Aerosol, chemical and physical properties of dry powder synthetic lung surfactant for noninvasive treatment of neonatal respiratory distress syndrome

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Inhalation of dry powder synthetic lung surfactant may assist spontaneous breathing by providing noninvasive surfactant therapy for premature infants supported with nasal continuous positive airway pressure. Surfactant was formulated using spray-drying with different phospholipid compositions (70 or 80 total weight% and 7:3 or 4:1 DPPC:POPG ratios), a surfactant protein B peptide analog (KL4, Super Mini-B, or B-YL), and Lactose or Trehalose as excipient. KL4 surfactant underperformed on initial adsorption and surface activity at captive bubble surfactometry. Spray-drying had no effect on the chemical composition of Super Mini-B and B-YL peptides and surfactant with these peptides had excellent surface activity with particle sizes and fine particle fractions that were well within the margins for respiratory particles and similar solid-state properties. Prolonged exposure of the dry powder surfactants with lactose as excipient to 40 °C and 75% humidity negatively affected hysteresis during dynamic cycling in the captive bubble surfactometer. Dry powder synthetic lung surfactants with 70% phospholipids (DPPC and POPG at a 7:3 ratio), 25% trehalose and 3% of SMB or B-YL showed excellent surface activity and good short-term stability, thereby qualifying them for potential clinical use in premature infants.

Surfactant deficiency and lung immaturity are the main causes of respiratory failure in premature infants. Morbidity and mortality of this neonatal respiratory distress syndrome has improved tremendously by the clinical introduction of animal-derived lung surfactant preparations1. These surfactants consist of a mixture of phospholipids, especially dipalmitoylphosphatidylcholine (DPPC) and phosphatidylglycerol (PG), and the hydrophobic surfactant proteins B and C (SP-B and SP-C).

Current research has focused on the design and development of a fully synthetic lung surfactant2, in which the essential function of native SP-B has been taken over by SP-B and SP-C peptide mimics, e.g., KL4, Mini-B (MB)4, Super Mini-B (SMB)5–7, and B-YL7 for native SP-B and SP-C33,34 and SP-Css ion-lock 110 for native SP-C. KL4 is a 21-amino acid peptide with repeating subunits of one lysine (K) and four leucine (L) residues3. MB is a 34-amino acid peptide based on the sequence of the N- and C-terminal α-helices of human SP-B and a short loop4. In contrast with MB, SMB and B-YL contain an insertion sequence and are 41-amino acid peptides. SMB contains 3 disulfide bridges5, whereas B-YL is sulfur-free by replacing the cysteine residues of SMB with tyrosine and less sensitive to oxidation by replacing the methionine residues with leucine7 (Fig. 1). The tyrosine for cysteine substitution in B-YL eliminates the need for an oxidation step to secure disulfide linkage in SMB5–7, thereby sharply reducing the production costs. Whereas SP-B deficiency through knockout or mutation is lethal in mice and humans11, SP-C is less essential than SP-B to maintain lung surfactant activity in vivo12.

The primary aim of our study was to develop an advanced dry powder (DP) synthetic lung surfactant for aerosol delivery to preterm infants with respiratory distress syndrome who need noninvasive respiratory support with nasal (continuous positive airway pressure) CPAP in a low technical setting. This surfactant would need to replicate the surface activity of clinically used liquid animal-derived lung surfactant and generate a physically and

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chemically stable dry powder form that is fit for aerosol delivery. Therefore, we screened various phospholipid quantities (70% and 80% total) and ratios (7:3 and 4:1) of DPPC and palmitoyl-oleyl-PG (POPG), and tested the use of various SP-B peptide mimics (KL4, SMB, B-YL) and stabilizing excipients (Lactose and Trehalose) towards down-selection of a dry powder synthetic lung surfactant for a preclinical trial in surfactant-deficient animal models. Although lactose is an approved excipient for inhaled pharmaceutical products, trehalose was included in the excipient screening procedure as this disaccharide protects labile macromolecules and lipid membranes.

