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Mechanical detection of liposomes using piezoresistive cantilever

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Abstract

This paper firstly reports the mechanical and real-time detection of liposomes using the highly sensitive microfabricated piezoresistive cantilever sensor chip and liposome–protein interaction. The cantilevers were functionalized with the chemically modified protein C2A which recognizes the phosphatidylserine (PS) exposed on the surface of liposome. The attachment of liposome induced the bending motion of the piezoresistive cantilever and consequently generated the electrical signal changes. The sensitivity of the sensor was determined from the relationship between electrical signal and the number of liposome immobilized on the cantilever surface. This result will help challenge a new detection method to monitor various micro-sized organisms such as bacteria and animal cells.

Keywords: Piezoresistive cantilever; Liposome; Protein C2A

1. Introduction

Analysis of biochemical events such as biomolecular interactions, enzyme reactions and programmed cell death play a pivotal role in understanding the biological systems. Thus, ultra-sensitive detection schemes and analytical methods have been central to biochemistry and biomedical sciences. These methods are directly applicable to clinical diagnostics for the early detection of cancer. Mechanical detection scheme using silicone-based and microfabricated cantilevers have been recently demonstrated as a powerful technique by measuring the difference created between the free and complex state of biomolecules in an aqueous condition. It has been, for instance, reported that DNA hybridization [1] and antibody–antigen interaction [2] could be analyzed by the mechanical bending of a solid surface in association with the optical deflection measurement. For diagnosis of enzyme-related diseases such as cancer and diabetes, the activity of telomerase was monitored by magneto-mechanical detection method [3] and glucose concentrations were micromechanically determined by immobilized glucose oxidase [4]. In addition, cantilevers have been used to detect microorganisms such as bacteria and yeast through piezorelectric oscillators [5] and optoelectric method [6], respectively. For *Escherichia coli* bacteria detection the cantilever surface was coated by antibody whereas poly-L-lysine was immobilized to capture yeast cells.

Liposomes are microscopic spherical vesicles that form when phospholipids are hydrated and have been widely used for biochemical assay [7], drug delivery [8] and molecular imaging [9]. In particular, they are well known for artificial cell membranes to study cellular functions such as programmed cell death [10] and cell fusions [11]. Although surface plasmon resonance (SPR) detection [12] and isothermal titration microcalorimetry (ITC) [13] have been established for the detection of liposome–protein interactions, yet there has been no attempt to detect the number of liposome by nanomechanical technique. Here, we firstly report liposome detection method in which piezoresistve cantilever sensor system was accomplished with electro-mechanical detection scheme. As shown in Fig. 1 phosphatidylserine (PS) exposed on the surface of liposomes binds to phosphatidylserine recognition protein C2A which is chemically immobilized on the gold surface of the cantilever sensor tips. The main idea of this work is detection of electrical signal change caused by surface stress after the bending of cantilever beam.

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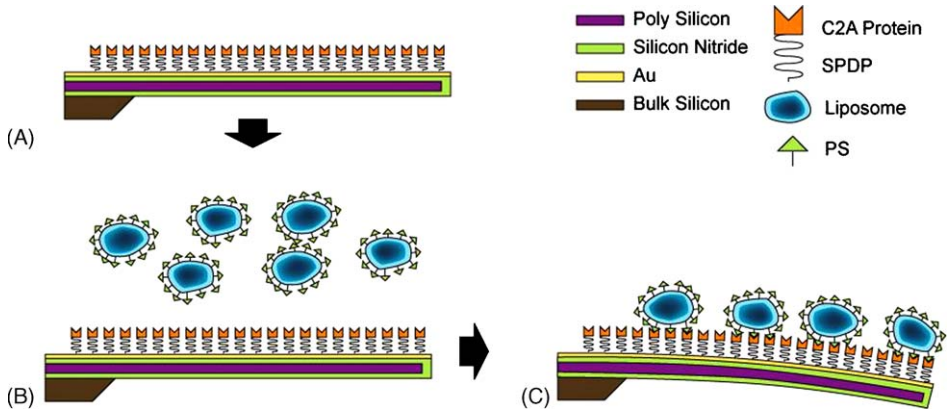

Fig. 1. Illustration of attachment of liposomes on gold-coated cantilever beam. Phosphatidylserine (PS)-recognition protein C2A was immobilized by chemical cross-linker SPDP (A). Liposome solution was loaded on the cantilever surface (B). Association of liposome with C2A protein was shown resulting in bending the cantilever beam (C).

