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Sinapultide-loaded lipid microbubbles and the stabilization effect of sinapultide on the shell of lipid

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Microbubbles (MBs) hold promise in various biomedical applications due to their ultrasonic-responsive properties. However, the stability and contrast enhancement duration time of gas encapsulated MBs are still challenging. The aim of this study is to fabricate a novel sinapultide, the synthetic pulmonary surfactant, stabilized MBs as ultrasound contrast agents. The optimized MBs generated from a mixture of phospholipid composition and sinapultide exhibit an average diameter of 1.82 ± 0.15 μm and zeta potential of -55.2 ± 3.9 mV. Over 95% of the MBs had a mean diameter less than 8 μm, indicating that the size of MBs is appropriately applied as ultrasound contrast agent used in clinic. Furthermore, the interaction between sinapultide and lipid molecules as well as the stabilization mechanism of sinapultide on the shell of MBs were investigated by molecular dynamics simulation. The results demonstrate that the stability of MBs was increased effectively when the appropriate amount of sinapultide was added due to the decrease of the surface tension. Accordingly, the acoustic accumulation imaging analysis in vitro indicates that the stable gas encapsulated sinapultide loaded MBs can provide a high scattering intensity resulting in better echogenicity. And the optimized concentration of sinapultide-loaded MBs can improve the contrast enhancement effect obviously compared with non-sinapultide formulation. Therefore, sinapultide-loaded lipid MBs may be designed as a novel ultrasound contrast agent based on synthetic pulmonary surfactant and used for clinical application in the future.

1 Introduction

Gas-filled microbubbles (MBs) have been widely used as ultrasound contrast agents (UCAs) in biomedical applications.\textsuperscript{1,2} Doppler signals from small and/or deep-lying vessels were enhanced and the difference in echo texture between normal as well as adjacent abnormal tissues could be further increased by UCAs.\textsuperscript{3-6} Compared with radioactive diagnosis, ultrasonic imaging has been considered as a promising diagnostic tool due to the advantages of non-invasive, real-time detection, freely utilizing, cost-efficient, and a higher priority of biological safety.\textsuperscript{6,7} Apart from their usage in diagnosis, gas-filled MBs have also been used more and more for their excellent potentiality as ultrasound-mediated drug and gene delivery systems.\textsuperscript{8-10} Therefore, novel UCAs for applications in ultrasound medicine and drug delivery hold great promises.

Typical MBs used as UCAs commonly prepared by various methods including sonication,\textsuperscript{11} emulsification,\textsuperscript{12} microfluid,\textsuperscript{13} inkjet printing\textsuperscript{14} and coaxial electrohydrodynamic atomization.\textsuperscript{15} Conventionally, they are composed with gaseous core and stabilizing shells. The gas inert and sparingly soluble in blood such as SF\textsubscript{6} or perfluorocarbon (PFC) is selected to substitute air as the gas component.\textsuperscript{16} In order to stabilize against the gas dissolution and coalescence, the shell materials are elaborately designed. Nowadays, the proposed shell materials are always composed of lipid, polymer, protein, and surfactant.\textsuperscript{17-19} Among them, lipids and surfactants composed shell offer the excellent structural characters and acoustic response.\textsuperscript{20} Such MBs readily expanded, compressed, as well as ruptured under ultrasound exposure. However, the novel preparation technique and shell materials still need to be developed in order to satisfy the better biosafety.\textsuperscript{21}