Surface activity of these dry powder surfactant formulations was determined by captive bubble surfactometry (CBS) and integrity of the SP-B peptide mimics by MALDI TOF mass spectrometry. Aerosol and solid-state properties were investigated with particle sizing, X-ray diffraction (XRPD), thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC). Samples of these surfactant formulations were aged for up to 6 months at 40 °C and 75% humidity and re-tested to determine whether the powders were viable for long-term storage.

### Methods

#### Production and formulation of DP synthetic lung surfactant.

Dry powder synthetic lung surfactant was designed and formulated at Acorda Therapeutics Inc. utilizing its ARCUS® pulmonary dry powder technology. The peptide mimics of SP-B (KL4, SMB, and B-YL) were synthesized using standard Fmoc protocols, cleaved and purified as detailed previously. Amino acid composition of these peptides is depicted in Fig. 1. Dry powder synthetic lung surfactant was formulated by adding the following components to the organic solvent used for spray drying: DPPC and POPG-Na (Corden Pharma, Liestal, Switzerland); an excipient, i.e., Lactose or Trehalose; NaCl; and a SP-B peptide mimic (KL4, SMB, B-YL). Respirable dry synthetic surfactant particles were produced using a GEA Niro PSD-1 spray dryer (Niro Inc., Copenhagen, Denmark) or Buchi B-290 mini (Buchi Corporation, New Castle, DE 19720) spray dryer. After spray drying, size 00 capsules were filled with surfactant (~ 30 mg per capsule), and packaged in heat-sealable pouches with desiccant. Table 1 describes the composition of the two series of dry powder synthetic lung surfactant formulations studied here.

#### Captive bubble surfactometry.

Adsorption and surface tension lowering ability of dry powder synthetic surfactant preparations were measured with a captive bubble surfactometer, described and built by Schürch and coworkers. In this leak-proof system, filled with Goerke’s buffer with 10% sucrose, surfactant floats against a hydrophilic roof consisting of 1% agarose gel. After inserting an air bubble into the chamber, adsorption of the surfactant to the bubble’s air–liquid interface is measured. The bubble chamber is then sealed and quasi-static compression and expansion of the air bubble is done in discrete steps at a rate of 5% of the bubble volume every 10 s for 4–10 cycles. Quasi-static cycling is followed by dynamic compression and expansion cycling between 10 and 110% of the original bubble area at a physiologic cycling rate of 20 cycles/min. Both cycling modalities show extreme flattening of the air bubble in surface-active surfactant preparations.

### Table 1. Composition of the two series of dry powder synthetic lung surfactants tested.

| Surfactant  | Formulation          | Weight% |
|-------------|----------------------|---------|
| First series |                      |         |
| PL70[7:3]-SMB | DPPC:POPG:SMB:Lactose:NaCl | 49:21:3:25:2 |
| PL70[7:3]-KL4  | DPPC:POPG:KL4:Lactose:NaCl  | 49:21:3:25:2 |
| PL80[7:3]-SMB | DPPC:POPG:SMB:Lactose:NaCl  | 56:24:3:15:2 |
| PL80[7:3]-KL4  | DPPC:POPG:KL4:Lactose:NaCl  | 56:24:3:15:2 |
| PL80[4:1]-SMB | DPPC:POPG:SMB:Lactose:NaCl  | 64:16:3:15:2 |
| PL80[4:1]-KL4  | DPPC:POPG:KL4:Lactose:NaCl  | 64:16:3:15:2 |
| Second series |                      |         |
| SMB: Lactose   | DPPC:POPG:SMB:Lactose:NaCl  | 49:21:3:25:2 |
| B-YL: Lactose   | DPPC:POPG:B-YL:Lactose:NaCl  | 49:21:3:25:2 |
| SMB: Trehalose  | DPPC:POPG:SMB:Trehalose:NaCl  | 49:21:3:25:2 |
| B-YL: Trehalose | DPPC:POPG:B-YL:Trehalose:NaCl  | 49:21:3:25:2 |

**Figure 1.** Amino acid composition of surfactant protein B (SP-B) peptide mimics KL4, Super Mini-B (SMB) and B-YL.
out each experiment, bubble shapes are monitored with continuous video recording and surface tension was analyzed with custom-designed software.

