Label-free discrimination of membrane-translocating peptides on porous silicon microfluidic biosensors

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A label-free optical sensor was constructed by integrating electrochemically etched porous silicon (pSi) and supported phospholipid bilayers in a microfluidic chip. The translocation of peptides through the phospholipid bilayers could induce a red shift in effective optical thickness of the pSi layer, which could be monitored by reflective interferometric Fourier transform spectroscopy. By measuring the kinetic data of membrane translocating on the phospholipid bilayers/pSi chip, the relationship between the behavior of membrane-translocating peptides (MTPs) and translocating mechanism was established. With these optical data, MTPs with different action modes on the cell membrane can be correctly discriminated. The bio-functionalized microfluidic sensor will provide a reliable and cost-effective platform to study the transmembrane behavior of peptides, which is of great importance in the MTP screening and peptide function study. *Published by AIP Publishing.*

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I. INTRODUCTION

During the last decades, a number of peptides presenting the ability to translocate across biological membranes were identified and thoroughly studied.¹ Membrane-translocating peptides (MTPs) with different structures displayed specific functions due to their different action modes with the cell membrane.^{1,2} Some of the peptides may just penetrate into the cytoplasm without disrupting the cell membrane, while others can severely interact with the cell membrane, resulting in pore formation or disruption of the cell membrane. The latter ones were usually classified as antimicrobial peptides (AMPs) due to their bacterial killing effect. On the contrary, peptides without the strong ability of membrane perturbation have been successfully used for intracellular delivery of different cargoes including nanoparticles, proteins, liposomes, and nucleic acids.³ Therefore, studying the action mode of peptide on the cell membrane is a very important step in the screening and discovery of new functional peptide.

Until now, the interaction of MTPs with the cell membrane was mostly studied by cell or vesicle-based and fluorescent probe labelled methods.⁴ However, fluorescent labels might influence the natural peptide structure or membrane permeability. On the other hand, the active defence system of real cell lines may slow down the rate of peptide-membrane interaction, resulting in a low screening efficiency. Artificial cell membranes (ACMs) have been extensively used to mimic the real cell membrane and study the interaction and transmembrane behaviour of molecules.^{5,6} Black phospholipid bilayers and vesicles coupled with fluorescent and electrical detection have been used in MTP screening.^{7,8} However, to learn the mode of action on the cell membrane, kinetic translocation data are needed. Currently, dynamically observation of the transmembrane process of peptide usually relied on some complicated methods, such as circular dichroism (CD) spectroscopy, nuclear magnetic resonance (NMR),⁹ and X-ray.¹⁰ Phospholipid bilayers supported or tethered on the solid surface coupled to biosensors have also been used to study the transmembrane behaviour.^{11,12}

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Porous silicon (pSi) has been used as the solid support for phospholipid bilayers due to its good biocompatibility and sensing ability.^{13–21} pSi with a well-controlled pore structure can produce an interferometric optical response when substances enter into the pSi, due to the change of refractive index in this layer.²² Meanwhile, the pSi layer can also provide an internal environment, allowing the transportation, diffusion, and exchange of ions or molecules across the ACMs. In the present work, phospholipid bilayers were self-assembled on the pSi chip, and the membrane translocation behavior of peptides through phospholipid bilayers was studied on the platform. With the assistance of reflective interferometric Fourier transform spectroscopy (RIFTS), the permeability and dynamics of peptide translocation through the phospholipid bilayers can be studied by real-time monitoring the change of effective optical thickness (EOT) of the pSi layer. The purpose of this work is to develop a label-free, cost-effective, and reliable system to establish a relationship between the kinetic data of peptide translocation and their action mode on cell membranes.

II. MATERIALS AND METHODS

A. Preparation of pSi sensing layer

Single-crystal, polished p^{++} -type silicon wafers (boron doped, $0.0012\ \Omega\text{cm}$ resistivity, (100)-oriented) were purchased from Siltronix Corp. (France). The wafer was first cleaned with piranha solution at 90°C for 1 h. Then, the wafer was rinsed with deionized water, followed by ultrasonic processing in ethanol and deionized water for 15 min, respectively. The schematic procedure for preparing the sensing layer is illustrated in Fig. 1. Briefly, pSi was prepared by electrochemically etching a cleaned silicon wafer in a 4:1 (v/v) solution of 40% aqueous hydrofluoric acid (CAUTION: HF is highly toxic and contact with skin should be avoided) and ethanol. The Si wafer with an exposed area of $1.13\ \text{cm}^2$ (diameter = 12 mm) was placed in contact with a strip of aluminum foil and mounted in a Teflon etching cell with a platinum counter electrode. The wafer was etched at a constant current of 77 mA for 180 s. The freshly prepared