Pulmonary surfactant (PS) is a complicated mixture of lipids and proteins that covers at the gas/liquid interface of the lung bronchioles.\textsuperscript{22} The main function of PS lies in maintaining normal respiratory mechanics by reducing alveolar surface tension to prevent alveolar collapse.\textsuperscript{22} Besides, it also plays important roles in providing uniform lung inflation, improving efficiency of airway clearance, and alleviating lung inflammatory reaction. At present, exogenous surfactants derived from animal and/or artificial synthesis are wildly used for neonatal respiratory distress syndrome (NRDS) as an effective complementary therapy.\textsuperscript{23-25} Apart from pure animal-origined extracts such as Curosurf\textsuperscript{®} and Infasurf\textsuperscript{®}, there are many other kinds of lung surfactant compositions on the market. For instance, partial synthetic PS formulation such as Survanta\textsuperscript{®} and Surfactant-TA\textsuperscript{®}, as well as chemically synthesized pure PS
component like Surfaxin®. In addition, the PS-based microbubble formulations have also been developed. R. E. Pattle first discovered stable lung surfactant MBs derived from the observation of lung lavage in 1955. In the follow-up studies, the researchers found that MBs prepared by animal-sourced lung surfactant could be used as ultrasound imaging contrast agents and drug delivery carriers due to the better stability. However, current studies mostly focus on the lung surfactant derived from animals, which the safety issues like the risk of anaphylaxis remains big challenging. Furthermore, the ratio of surface-active proteins to lipid was uncontrollable because the animal individual differences may be variable from batch to batch. Therefore, novel surfactant preparations composed of essential surfactant protein analogs and synthetic phospholipids were developed to substitute animal-derived surfactants. Surfacin®, the first peptide-based synthetic pulmonary surfactant used in clinical application, has been approved by Food and Drug Administration (FDA) in 2012 for the treatment of NRDS. Sinapultide (KL4), a novel 21-amino-acid peptide, was used as surfactant protein analog in the formulation to mimic the function of the critical human pulmonary surfactant protein (SP-B). Compared with animal-derived pulmonary surfactant, such synthetic surfactant has a higher bio-safety and batch homogeneity. Therefore, in this study, based on the commonly used therapeutic surfactant replacement formulation, Surfacin® (Discovery Laboratories, Inc.), we have developed novel sinapultide loaded lipid MBs, which were filled with sulfur hexafluoride (SF6) in the core of MBs. It is first reported that the stable synthetic pulmonary surfactant MBs in high yields have been successfully prepared. Moreover, the effects of sinapultide concentration on acoustic imaging in vitro and stabilization mechanism of sinapultide MBs were also investigated.

### 2 Experimental

#### 2.1 Materials

Sinapultide (purity> 98%) was purchased from Paichage Pharmaceutical Technology Co., Ltd. (Zhengzhou, China). Fluorescein isothiocyanate-sinapultide (FITC-Sinapultide) (purity>98%) was purchased from GenScrip Biotechnology Co., Ltd. (Nanjing, China). Dipalmitoyl phosphatidylcholine (DPPC, purity >99%) was obtained from Southeast Pharmaceuticals Co., Ltd. (Suzhou, China). Phosphatidyl glycerol monosodium salt (POPG, Na, purity >99%) was purchased from Shanghai Advanced Vehicle Technology Pharmaceutical Ltd., Co. (Shanghai, China). Palmitic acid (PA, purity >98%) and dioctadecyl-tetramethylindocarbocyanine perchlorate (Dil C18, purity >98%) was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). Sulfur hexafluoride (SF6) with the purity of 99.99% was obtained from Qiangyuan Gas Co., Ltd. (Wuhu, China). All other solvents and reagents were analytical purity.

#### 2.2 Fabrication of microbubbles

The film dispersion and sonication method was used to prepare sinapultide loaded lipid MBs. Briefly, the optimized sinapultide loaded MBs formulation was composed of 0.1 mg of sinapultide, 23.22 mg of DPPC, 7.68 mg of POPG, Na, 4.21 mg of PA. The prescribed ingredients were synchronously dissolved in 2 mL of chloroform with ultrasound. Then, the organic solvent was evaporated under reduced pressure in a rotary evaporator for 2 h with a rotation speed of 110 rpm, and the water bath temperature was maintained at 25 °C. After all the organic solvent was removed from the mixture, the film was obtained after they were dried at 30 °C in a vacuum oven for 12 h. Phosphate buffered saline (PBS, 1 mL, pH=7.4 ± 0.1) was added to the sinapultide loaded lipid film in radius flask, after that, a water ultrasonic bath was carried for 15 minutes at 60 °C (30 kHz). And the precursor solutions for lipid-coated MBs were obtained. 1 mL aliquots of the precursor milk-white emulsion were placed in a 3 mL glass vial, and the vial was capped and sealed immediately. The collected emulsion was stored at 4 °C in tightly capped vials sealed with paraffin films for filling gas.

For SF6 was required as the filling gas, the air headspace of vial was replaced using a self-made gas exchange apparatus. MBs were produced by the homogenizer (Aoshen Instrument Co., Ltd., Bioprep-24, Hangzhou, China), and the vial was shaken at a speed of 4.0 m/s for 60 s. The shaking action brought the gas to the aqueous phase and obtained a suspension of MBs. The control MBs without sinapultide were prepared using the same procedures as those mentioned above just without adding sinapultide.