For each measurement, a capsule with ~30 mg of dry powder surfactant was opened and its contents were dissolved in 1 ml of distilled water. Two µL of this surfactant sample was then inserted into the bubble chamber, resulting in a surfactant concentration of ~50 µg/ml. All measurements were performed in triplicate at 37 °C.

Chemical stability of surfactant peptide in spray-dried preparations. Matrix-assisted laser desorption/ionization (MALDI) mass spectrometry was applied to determine the integrity of SP-B peptide mimics after exposure to the high temperatures associated with spray drying. One month old spray dried surfactant samples, dispersed in water at the same concentration used for surfactometry, were analyzed using an AB SCIEX TOF/TOF 5800 system (Sciex, Framingham, MA 01701). Spray-dried surfactant peptide-lipid samples containing approximately 50 pmoL of peptide were co-solvated with either α-cyano-4-hydroxycinnamic acid or sinapic acid (10 mg matrix/ml water: acetonitrile 1:1, v:v with 0.3% trifluoroacetic acid) by mixing 24 µl of matrix solution with 1 µl of peptide-lipid dispersion solution co-solvated with an equal volume of acetonitrile with 1% trifluoroacetic acid. 2 µl of this mixture was then deposited onto a metal MALDI sample plate and allowed to air dry before mass spectral measurement. The spectrometer was set for mid-mass positive linear mode detection and the resulting spectrum expressed as the mass/charge ratio (m/z) in daltons (Da). The mass spectra were analyzed using ABI SCIEX Analyst and Data Explorer software.

Physical stability. Average geometric particle size (gPSD) in microns (d50) was measured using laser diffraction (HELOS, Sympatec, Clausthal-Zellerfeld, Germany). The fine particle fraction (FPF) was measured with a three-stage Andersen Cascade Impactor (ACI-3) operated at a volumetric flow rate of 28.3 and/or 60 l/min (Copley Scientific, Nottingham, UK). The FPF of the total dose was than calculated using 5.8 and 3.3 microns as upper limits at a flow rate of 28.3 l/min. XRPD was used for structural powder characterization with special attention to the characteristic diffraction peak at 21° 2θ that indicates the presence of phospholipids in a bilayer structure. TGA was used to measure the mass loss of a powder sample as a function of temperature and to determine the percentage of volatiles loss up to 120 °C, i.e., residual solvent (ethanol and water). DSC was used to measure temperatures at phase transitions. Low T1 is the characteristic temperature of the first thermal event(s) observed and Low T2 the second set of thermal event(s) observed during a DSC scan at 20 °C/min.
**Statistical analysis.** All data are expressed as mean ± standard error of the mean (SEM). Student's t-tests were used for comparisons of discrete data points and functional data were analyzed with one-way analysis of variance with Tukey's post-hoc test using SPSS software. Differences with a p value < 0.05 were considered to be statistically significant.

**Results**

**Captive bubble surfactometry.** The first series of six dry powder synthetic lung surfactant preparations focused on phospholipid ratio (DPPC:POPG ratio of 7:3 or 4:1) and quantity (70 or 80 wt%) and SP-B peptide mimic selection (3 weight% of KL4 or SMB), all powders contained lactose as sugar excipient. Initial adsorption (IA) of SMB surfactants was significantly better than that of the KL4 surfactants (p < 0.02) (Fig. 2), but there were no differences in post-expansion adsorption (PEA). IA was higher than PEA for all surfactant formulations, except for PL70[7:3]-SMB and PL80[4:1]-SMB. Average ± SEM minimum surface tension of the three KL4 surfactants during the 1st quasi-static cycle was 5.1 ± 1.7 mN/m versus 1.3 ± 0.3 mN/m in the three SMB surfactants (p = 0.0442), but all surfactants reached average minimum surface tension values < 2 mN/m during the next cycles.

Figure 3 shows minimum and maximum surface tension values of these surfactants during quasi-static cycle 4 and dynamic cycle 10. Phospholipid composition (both quantity and ratio) and SP-B peptide mimic selection did not affect minimum surface activity. Average area reduction during compression to reach minimum surface tension varied between 25 and 32% during quasi-static cycle 4 and between 22 and 36% during dynamic cycle 10 (Fig. 2).

