ± 1	24 ± 1	24 ± 1	1.3 ± 0.1	14.0 ± 1.1	26.5 ± 0.8	1.5 ± 0.3	14.4 ± 1.7	26.3 ± 0.9
Organic extract	NA	24 ± 1	23 ± 1	26 ± 1	25 ± 1	1.4 ± 0.2	17.3 ± 2.8	28.7 ± 2.0	1.5 ± 0.2	15.5 ± 1.6	29.9 ± 2.5
Organic extract (reassembled)	NA	27 ± 1	25 ± 1	27 ± 1	25 ± 1	1.4 ± 0.2	16.1 ± 1.3	27.2 ± 1.6	1.6 ± 0.4	15.2 ± 1.1	28.5 ± 2.8

Protein (%): protein content % with respect to phospholipids (w/w)

γ_i (mN/m): surface tension 20 seconds and 5 minutes after surfactant contact with air-liquid interface (initial film formation)

γ_{post} (mN/m): surface tension 20 seconds and 5 minutes after rapid film expansion

γ_{min} QS4 (mN/m): minimum surface tension at 4th quasi-static compression

Area change QS4 (%): area change of film from fully expanded state (100% area) to achieve minimal surface tension (4th quasi-static cycle)

γ_{max} QS4 (mN/m): maximum surface tension at the end of the 4th quasi-static compression-expansion cycle.

γ_{min} D20 (mN/m): minimum surface tension at 20th dynamic compression

Area change D20 (%): area change of film from fully expanded state (100% area) to achieve minimal surface tension (20th dynamic cycle)

γ_{max} D20 (mN/m): maximum surface tension at the end of the 20th dynamic compression-expansion cycle

Supplementary Table 2:

Influence of SP-B and SP-C on surfactant film stability

Sample	Protein concentration	$\gamma_{\min \text{ QS1}}$ (mN/m)	$\Delta \gamma_{\text{QS1}}$ (mN/m)	$\gamma_{\min \text{ post}}$ (mN/m)	$\Delta \gamma_{\text{post}}$
Lipid extract (protein depleted)	0	2.3 ± 0.8	4.8 ± 4	2.1 ± 0.2	1.9 ± 0.3
Lipid extract + SP-B	0.4	1.7 ± 0.1	2.4 ± 0.3	1.7 ± 0.1	1.6 ± 0.2
	1.0			1.6 ± 0.2	1.5 ± 0.1
	1.2			1.8 ± 0.2	1.5 ± 0.1
Lipid extract + SP-C	0.6	2.3 ± 0.3	4.5 ± 0.4	2.2 ± 0.3	2.2 ± 0.2
	1.0			2.0 ± 0.2	2.1 ± 0.3
	1.2			2.1 ± 0.2	2.2 ± 0.1
	1.6			2.4 ± 0.3	2.5 ± 0.2
Lipid extract + SP-B, SP-C	0.4, 0.6	1.6 ± 0.2	2.5 ± 0.6	1.7 ± 0.2	1.7 ± 0.2
	1.2, 1.2			2.0 ± 0.3	2.1 ± 0.2
	1.2, 1.6			2.1 ± 0.2	2.3 ± 0.3
Native surfactant	NA	1.3 ± 0.1	1.6 ± 0.2	1.2 ± 0.1	1.3 ± 0.1
Organic extract	NA	1.4 ± 0.2	1.7 ± 0.3	1.1 ± 0.2	1.4 ± 0.2
Organic extract (reassembled)	NA	1.4 ± 0.2	1.8 ± 0.2	1.2 ± 0.2	1.4 ± 0.2

Protein concentration: % w/w total phospholipids (concentrations featured in Fig 5 in bold)

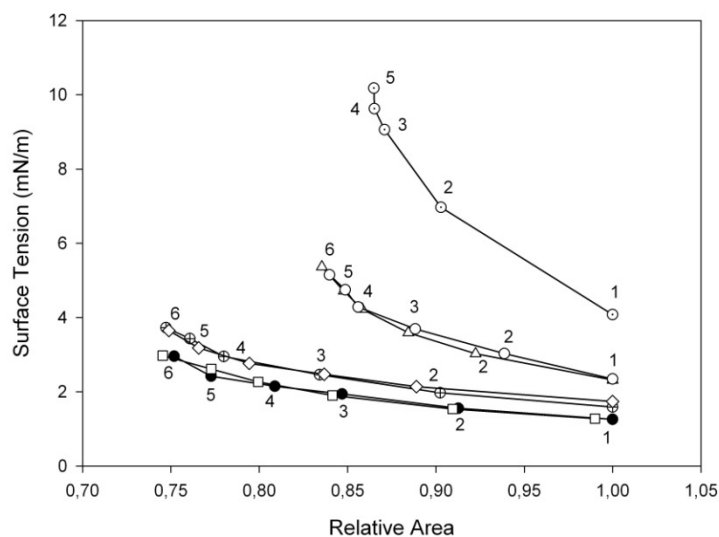
$\gamma_{\min \text{ QS1}}$: minimum surface tension after 1 Quasi-static compression

$\Delta \gamma_{\text{QS1}}$: change in surface tension from $\gamma_{\min \text{ QS1}}$ after perturbation

$\gamma_{\min \text{ post}}$: minimum surface tension (quasi-static compression) after full experimental protocol: 4 quasi-static + 20 dynamic cycles

$\Delta \gamma_{\text{post}}$: change in surface tension from $\gamma_{\min \text{ post}}$ after perturbation

Supplementary Figure S1



Surface tension/area relationship for bubbles compressed to minimum surface tension and subjected to consecutive mechanical perturbations

Change in surface tension and area for 5-6 successive perturbations introduced with a pendulum hammer. Numbers indicate tension/area situation after the corresponding perturbation and symbols are as in figure 2. Curve shapes are representative for each sample (after repeating 4-6 independent experiments with qualitatively similar results). Pure lipid-coated bubbles showed a somehow more variable stability, and two different experiments have been included (open and dotted circles, respectively) showing extreme behaviors.