### 2.3 The sinapultide loading efficiency measurement

In order to measure the amount of sinapultide encapsulated in the MBs, the centrifugation method was used. 500 μL of sinalpultide MBs for different prescriptions were dispersed in PBS (5 mL, pH 7.4 ± 0.1), initially by centrifuging at 3000 rpm for 5 min in syringe. Then the upper layer of foam was obtained, after which 2 mL of 60% Acetonitrile/ water was added to the separated MBs to destroy and dissolve the loaded sinapultide. After separation, the amount of sinapultide loaded lipid MBs was measured by high performance liquid chromatography (HPLC) using a reverse-phase C18 column (250 mm× 4.6 mm, 5 μm particle size; Dikma Corp., Beijing, China). The mobile phase conditions were Acetonitrile/ water solution (60:40, v/v) with 0.1% trifluoroacetic acid (TFA) at 30 °C at a flow-rate of 1.0 mL/ min. The entrapment efficiency (EE) was calculated as equation (1):

\[
EE (%) = \frac{W_L}{W_T} \times 100\% \tag{1}
\]

where \( W_L \) was the amount of sinapultide entrapped in MBs, and \( W_T \) was the total amount of sinapultide added in the solution.

The loading capacity (LC) (mass %) was calculated as equation (2):

\[
LC = \frac{W_L}{W_T} \times 100\% \tag{2}
\]
2.4 Structural and physicochemical characterization

Samples in PBS solution were placed on glass slides and observed with an inverted microscope (Nikon, ECLIPSE-TS100, Tokyo, Japan). The morphological structure of ultimately obtained MBs was further observed by transmission electron microscopy (TEM) (JEOL, JEM-2100EX, Tokyo, Japan) and scanning electron microscopy (SEM) (Carl Zeiss, Ultra-Plus, Oberkochen, Germany). Samples were dispersed on copper grids followed with negative staining by 2% phosphotungstic acid for TEM analysis. For TEM analysis, sample solution was dispersed onto a silicon wafer and then be dried at room temperature. For a more detailed view of the assembled structure of sinalpultide on MBs, laser scanning confocal microscopy (LCSM) (Leica, TCS-SP8, Solms, Germany) was used to determine the morphology of Di labelled MBs. The mean diameter size of the bubbles was measured by the particle size analyser (Beckman Coulter, California, USA). The zeta potential of MBs were examined using Zeta PALS instrument (Brookhaven Instrument Corporation, Austin, TX). The MBs for zeta potential analysis were diluted with PBS (pH 7.4). Measurements were carried out at 25 ± 1 °C. Each sample was tested in triplicate.

2.4 In vitro ultrasound imaging evaluation

For acoustic imaging evaluation of sinalpultide MBs in vitro, we used a laboratory made agar phantom with the similar ultrasonic parameters to human soft tissue, which was composited with 3.1 % agar, 92.7 % distilled degassed water, and 4.2 % glycerol. A round hole with a depth of 20 mm was prepared in the gel phantom to load samples. The testing samples were imaged by Visual Sonics micro imaging Vevo 2100 systems (FUJIFILM Visual Sonics, Inc., USA) and a transducer of MS-250 was used. Transmit frequency was set at 18 MHz and the acquisition contrast gain was 35 dB. All parameters were unchanged throughout the imaging process, and degassed water was tested to confirm a clear background signal before sampling. Then sinalpultide MBs with different concentrations were injected into the gel phantom to be imaged. The mean power intensity under B-mode and contrast mode were measured in the Region of Interest (ROI).

2.5 Molecular dynamics simulations

In order to study the interaction between the sinalpultide molecules and lipid agents, the molecular dynamic simulation was applied. We used a coarse grained (CG) force field, MARTINI, for the larger length-scale and longer time-scale system. In our system, two symmetric monolayers were at the two vacuum-water interfaces. Each monolayer was constructed by 300 DPPC lipids and 92 POPG lipids according to the ratio of prescription in experiments (Table S1). POPG lipid contained a glycerol group (P4 bead) instead of a choline moiety (Q4 bead) in DPPC (Fig. 1A, B). Therefore, we replaced 184 water beads by cations (NA). Sinapultide (KL4) was a synthetic product containing a 21-amino acid. Mapping of helical sinalpultide was in line with new Martini force fields. We chose Qd bead for the positively charged Lys in neutral pH system because the pK value of Lys was ~10.40. Similarly, we also replaced the corresponding amount of water beads by anions. We ran 100 ns equilibrium simulation for DPPC and POPG bilayer, and a sinalpultide molecule (Fig. 1C) in bulk water to obtain an appropriate structure with lower energy.