Surfactometry of the second series of dry powder synthetic lung surfactant demonstrated that the four dry powder surfactant formulations with 70% phospholipids at a 7:3 DPPC:POPG ratio and 3% of SMB or B-YL as SP-B peptide mimic and 25% of Lactose or Trehalose as excipient were highly surface-active. Surface activity was measured with captive bubble surfactometry shortly after production (0 months) and after 1 and 3 months of exposure to 40°C and 75% relative humidity (1 and 3 months). Initial adsorption at 0 months was in general higher than PEA (p < 0.001, Fig. 4). Average minimum surface tension was < 2 mN/m during quasi-static cycle 4 with a maximum surface tension of 29–35 mN/m (Fig. 5). During dynamic cycle 10, all four formulations also reached average minimum surface tension values < 2 mNm and average maximum surface tension varied between 38 and 41 mN/m (Fig. 5). Average area reduction during compression to reach minimum surface tension varied between 19 and 24% during quasi-static cycle 4 and between 26 and 34% during dynamic cycle 10 (Fig. 4). Although % compression was on average higher during dynamic than quasi-static cycling, the differences were not statistically significant.
Exposure to 40 °C and 75% relative humidity for 1 or 3 months did not significantly affect the surface tension lowering capabilities of these four surfactant formulations. After 1 and 3 months of stress exposure, average minimum surface tension values during quasi-static and dynamic cycling were still < 3 mN/m (Fig. 5). However, minimum surface tension at quasi-static cycle 4 of SMB:Trehalose was higher at 1 than at 3 months (p = 0.024) and minimum surface tension of SMB:Lactose was higher at 3 months than at 0 months (p = 0.021) (Fig. 5). Minimum surface tension during dynamic cycling decreased during heat and humidity stress in both Trehalose surfactant formulations (p < 0.03). Maximum surface tension values during dynamic cycling varied between 27 and 45 mN/m (Fig. 5). Area reduction secondary to compression during quasi-static and dynamic cycling varied considerably under stress exposure, except for B-YL:Trehalose surfactant (Fig. 4).

B-YL:Trehalose surfactant stood out at 1 and 3 months of stress exposure with minimum surface tension values << 2 mN/m during quasi-static and dynamic cycling (Fig. 5). Figure 6 shows that surface activity of a repeat production of B-YL:Trehalose surfactant did not change after aging for 9 months. These values are comparable to those of the clinical porcine surfactant Curosurf®.

Physical stability. Table 2 summarizes the aerosol and solid-state properties of the first series of dry powder surfactant formulations at baseline and after exposure for 2 weeks or 1 months to 40 °C and 75% relative humidity. gPSD and FPF were well within the margins for respiratory particles at baseline, but the KL4 and SMB powders with 70% phospholipids and 25% lactose (PL70[7:3]-KL4 and PL70[7:3]-SMB) had a more stable FPF than the powders with 80% lipids and only 15% lactose. Solid state properties were similar among the various surfactant formulations. They were semi-crystalline with a characteristic diffraction peak at 21° 2θ, which can be attributed to the presence of phospholipids in a bilayer structure. Residual solvent content (TGA-120) was less than 2.2%. The dry powder formulations contained phase transitions at ~ 38–44 °C, calculated as the intercept of a step transition, and 49–63 °C, calculated as a peak extremum of an endotherm.

Table 3 provides the same data for the second series of dry powder surfactant formulations at baseline and after exposure for 2 weeks, 1, 3, and 6 months to 40 °C and 75% relative humidity. gPSD and FPF were again well within the margins for respiratory particles. Solid state properties were similar among the various dry powder surfactant formulations that were semi-crystalline with a characteristic diffraction peak at 21° 2θ due to the
presence of phospholipids. Residual solvent content was less than 1.5%. The dry powder formulations contained phase transitions at ~45–53 °C, calculated as the intercept of a step transition, and 61–67 °C, calculated as a peak extremum of an endotherm.

Chemical stability of surfactant peptides in spray-dried preparations. MALDI mass spectrometry confirmed the appropriate masses for SMB and B-YL in the second series of dry powder surfactant formulations (Fig. 7). No degradation products of these SP-B peptide mimics were detected in samples stored at 5 °C for 1 month before analysis by mass spectrometry, i.e., spray drying during surfactant production did not affect the integrity of these peptides in phospholipid mixtures containing either Trehalose or Lactose as excipient.