FIG. 1. Schematic procedure for the preparation of the porous silicon microfluidic sensor integrated with supported phospholipid bilayers. (Left) Preparation of the PDMS film patterned with the microchannel (1.5 cm in length, $450\ \mu\text{m}$ in width, and $250\ \mu\text{m}$ in depth) and microwell acting as the detection zone (2 mm in diameter). (Middle) Procedure for the preparation of the pSi sensing layer. The pSi and PDMS film were sealed together by plasma treatment. (Right) Procedure for preparation of Small Unilamellar Vesicles (SUVs). (Bottom) The SUV suspension was injected into the microchip and self-assembled on the pSi substrate to form phospholipid bilayers.

pSi was thermally oxidized at 800 °C for 20 min in a muffle furnace to form a hydrophilic surface. The porosity of pSi was measured by the spectroscopic liquid infiltration method (SLIM).²³ The thickness and surface roughness of the pSi layer were measured by scanning electron microscopy (SEM) and atomic force microscopy (AFM), respectively.

B. Preparation of the microfluidic chip

The channel patterned polydimethylsiloxane (PDMS) film was fabricated with the commonly used method²⁴ shown in Fig. 1. A layer of SU-8 gel (MicroChem Corp., USA) was spin-coated on a cleaned silicon wafer. After being soft baked at 95 °C for 1 h, the SU-8 layer was covered with a photo mask designed with CorelDraw software (Corel Inc.) and exposed to ultraviolet light for photo-polymerization. Then, the SU-8 was baked at 95 °C for 30 min followed by development to obtain SU-8 mold. PDMS (Sylgard 184, Dow Corning) was mixed with the as supplied cross-linking reagent in its standard volume ratio (10:1) and poured on the SU-8 mold. The wafer was baked at 75 °C for 90 min to completely crosslink the PDMS. At last, the PDMS film patterned with microchannels and holes was peeled off. Then, both the PDMS film and the pSi chip were treated with oxygen plasma and sealed with each other.

C. Preparation of supported phospholipid bilayers in microfluidic chip

The preparation of supported phospholipid bilayers (PLBs) in the microfluidic chip was similar to that of our previously reported work.²⁵ Dioleoyl Phosphatidylcholine (DOPC) was purchased from A.V. T. Pharmaceutical, Ltd. (China). N-(7-Nitrobenz-2-Oxa-1,3-Diazol-4-yl)-1,2-Dihexadecanoyl-sn-Glycero-3-Phosphoethanolamine (NBD-PE) was purchased from Invitrogen (USA). 10 mg DOPC was dissolved in 200 μ l chloroform. After being evaporated under nitrogen, the lipids were kept in a vacuum for 2 h to ensure no residue chloroform. Then, the lipids were suspended in 1 ml phosphate-buffered saline solution (1 $\times$ PBS, pH 7.4). The mixture was sonicated for another 30 min to obtain small unilamellar vesicles (SUVs). The fluorescent SUVs were prepared by the same method except that the DOPC was mixed with 1% NBD-PE. As shown in Fig. 1, SUV suspension was injected into the microfluidic channels and heated at 50 °C for 30 min to break up the vesicles into lipid fragments. Then, the chip was kept at 37 °C for another 3 h to let the lipids fuse on the hydrogel surface. All processing must be undertaken in a water bath environment. The surface assembled with bilayers was also measured by AFM.

D. Fluorescence recovery after photobleaching (FRAP)

Fluorescence recovery after photobleaching (FRAP) experiment was taken to verify the mobility of the bilayers supported on pSi. 10.0 mg/ml DOPC spiked with 1% NBD-PE was self-assembled on pSi to form fluorescent phospholipid bilayers through the same method described in the above paragraph. A fluorescence microscope (DFM-55C, Caikon Co.) was coupled to a CCD camera (MC-20, Tucson Co. China) and a miniature spectrometer (Ocean Optics USB 2000+). The excitation and emission wavelengths were selected at 480 nm and 532 nm, respectively. Photo-bleaching processing was carried after the fluorescent phospholipid bilayers was exposed to the light irradiation through a 40 $\times$ objective lens until the fluorescence of phospholipid bilayers was totally bleached. After removing the light source, the recovered fluorescent signal was measured at a time interval of 0.5 min.