Subsequently, 0, 2, 4, 8, 16 and 24 energy-optimized sinalpultide was inserted into the interface between water slab and each monolayer respectively. Then, we performed 400 ns equilibrium simulation for every systems to relax any steric conflicts. We observed all of the sinapultide laid parallel to the polar head groups of monolayer and were wrapped by lipids partially (Fig. 1D), which was consistent with the previous experimental research. Finally, we used NVT ensemble for our simulations. The z-direction scale of box kept constant, while the x/y-direction scale of box was expended and compressed at the same ratio. We obtained the surface tension under different monolayer areas and the critical areas where some lipid pores formed, which would simply illustrate effects of the sinalpultide concentration on the stability of monolayer (DPPC/POPG).

Fig. 1 Martini-based snapshots of DPPC (A) and POPG (B) molecules. Two molecules were composed of amine groups (blue), phosphate groups (brown), glycerol groups (pink) and carbon chains (cyan). (C) Snapshot of a coarse-grained sinalpultide (KL4) including helical backbone structure (green) and amino acid side chains (yellow). (D) Snapshot of a sinalpultide wrapped by lipids partially on the monolayer.

All simulations were performed with GROMACS 4.5.4 simulation package. The cutoff of van der Waals interactions was 1.2 nm. In order to reduce the cutoff noise, both Lennard-Jones potential and Columbic potential were smoothly shifted to zero between 0.9 nm and 1.2 nm. Lipids, water/neutralizing counterions and sinalpultide were coupled separately to Berendsen heat baths at T = 300 K with a coupling constant τ = 1 ps. Berendsen coupling schemes for both pressure (semi-isotropic, coupling constant of 4.0 ps, lateral reference pressure of -16 bar, compressibility in the x-y plane of 3 × 10^{-5} bar^{-1} and in the z-axis of zero) were used to establish a NPT ensemble for equilibrium simulations. 200 ns separated MD simulations for different x-y scale systems were carried out using NVT ensemble.

2.6 Statistical analysis

The data obtained were expressed as mean ± SD (standard deviation). Statistical analysis was performed by Student’s t-test for two groups and one-way analysis of variance for multiple groups. All experiments were conducted at least in triplicate.

3 Results and discussion

3.1 Characterization of sinalpultide-loaded MBs at different concentrations
The construction of sinapultide loaded lipid MBs is illustrated in Fig. 2A. In this study, we first used film-dispersion and sonication method to prepare suspension for MBs, and then SF₆ was used as the filling gas to obtain the sinapultide loaded MBs. Since both sinapultide and phospholipid components indicate the superior biocompatibility, the sinapultide-based MBs can be potentially used for in vivo application. The hydrophobic sinapultide could be efficiently encapsulated in the MBs. When sinapultide concentrations in prescription were 0.05, 0.1, 0.2, 0.4 and 0.8 mg/mL, the encapsulation efficiencies were 39.5 ± 2.1%, 46.3 ± 1.6%, 35.4 ± 0.9%, 21.3 ± 1.5%, 15.6 ± 1.7% in turn, and the LCs were 3.16 ± 0.3%, 3.67 ± 0.2%, 3.92 ± 0.4%, 4.33 ± 0.2%, 4.79 ± 0.5%, respectively.

Fig. 2 (A) Schematic representation of the lipid microbubble with encapsulated sinapultide. Results of (B) mean diameter, (C) zeta potential, and (D) microbubble yields of different formulations with the concentrations were set at 0.0, 0.05, 0.1, 0.2, 0.4 and 0.8 mg/mL.