Discussion
In this study, we investigated the aerosol, chemical and physical properties of two series of dry powder lung surfactant formulations that differed with respect to phospholipid composition, choice of SP-B peptide analog used as active pharmaceutical ingredient (API), and the sugar-based excipient.

The effect of phospholipid composition was investigated using captive bubble surfactometry in the first series of dry powder surfactants. Total quantity (70 or 80 wt%) and ratio (7:3 or 4:1) of DPPC and POPG did not directly affect surface activity, but initial adsorption was significantly higher in surfactant formulations with KL4 as SP-B peptide mimic. Considering the relatively high costs of POPG over DPPC and advancing viscosity at higher concentrations of DPPC, dry powder surfactants in the second series were produced with 70% of phospholipids at a 7:3 DPPC:POPG ratio. This choice was enforced by the finding that a higher phospholipid concentration led to a lower concentration of sugar excipient (15% instead of 25%) and a less stable FPF (Table 2).

In the first series of surfactants, KL4 surfactant underperformed with regard to initial adsorption and surface activity during the first quasi-static cycle when compared to SMB. In the second series of surfactants, surface activity, particle size and FPF, chemical and physical properties of SMB and its sulfur-free derivative B-YL were similar (Table 3). Lower production costs of B-YL peptide by avoiding the oxidation step in the production of SMB therefore warranted a choice for B-YL as lead API.

The choice between lactose and trehalose for sugar excipient was based on the reduction in hysteresis area seen in lactose formulations during dynamic cycling at captive bubble surfactometry after 1 and 3 months of
exposure to 40 °C and 75% relative humidity (Fig. 5). These downsides were not found in the SMB:Trehalose and B-YL:Trehalose formulations.

Viability of dry powder surfactant for long-term storage will require further studies. We know that the material may still have some surface activity; however, we also know that there will be chemical degradation over time, especially of the phospholipids. Therefore, it is important to focus on both surface activity and chemical assay/potency after long-term storage.

Limitations of this study included the need to reconstitute the surfactant formulations and the occurrence of overcompression in some dynamic cycling runs. We considered aerosolization of the dry powder surfactants into the sample chamber, but this was not practical as it was not possible to blow the dry powder through a 1 µm needle into the sample chamber filled with 10% Goerke's sucrose solution. Overcompression is an artefact that can occur because the captive bubble software is set to perform dynamic compression and expansion cycling between 10 and 110% of the original bubble area.

Dry powder synthetic lung surfactant has a 40-year history. Morley et al. prepared a protein-free dry artificial lung surfactant (ALEC) consisting of DPPC and PG at a ratio of 7:3 and blew it down an endotracheal tube into the lungs of 22 very premature babies at birth. These infants required less ventilatory support in the first six hours and had a lower neonatal mortality than a group of 33 untreated controls. The introduction of intratracheal administration of bovine and porcine lung surfactant suspensions in the 1980s quickly overshadowed the early work with protein-free dry powder surfactant formulations. Recently, Boc et al. did in vitro testing of a dry powder aerosol formulation of Survanta, a clinical bovine-derived lung surfactant, whereas Bianco et al. investigated aerosol delivery with a vibrating mesh nebulizer of the porcine surfactant suspension Curosurf in spontaneously breathing surfactant-deficient rabbits supported with nasal CPAP. The latter study demonstrated a similar improvement in oxygenation and ventilation efficacy index compared to intratracheal surfactant administration. These findings are in line with our studies using fully synthetic surfactant formulations. The increased interest in aerosol delivery of surfactant and medications to premature infants supported with noninvasive ventilation reflects an unmet need in clinical neonatology and may further improve outcome of this fragile population.