E. Detecting the optical signal induced by the translocation of peptides

White light from a tungsten lamp (Ocean Optics R-LS-1) was fed through one end of a bifurcated fiber-optic cable and focused through a lens onto the detection zone (diameter $\sim$ 2 mm) of the microchip (Fig. 2(a)). The light spot size is approximately 1 mm in diameter. Light reflected from the film was collected through the same optics and input into a miniature spectrometer (Ocean Optics USB 2000+). The light path on porous Si (Fig. 2(b)) and resulting reflectivity spectra were recorded in the wavelength range of 200–850 nm (Fig. 2(b)) with

FIG. 2. (a) Schematic setup for the measurement of peptide translocation on the optical biosensor integrated with phospholipid bilayers. (b) The light path of RIFTS measurement on the pSi chip. (c) The reflectometric interference spectrum of the pSi displays a Fabry-Pérot interference spectrum. (d) The corresponding Fourier transformed spectrum, whose peak position corresponds to the $2nL$ value of the pSi layer.

Spectrasuit software (Ocean Optics Inc.). The effective optical thickness (EOT) of the pSi sensing layer was calculated with the Fabry-Pérot relationship

$$2nL = m\lambda,$$

where λ is the wavelength of maximum constructive interference for a spectral fringe of order m . n and L represent the average refractive index and thickness of the sensing layer, respectively. The quantity of $2nL$, or the EOT, was determined by Fourier transformation of the reflectance spectrum calculated with the IGOR program (Wavemetrics, Inc.). Fourier transformation resulted in a sharp peak whose position was equal to the product value of $2nL$ (Fig. 2(d)). Solutions were driven by a syringe pump that was connected to the microfluidic chip through polytetrafluoroethylene (PTFE) tube. $1 \times$ PBS buffer (pH 7.4) was introduced into the microfluidic chip at a flow rate of $20 \mu\text{l}/\text{min}$ until a stable base line reached. After 20 min, peptide solution was pumped through the flow cell. Interference spectra were collected every 10 s and the time course of EOT value was obtained with IGOR software. With this device, the signal of peptide translocation through phospholipid bilayers could be measured.

F. Study the transmembrane behaviour of cecropin A

Cecropin A and scrambled peptide with the same amino acid component but different sequences were synthesized by ChinaPeptides Co., Ltd. The peptides were stored at -20°C and freshly dissolved in PBS to form $500 \mu\text{g}/\text{ml}$ solution before use. Before phospholipid bilayers formed, control peptide was injected into the channel after equilibrium with $1 \times$ PBS buffer (pH 7.4). After phospholipid bilayers formed, the phospholipid bilayers inserted chip was first equilibrated with PBS and then injected with control peptide solution or cecropin A solution.

For contrast, anti-bacterial activity of cecropin A was tested by *E. coli*. Cecropin A and scrambled peptide with a concentration of $500 \mu\text{g}/\text{ml}$ was each 1:1 (v/v) mixed together with

suspending bacterial solution with a concentration of 1.0×10^8 cfu/ml, respectively. After interaction for 1 h, the bacteria viability was evaluated by the plate counting method.

G. Study different interaction behaviour of cell membrane and peptides

Cecropin A, hIAPP, and TP2 peptides with >90% purity were synthesized by ChinaPeptides Co., Ltd. The peptides were stored at -20°C and freshly dissolved in PBS to form 50, 100, 200, and 500 $\mu\text{g/ml}$ solution before use. A synthetic peptide that has the same amino acid composition but with a scrambled sequence as cecropin A was used as a negative control, while dermaseptin was used as a test peptide. Before phospholipid bilayers formed, control peptide was injected into the channel after equilibrium with $1 \times$ PBS buffer (pH 7.4). After phospholipid bilayers formed, the pSi/phospholipid bilayers' chip was first equilibrated with PBS and then injected with control peptide to make sure the integrity of phospholipid bilayers and lastly injected with each peptide solution. The RFTS signal of each chip was recorded.