Based on the fixed input amount of gas and lipids, formulation with different concentrations of sinapultide were screened. The average size, zeta potential, as well as concentrations of MBs were tested. Experimental results shown in Fig. 2B-D demonstrate that the optimal concentration of sinapultide was 0.1 mg/mL. With the optimized formulation, the sinapultide MBs are with mean diameter of 1.82 ± 0.15 μm, zeta potential of -55.2 ± 3.9 mV, and concentration of 6.37 × 10⁹ MBs per mL. There was no significant difference in the diameters of the different concentrations of sinapultide MBs. Therefore, it can be concluded that the sinapultide content changes will not significantly affect their size. Besides, the high negative charges of sinapultide loaded MBs can endow the MBs with good dispersion stability as proved in previous studies. Furthermore, the stability of sinapultide microbubble formulation was tested. The parameters of zeta potential, mean diameter, as well as microbubble yields were analyzed. The results showed that the mother liquor of sinapultide suspension can keep good stability in 3 months (Fig. S1).

Inverted microscopy images, TEM images, and LCSM images of sinapultide MBs, FITC labeled sinapultide MBs were shown in Fig. 3A-C, respectively. Fluorescent images of FITC-labeled MBs showed that sinapultide was distributed in lipid shell (Fig. 3B). In order to further quantitatively confirm that sinapultide was carried by lipid layer successfully, fluorescence intensity was tested by flow cytometer. The results showed that sinapultide labelled by FITC indicated more than one hundred times of fluorescence intensity compared with non-sinapultide MBs, as shown in Fig. 3D, E. Moreover, the SEM images and elemental analysis to sinapultide MBs were displayed in Fig. 4A, B. From results of elemental analysis, we can see that nitrogen, sulfur, and fluorine etc. elements are existed in sinapultide MBs.

Fig. 3 Microscopy image of sinapultide (A) and FITC-labeled sinapultide-loaded lipid microbubbles (B) in bright fields. Insets in (A) was the TEM image of microbubbles loaded with sinapultide, and the scale bar was 500 nm. C) Laser Confocal Scanning Microscope (LCSM) images of sinapultide-loaded lipid microbubbles under excitation wavelength of 543 nm (in red color) and 488 nm (in green color), and yellow picture was the fusion image. (D) Fluorescence intensity to microbubbles was analyzed by flow cytometer. (a) Microbubbles without sinapultide, (b) sinapultide-loaded lipid microbubbles, (c) FITC-labeled sinapultide-loaded lipid microbubbles. (E) Quantitative diagram of fluorescence intensity.
3.2 Ultrasound imaging evaluation of sinapultide-loaded MBs in vitro

In vitro ultrasound imaging was performed using the MBs coated with sinapultide at different concentrations. Since the dynamic behavior of MBs in ultrasonic field was affected by the viscoelastic characteristics of their shells, which depends on the composition of the membrane shells to coated MBs, the assembly of sinapultide onto the surface of the MBs at different concentrations may change the membrane shell composition, structure and stability. Thus, the signal in the US images may be changed with the increase of sinapultide concentration.

Fig. 5A is the typical US images at 0, 5, 10, 15, 20 and 30 min after different sample addition into the phantom. The acoustic imaging analysis in vitro indicated that ultrasound imaging enhancement also could be acquired under B-mode ultrasonography, as shown in Fig. 5B. Furthermore, in order to study the effect of sinapultide concentration on the US enhancement effect, both the contrast mean power and B-mode mean power were calculated quantitatively from the average grayscale values of the ROIs of the images, which were recorded at 0, 5, 10, 15, 20 and 30 min. The plotted curves in Fig. 5B indicate that the US enhancement level and duration time were related with the concentrations of sinapultide. And the group of sinapultide MBs with 0.1 mg/mL has the brightest US imaging. The quantitative analysis according to the Fig. 5A, Fig. 5D indicates that sinapultide loaded MBs remain better US imaging enhancement within 30 min observation time except the group of MBs with 0.8 mg/mL sinapultide. Based on the US imaging, it is indicated that sinapultide MBs with 0.1 mg/mL concentration has the longest duration time compared with other groups and this optimized sinapultide MBs could last for more than 30 min in vitro. Thus it is demonstrated the potential of its clinical application as a contrast agent.