Figure 6. Quasi-static (left) and dynamic cycling (right) isotherms at captive bubble surfactometry of a B-YL:Trehalose surfactant (DPPC:POPG:B-YL:Trehalose:NaCl 49:21:3:25:2) at baseline and after 9 months of exposure to 40 °C and 75% relative humidity. Differences in minimum surface tension at quasi-static cycling and % compression area to reach minimum surface tension at dynamic cycling were not statistically significant.
### Table 2. Aerosol properties and solid state properties of each of the first series of dry powder surfactant formulations tested. Powders vary according to phospholipid ratio (DPPC:POPG 7:3 or 4:1) and quantity (70 or 80 wt%) and SP-B peptide mimic (SMB or KL4), but all contain lactose as excipient. *RH: Relative Humidity; **NT: not tested; n.c.: not calculated.

| Surfactant Description | Condition | gPSD (µm) | ACI 60 LPM Size 00 FPF < 5.6 µm (%) | Size 00 FPF < 3.4 µm (%) | Size 00 FPF < 5.6 µm (%) | Size 00 FPF < 3.4 µm (%) | XRPD | TGA-120% | DSC | Low T1 (°C) | Low T2 (°C) |
|------------------------|-----------|-----------|---------------------------------|------------------------|------------------------|------------------------|----------------|----------|-----|------------|------------|
| PL70[7:3]-SMB          | t = 0     | 5.1       | 68                               | 48                     | 50                     | 28                     | SC-D           | 2.12     | 40.4 | 49.3       |            |
|                        | 40°C/75% RH 2 week | 60       | 37                               | 34                     | 16                     | 19                     | SC-D           | 1.91     | 43.8 | 51.9       |            |
|                        | 1 month    | 55       | 34                               | 29                     | 12                     | 11                     | SC-D           | 1.94     | 43.3 | 50.3       |            |
| PL70[7:3]-KL4          | t = 0     | 4.5       | 71                               | 52                     | 60                     | 37                     | SC-D           | 2.11     | 39   | 50.3       |            |
|                        | 40°C/75% RH 2 week | 50       | 34                               | 21                     | 9                      | 8                      | SC-D           | 1.96     | 43.3 | 52         |            |
|                        | 1 month    | 47       | 29                               | 18                     | 8                      | 7                      | SC-D           | 2.02     | 43.9 | 52.5       |            |
| PL80[7:3]-SMB          | t = 0     | 5.3       | 61                               | 41                     | 44                     | 23                     | SC-D           | 2.01     | 38.8 | 59.1       |            |
|                        | 40°C/75% RH 2 week | 33       | 23                               | 10                     | 5                      | 4                      | SC-D           | 1.78     | 40.9 | 59.9       |            |
|                        | 1 month    | 29       | 20                               | 9                      | 5                      | 4                      | SC-D           | 1.81     | 41.7 | 59.6       |            |
| PL80[7:3]-KL4          | t = 0     | 5.0       | 67                               | 48                     | 53                     | 31                     | SC-D           | 1.86     | 39.6 | 59.5       |            |
|                        | 40°C/75% RH 2 week | 33       | 23                               | 7                      | 2                      | 1                      | SC-D           | 1.71     | 43.2 | 58.8       |            |
|                        | 1 month    | 23       | 14                               | 7                      | 3                      | 1                      | SC-D           | 1.66     | 41.8 | 59.2       |            |
| PL80[4:1]-SMB          | t = 0     | 5.3       | 64                               | 42                     | 44                     | 25                     | SC-D           | 1.95     | 40.2 | 62.5       |            |
|                        | 40°C/75% RH 2 week | 49       | 30                               | 1                      | 0                      | SC-D                   | 1.83           | NT**      | NT**        |            |
|                        | 1 month    | 43       | 28                               | 17                     | 7                      | 6                      | SC-D           | 1.86     | 41.7 | 62.9       |            |
| PL80[4:1]-KL4          | t = 0     | 5.5       | 66                               | 47                     | 54                     | 29                     | SC-D           | 1.87     | 39.9 | 62         |            |
|                        | 40°C/75% RH 2 week | 35       | 20                               | 1                      | 0                      | SC-D                   | 1.81           | 42.4     | 62         |            |
|                        | 1 month    | 27       | 16                               | 2                      | 0                      | SC-D                   | 1.82           | 42.2     | 62.5       |            |

### Table 3. Aerosol properties and solid state properties of each of the second series of dry powder surfactant formulations tested. Powders have the same phospholipid composition, but differ in regard with SP-B peptide mimic (SMB or B-YL) and excipient (Lactose or Trehalose). Samples have desiccant incorporated in the packaging. *NT: Not Tested; **RH: Relative Humidity; n.c.: not calculated.