H. Cell culture and stain experiment

HepG2 cell lines (ATCC CRL-11997) were cultured in standard condition. Dual dyes, 20 mg/ml fluorescein diacetate (FDA, purchased from Aladdin Industrial Inc.) and Dil (purchased from Beyotime), were dissolved in dimethyl sulfoxide (DMSO) as stock solution. Before use, the dyes were freshly 1:1000 diluted into colorless cell culture medium. The cells were first stained with FDA for 10 min and then with Dil for another 10 min. After being washed with colorless cell culture medium, 500 $\mu\text{g/ml}$ cecropin A, hIAPP, and TP2 peptide solution was added into the culture medium and co-cultured for 24 h. While dermaseptin was co-cultured for 5 min. Colorless culture medium was used as blank control. Images were captured by a confocal laser scanning microscope (CLSM) (Nikon A1).

III. RESULTS AND DISCUSSION

A. Characterization of pSi supported phospholipid bilayers' microfluidic chip

Scanning electron microscopy (SEM) shows that the pore diameter and thickness of pSi layer are ~ 14 nm and $5.7 \mu\text{m}$, respectively (Fig. S1, [supplementary material](#)). The SLIM measurement indicates that the porosity of pSi is $\sim 60\%$. Atomic force microscopy (AFM) in the tapping mode indicated that the average roughness of the pSi surface was 0.752 nm (Fig. S2(a), [supplementary material](#)). The roughness on this scale had been assumed to not drastically modify the membrane behaviour.¹⁹ Phospholipid bilayers on pSi were characterized with optical and electrochemical methods. As shown in the AFM image (Fig. S2(b), [supplementary material](#)), the average roughness of the pSi surface changed from 0.752 nm to 0.524 nm after being coated with phospholipid bilayers. The electrochemical method can also give clear evidence of phospholipid bilayers assembling on pSi, which acted as a working electrode for electrochemical study. Steady state current measurements were performed in a $1 \times$ PBS (pH 7.4) electrolyte containing 0.02 M $\text{Fe}(\text{CN})_6^{3-}$ as a redox probe (Fig. S3, [supplementary material](#)). The results showed that the reductive current of $\text{Fe}(\text{CN})_6^{3-}$ reduced more than 90% after the assembly of phospholipid bilayers on pSi, indicating a significant blocking effect of phospholipid bilayers to redox species. To clarify whether the phospholipid bilayers retain mobility on pSi, green fluorescent probe *N*-(7-Nitrobenz-2-oxa-1,3-diazol-4-yl) (NBD) labeled phospholipid bilayers were self-assembled on the surface of the pSi chip. The fluorescent image clearly showed the process of photo-bleaching and fluorescent recovery during the whole experimental time. The fluorescence recovery after photobleaching (FRAP) results proved that the phospholipid bilayers supported on pSi still displayed good fluidity on the pSi chip (Fig. S4, [supplementary material](#)).

B. The principle of peptide translocation biosensor based on pSi supported phospholipid bilayers' chip

To check the integrity of phospholipid bilayers assembled on pSi, the signal of peptide translocation across the phospholipid bilayers was detected by RIFTS (Fig. 2). Insulin is a non-transmembrane peptide and thereby selected as a probe to test the integrity of phospholipid bilayers, which do not allow the translocation of the peptide. As a control experiment, the RIFTS measurements were also conducted on a bare pSi chip (without phospholipid bilayers). The microfluidic chip was in pre-equilibrium with PBS buffer, until the EOT value reached a constant value. After the insulin sample was loaded into the microfluidic channel, a large increase in EOT value (>30 nm) could be observed (Fig. 3, black trace), because the insulin molecules could freely enter into the bare pSi layer. In contrast, for the pSi with phospholipid bilayers layer, there was no obvious EOT shift after the PBS buffer was replaced by the insulin sample (Fig. 3, red trace). The results indicate that the phospholipid bilayers self-assembled on the pSi surface can effectively block the mass transfer of insulin from the buffer solution to the pSi layer, confirming the good integrity of the self-assembled phospholipid bilayers on the pSi surface.

Reflectometric interference spectroscopy provides a novel method to monitor the infiltration of peptides into the porous layer.^{26,27} In the context of the present work, the value of $2nL$ measured from the pSi chip will increase when peptide infiltrates the film, indicating the peptide translocating through phospholipid bilayers.