3.3 Stabilization mechanism of sinapultide MBs

In order to fully understand the stabilization mechanism of sinapultide for MBs, the simulation results in Fig. 6A exhibits the distribution of 0, 2, 4, 8, 16, 24 sinapultide on the lipid monolayer respectively. All of them lay parallel on the polar surface of monolayer. Part of each peptide was wrapped by lipids. Each of them occupied a relatively independent space. It was hard to overlap along the vertical direction with each other. They maintained steadily helical structure and cylinder shape. There was no apparent deformation though the system with 24 sinapultide was very crowded. The surface tension of the monolayer was calculated according to the equation:

\[ \gamma_m = (P_N - P_L) \cdot L_x / 2 \]

where \( P_N \) and \( L_x \) were the normal pressure and size of the box. The lateral pressure \( P_L \) was the average of \( P_{xx} \) and \( P_{yy} \). After NPT equilibrium simulations at lateral reference pressure of -16 bar, we expanded x and y of the box by 0.25 nm and performed 200 ns NVT simulation with each iteration, until a pore formed on the monolayer. We calculated all the surface tension at different areas of monolayer. The profiles of surface tension-area of monolayer for different number of sinapultide was shown in Fig. 6B. The surface tension increased as the area of monolayer enlarged during the first stage. The trend was similar to the results in previous reports. The right shift of curve with the increase of sinapultide indicated they could reduce the surface tension at the same area. This was consistent with SP-B which could decrease the surface tension of pulmonary alveolus. It was also reported that the sinapultide spatial structure resembles an amphiphatic domain of SP-B. However, after the peak of the curve, the surface tension would decline with larger area abnormally corresponding to the end dash point in Fig. 6B. It was because the monolayer ruptured and a pore formed at this point. We defined the area as critical area and the surface tension as critical surface tension corresponding to the last solid point, where the monolayer was about to rupture but not yet. These could be considered as the maximum area and surface tension that the system could be expanded to at a stable state.

More amount of sinapultide induced larger critical areas when the number of sinapultide was less than 16, but the critical area decreased with 24 sinapultide on a monolayer in Fig. 6C. The results
of simulations illustrated the monolayer could be expanded to larger area without breaking at lower concentration of sinapultide, thus improving the stability of MBs efficiently. However, the overdose of sinapultide would result in rupture of monolayer at smaller area. The two effects were very consistent with the inference in experiments. Besides, we observed the critical surface tensions with 0-16 sinapultide were within the range of 46-50 mN/m and the difference was very small in Fig. 6B. The right shift of curve would lead to the larger critical area when the monolayer reached to the similar critical surface tension with more sinapultide. On the contrary, 24 sinapultide could also shift the curve to right, but the critical surface tension was much less than them of 0-16 sinapultide. Therefore, the rupture occurred at smaller area, and the stability of MBs will fall into decline. The mechanism and experimental results also have the meaning of theory and practice for the preparation of other polypeptides MBs.

The plot of the number of sinapultide – critical area of monolayer. The end point of each profile was marked by dash to illustrate the critical area where lipid pores formed initially. Black, blue, cyan, green, yellow and red colour was used to distinguish the 0, 2, 4, 8, 16 and 24 sinapultide on one monolayer respectively. (C) The plot of the number of sinapultide – critical area of monolayer. Overhead view of 0, 2, 4, 8, 16 and 24 sinapultide on the monolayer corresponding to (a-f) snapshots respectively.

**4 Conclusions**

In this study, novel sinapultide stabilized MBs were developed by film-ultrasonic method based on the phospholipid and synthetic lung surfactant formulation. By studying the structure and physicochemical properties of the sinapultide MBs, it is found that the appropriately sized MBs with regular morphological character could be prepared. The introduction of sinapultide does not significantly affect the size and zeta potential of the MBs. The *in vitro* ultrasound imaging evaluation demonstrated that the optimized sinapultide concentration (0.1 mg/ mL) could maintain the better stability and US imaging enhancement. Moreover, results of molecular dynamics simulations show that the addition of sinapultide can efficiently decrease surface tension to MBs. Accordingly, the stability of the MBs has been greatly improved. Compared with MBs prepared by animal-sourced pulmonary surfactant, sinapultide MBs, fabricated by synthetic pulmonary surfactant not only possess the satisfied immunogenicity, but also have good batch homogeneity. Overall, synthetic lung surfactant microbubbles show a promising clinical potential as an ultrasound contrast agent. It should be possible to use the sinapultide MBs as drug delivery system and as a potential theranostic agent in biomedical applications.55, 56

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Sinapultide-loaded lipid microbubbles and the stabilization effect of sinapultide on the shell of lipid

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Sinapultide-loaded lipid microbubbles are fabricated for ultrasound imaging, and the stabilization mechanism was investigated by molecular dynamics simulation.