| Surfactant Description | Condition | gPSD (µm) | Relative to emitted powder ACI 28.3 LPM FPF < 5.6 µm (%) | FPF < 3.4 µm (%) | FPF < 5.6 µm (%) | FPF < 3.4 µm (%) | XRPD | TGA-120% | DSC | Low T1 (°C) | Low T2 (°C) |
|------------------------|-----------|-----------|--------------------------------------------------------|-----------------|-----------------|-----------------|----------------|----------|-----|------------|------------|
| SMB: Lactose           | t = 0     | 4.2       | 49.7                                                   | 30.1            | 49.3            | 29.9            | SC-D           | 2.11     | 40.2 | 51.5       |            |
|                        | 40°C/75% RH 2 week | 53       | 30                                                   | 52.5            | 29.7            | 61              | SC-D           | 0.61     | n.c. | 60.5       |            |
|                        | 1 month    | 47.7      | 28.2                                                   | 41.7            | 24.7            | NT              | SC-D           | 0.98     | 61.7 |            |            |
|                        | 3 month    | 44.8      | 26.8                                                   | 44.3            | 26.5            | SC-D           | 0.98           | NT       | 38.3 | 51.3       |            |
|                        | 6 month    | 42.2      | 22.2                                                   | 43.5            | 20.3            | SC-D           | 0.83           | n.c.     | 64.2 |            |            |
|                        | 3 month    | 54        | 31.2                                                   | 50.1            | 29              | SC-D           | 0.80           | n.c.     | 64.2 |            |            |
|                        | 6 month    | 54.7      | 30.3                                                   | 54.2            | 30              | SC-D           | 0.75           | n.c.     | 63.1 |            |            |
| B-YL: Lactose          | t = 0     | 3.8       | 42.8                                                   | 25.1            | 43.5            | 20.3            | SC-D           | 1.54     | 42.1 | 51.9       |            |
|                        | 40°C/75% RH 2 week | 42.6     | 22.2                                                   | 41.3            | 19.4            | SC-D           | 0.91           | n.c.     | 59.6 |            |            |
|                        | 3 month    | 47.9      | 23.5                                                   | 43.5            | 20.3            | SC-D           | 0.93           | n.c.     | 59.3 |            |            |
|                        | 6 month    | 41.6      | 20.4                                                   | 41              | 20.2            | SC-D           | 0.91           | n.c.     | 59.3 |            |            |
Figure 7. Maldi TOF mass spectra of the four dry powder surfactant preparations from the 2nd series (SMB or B-YL peptide with Lactose or Trehalose as excipient). Figure A on the left: (A) SMB: Lactose surfactant (DPPC:POPG:SMB:Lactose:NaCl 49:21:3:25:2) and (B) SMB: Trehalose surfactant (DPPC:POPG:SMB:Trehalose:NaCl 49:21:3:25:2). Figure B on the right: (A) B-YL: Lactose surfactant (DPPC:POPG:B-YL:Lactose:NaCl 49:21:3:25:2) and (B) B-YL: Trehalose (DPPC:POPG:B-YL:Trehalose:NaCl 49:21:3:25:2). Samples had been stored at 5 °C for 1 month before analysis by mass spectrometry. Spray drying during surfactant production did not affect the integrity of the SMB peptide in phospholipid mixtures containing either Trehalose or Lactose as excipient.

In summary, surface activity, chemical stability, and aerosol and physical properties are in favor of dry powder lung surfactant with 49% DPPC, 21% POPG, 3% B-YL, 25% Trehalose, and 2% NaCl as a leading candidate for potential clinical use in noninvasive synthetic lung surfactant administration. Preclinical studies in surfactant-deficient animal models will have to confirm whether these in vitro findings hold true in vivo.

Data availability
All data generated or analyzed during this study are included in this article.

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Author contributions
FW, HC and AW conceptualized and designed the study. All authors participated in the data collection. FW, HC and AW performed the data analysis. FW wrote the first draft of the manuscripts. All authors reviewed and revised the manuscript. All authors read and approved the final manuscript.

Competing interests
The authors declare no competing interests.

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