C. Detection of transmembrane signal on pSi supported phospholipid bilayers' chip

To test the applicability of our sensing platform in screening of membrane translocating peptides, the translocation of cecropin A (amino acid sequence shown in Table S1, [supplementary material](#)) across phospholipid bilayers supported on the pSi was observed by RIFTS.

Cecropin A²⁸ belongs to a family of antimicrobial peptide active against both Gram-positive and Gram-negative bacteria. The peptide can induce bacterial death by permeating their plasma membrane. This permeation occurs following the adhesion and insertion of the peptides to the bacterial lipid bilayer, finally forming transmembrane pores. For contrast, control peptide

FIG. 3. (a) Scheme illustrating the mechanism of integrity test on the phospholipid bilayers supported on porous silicon (pSi). For blank pSi, insulin can directly enter into the pSi layer (A, upper), resulting in optical response (b, black trace). After phospholipid bilayers self-assembled on the surface of pSi, insulin was blocked by phospholipid bilayers (a, bottom) without EOT response (b, red trace).

with the same amino acid component (Table S1, [supplementary material](#)) but a scrambled sequence was employed. Without phospholipid bilayers, the EOT of the sensing layer quickly increased after the injection of cecropin A (Fig. 4(a), black) or the control peptide (Fig. S5, [supplementary material](#)). After the assembling of phospholipid bilayers on pSi, there was no obvious response upon injection of control peptide (Fig. 4(a), red). But for the cecropin A, the EOT value increased around 100 nm (Fig. 4(a), green), indicating the occurrence of peptide translocation across the phospholipid bilayers' membrane. Due to the blockage effect of phospholipid bilayers, the increasing rate and the shift of EOT on the pSi with phospholipid bilayers are significantly less than those on pSi without phospholipid bilayers. To confirm the bioactivity of the two samples, *E. coli* was treated with PBS (Fig. 4(b), upper), control peptide (Fig. 4(b), middle), and cecropin A (Fig. 4(b), bottom), respectively. Bacteria growth on each sample was measured by the standard plate count method. The bioactivity test proved that the cecropin A sample displayed strong bacterial killing ability, while no antimicrobial activity was found on the control peptide. The results obtained with *in-vitro* detection coincided with those of RIFTS measurements.

D. Dynamic membrane translocation behaviour of different peptides

Until now, there has been no real consensus regarding the mechanism of membrane translocation for peptide. Herein, we classified the membrane-translocating behaviour into three main entries: (i) direct penetration across the membrane; (ii) membrane damage-mediated entry; and (iii) membrane pore formation and translocation.

We used three types of peptides to represent their corresponding translocation mechanism (Table S1, [supplementary material](#)), in which the cecropin A,²⁸ TP2,²⁹ and hIAPP^{30,31} represent pore forming, direct membrane penetration, and membrane damage peptides, respectively. To obtain the kinetic information of peptide translocation across the phospholipid bilayers, the slope of the initial EOT response was calculated, since it is related to the initial transmembrane velocity of the peptide. For blank corrections, the slope of initial EOT response on pSi/phospholipid bilayers (S_1) and bare pSi (S_0) was measured, respectively (Fig. 4). In the present work, the slope ratio (S_1/S_0) was used as a parameter to represent the index of the initial transmembrane rate, whereas the τ value represents the time from initial EOT response to a constant EOT value when the translocation of peptide through the phospholipid bilayers reaches

FIG. 4. (a) The real-time RIFTS sensorgram of the pSi chip in response to cecropin A before (black trace) and after (green trace) being coated with phospholipid bilayers and the scrambled peptide (red trace) after being coated with phospholipid bilayers. The slope of initial EOT response on pSi/phospholipid bilayers (S_1) and bare pSi (S_0) was measured by linear fitting of the EOT increase in first 3 min. The τ value represents the time from initial EOT response to a constant EOT value when the translocation of peptide through the phospholipid bilayers reaches equilibrium. (b) *E. coli* colony after being treated with blank PBS (upper), control peptide (middle), and cecropin A (bottom). ΔEOT value meant the peak shift of the RIFTS spectrum shown in Fig. 1.

equilibrium (Fig. 4). Typically, τ is inversely correlated with the average rate of peptide translocation. A larger τ value means a slower average transmembrane speed.

For the direct penetration peptide (TP2), there is little interaction between peptide and phospholipid bilayers. Therefore, the peptide may directly cross over the phospholipid bilayers without any hindrance. As revealed by real-time RIFTS (Fig. 5), the slope of the initial EOT response on phospholipid bilayers/pSi and bare pSi is almost the same. In addition, a constant slope ratio ($S_1/S_0 \approx 1$) was observed within the tested peptide concentration. However, the τ value decreases with the increase of peptide concentration (Fig. 6(a)), because a higher peptide concentration usually displays a higher diffusion rate, thereby reducing the time needed for peptide accumulation in the porous layer. In contrast, the membrane damage mediated peptide (hIAPP) displays a smaller initial EOT response on phospholipid bilayers/pSi (S_1) than that on bare pSi (S_0), indicating that translocation of peptide was partially blocked by phospholipid bilayers supported on pSi (Fig. 5). With the increase of peptide concentration, the value of S_1/S_0 obviously increased, whereas the τ value significantly decreased after the concentration of peptide exceeded $100 \mu\text{g ml}^{-1}$ (Fig. 6(b)). The result is reasonable since the membrane damage rate depends on the concentration of the hIAPP peptide. A higher peptide concentration leads to a faster membrane damage rate. But, a small τ value was found on $50 \mu\text{g ml}^{-1}$ of hIAPP. Perhaps in low concentration range, the transmembrane behaviour of the peptide is different from that observed in a high concentration range. The detail mechanism behind the phenomenon is still unclear now. As to the pore-forming peptide, cecropin A, the S_1/S_0 value increased with the increase of peptide concentration, but a constant τ value was observed within the tested concentration range (Fig. 6(c)). We assume that initial slope ratio (S_1/S_0) is correlated with the pore forming rate, which depends on the concentration of cecropin A. Accordingly, the S_1/S_0 value increases with the increase of peptide concentration. However, the τ value is correlated with the pore density forming on the phospholipid bilayers. When the concentration of cecropin A exceeds a critical value, the pore density becomes constant. The initial peptide concentration ($50 \mu\text{g ml}^{-1}$) used in this work may be beyond this critical value; therefore, the peptide displayed a constant τ value at different concentrations. Based on the above

FIG. 5. The real-time RIFTS response of peptide translocation on the bare pSi chip (black trace) and PLBs/pSi chip (red trace). Three types of model peptides (TP2, Cecropin A, and hIAPP) were used to test the transmembrane behaviour at different concentrations ($50, 100, 200$, and $500 \mu\text{g ml}^{-1}$).

FIG. 6. Dependence of the slope ratio (S_1/S_0) and τ value obtained from real-time RIFTS spectrum on the concentration of (a) TP2, (b) hIAPP, (c) cecropin A, and (d) test peptide.

observation, hIAPP and cecropin A show different transmembrane behaviours although both of them are antimicrobial peptides.

As summarized in Table I, the three types of model peptides display different behaviors in their transmembrane process. Accordingly, discrimination of membrane translocating peptide or antimicrobial peptide can be potentially achieved based on the kinetic parameters measured by RIFTS. In order to validate the method, another peptide, dermaseptin, was employed as the validation peptide to test the method. As shown in Figure 6(d), the kinetic pattern of membrane translocation observed on dermaseptin is similar to that found on the pore-forming peptide. According to literature search, dermaseptin³² indeed has the similar amino acid length (Table S1, [supplementary material](#)) and the same action mode on cell membrane as that of cecropin A.

To further confirm the different membrane translocating behaviours of these peptides, the DOPC was mixed with 1% fluorescent labelled phospholipids (NBD-PE). Self-assembling of the phospholipids on the pSi surface followed the same methods described above. The fluorescent images were observed after the fluorescent labelled phospholipid bilayers interacted with different peptides, respectively. As shown in the fluorescent images (Figure S6, [supplementary material](#)), the phospholipid bilayers kept intact after the translocation of TP2 or cecropin A. In contrast, the phospholipid bilayers were obviously damaged after the hIAPP interacts with the membrane. However, the fluorescent observation fails to discriminate between TP2 and

TABLE I. The ratio of the Slope (S_1/S_0) and total interaction time (τ) in response to different concentrations (C) of peptide with different translocation mechanisms.

	Direct penetration	Pore-forming	Membrane-damage
S_1/S_0	≈ 1	$\propto C$	$\propto C$
τ	$\propto 1/C$	$= \text{Constant}$	$\propto 1/C$

cecropin A. The translocation of that each tested peptide through the phospholipid bilayers was also confirmed by Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF). After the phospholipid bilayers/pSi chip was subjected to the peptide translocating experiment as described above, the pSi chip was washed to remove phospholipid bilayers and peptides adsorbed on the outside surface. Then, the chip was then subjected to MALDI-TOF detection according to methods described in the [supplementary material](#). As shown in the mass spectra (Fig. S7, [supplementary material](#)), each type of model peptide (TP2, hIAPP, and cecropin A) and test peptide (dermaseptin) can be found in the pSi layer, indicating that these peptides indeed translocate through the phospholipid bilayers and infiltrate into the pore channel of the pSi chip. It should be noted that the hIAPP generates a complex mass spectrum compared with other types of peptides. The results also infer that only hIAPP can severely damage the phospholipid bilayers, resulting in the infiltration of phospholipids into the porous Si layer.

E. Study of membrane translocating behaviour with real cell stained with fluorescent dye

In order to test whether the results obtained on the label-free optical platform are consistent with the cell-based test, each type of peptide was cultured with HepG2, whose membrane and the cytoplasm were stained with red fluorescent dye (DiI) and green fluorescent dye, fluorescein diacetate (FDA), respectively. Fluorescent images in green, red, and merged channel were captured by a confocal laser scanning microscope (CLSM) to represent the status of cytoplasm, cell membrane, and whole cell. An intact cell structure was observed in the blank and the one treated with TP2 (Figs. 7(a1)–7(a3) and 7(b1)–7(b3)). Therefore, TP2 cannot be discriminated from blank control on CLSM platform. Both cecropin A and hIAPP caused partial cytoplasm leakage (Figs. 7(c1) and 7(d1)), but the cell membrane kept intact after they were treated with pore-forming cecropin A (Fig. 7(c2)). In contrast, the hIAPP broke the cell membrane structure and thereby weakened its fluorescent intensity (Fig. 7(d2)). The cell-based fluorescent measurement also classified the dermaseptin into pore-forming peptide due to the observed cytoplasm leakage (Fig. 7(e1)) and intact cell membrane (Fig. 7(e2)). In addition, cell-based test indicated that dermaseptin took less time (~ 5 min) than cecropin A (24 h) to display their bioactivity, even both of them belong to pore-forming peptide. This result has a similar trend to the data measured on our biosensor system, in which the τ value of dermaseptin is ~ 3 min (Fig. 6(d)),

FIG. 7. Fluorescent image of blank control (a), TP2 (b), cecropin A (c), hIAPP (d), and test peptide (e) in green, red, and merged channel of the confocal laser scanning microscope (CLSM).

while the τ value for cecropin A is ~ 30 min (Fig. 6(c)). Therefore, the cell-based results well fit the data obtained from the optical biosensing platform constructed from phospholipid bilayers/pSi microfluidics chip. Moreover, the discrimination of membrane translocating behaviour based on our biosensor platform is more distinct compared with the fluorescent method.

IV. CONCLUSIONS

In summary, this work demonstrated that the bio-microfluidic sensor based on phospholipid bilayers/pSi could well mimic the environment of the real cell membrane. With the simple and cost-effective system, the transmembrane behavior of membrane translocating peptides can be reliably acquired using real-time RIFTS. Further validation of this method by a library of membrane translocation peptide will lead to the practical application in the screening of peptide with membrane translocation ability. If combined with an optical imaging system, high throughput screening of cell penetrating peptides and antimicrobial peptides could be achieved. Furthermore, by constructing versatile bio-functional film on the pSi chip, the platform will find more applications in drug screening process.

SUPPLEMENTARY MATERIAL

See [supplementary material](#) for the scanning electronic microscopy image of porous silicon (Fig. S1), atomic force microscopy image (Fig. S2), steady state current measurements (Fig. S3), fluorescence recovery after photo-bleaching (Fig. S4), real-time RIFTS sensorgram of the pSi chip in response to cecropin A and control peptide (Fig. S5), fluorescent images of the NBD-labeled phospholipid bilayers supported on pSi (Fig. S6), MALDI mass spectra (Fig. S7), and the amino acid sequence of the peptides (Table S1).

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