2. Experimental

2.1. Fabrication of cantilever sensor chip

In order to fabricate pieozoresistive cantilever sensor with low cost and realize simple fabrication scheme, we used single crystalline ⟨1 0 0⟩ wafer instead of silicon on insulator (SOI) wafer and reduced fabrication process steps by direct contact between poly-silicon piezoresistor and Au/Ti signal line by using tetraethoxysilane (TEOS) as a protection layer. Through this work we could exclude additional insulation layer and via hole fabrication process. Piezoresistive cantilevers fabrication started on 4 in. ⟨1 0 0⟩ single crystalline silicon wafer. At first, 1 μm silicon nitride and 0.3 μm polysilicon are consecutively deposited using low pressure chemical vapor deposition (LPCVD). Secondly, boron doping and drive in processes are followed. After piezoresistor define using reactive ion etching (RIE) and electrical wiring processes using direct contact with Au/Ti, additional 1 μm TEOS layer was deposited to prevent surface roughening and failure of the front side during the bulk etching process. Finally, cantilever sensors were etch released through the bulk etching by the potassium hydroxide (KOH) and front side TEOS layer was removed using buffered oxide etchant (BOE) or RIE. The dimensions of fabricated cantilever sensors were varied from 300 μm (L) × 100 μm (W) to 600 μm (L) × 200 μm (W). Through the sheet resistance analysis, the resistivities of fabricated cantilever on the same wafer were confirmed uniform with the deviation of 2%.

2.2. Chemical modification and immobilization of protein C2A on the sensor chip

The first domain of Synaptotagmin I (protein C2A) has been known for phosphatidylserine-recognition protein [10], which was used to capture the phosphatidyleserine containing liposome in our work. The chemical modification of protein C2A to enhance its attachment onto the gold surface of cantilever was performed as follows. The protein was thiolated using an *N*-hydroxysulfosuccinimide (NHS) ester derivative of 2-pyridyl disulfide residue (sulfo-LC-SPDP, purchased from Pierce, USA), which modifies primary amine groups. Since the Ca^{2+}-dependent PS binding sites on the protein C2A contain two lys residues (lys200 and lys236) [14], we need to protect these by the first attachment of the protein onto an SP sepharose column. The protein binds tightly to the negatively charged SP sepharose resin in a Ca^{2+}-dependent manner, via binding sites that likely overlap those for PS. Purified protein C2A (6 mg/mL, 1 mL) was loaded onto an SP column (HiTrap SP FF, 1 mL volume, Amersham Biosciences) equilibrated with HEPES-buffered saline (HBS) containing 20 mM HEPES, pH 7.4, 150 mM NaCl, and 0.2 mM $CaCl_2$. After extensive washing, sulfo-LC-SPDP (Pierce) (1.0 mg/mL, 1 mL) in the same buffer was injected into the column and the flow-through fraction was collected and reinjected. After four cycles of reinjection, the column was left at 25 °C for 15 min and then washed with 10 bed volumes of HBS–Ca^{2+} buffer. The thiolated protein was eluted using buffer lacking $CaCl_2$ and containing 10 mM EDTA. The collected fractions were pooled and dialyzed against HBS for 24 h to remove EDTA. The collected fractions were pooled and washed against acetate buffer by using viva-spin column. To immobilize the thiolated protein 25 μl of DTT solution was added to 100 μl of the protein solution and incubated for 5 min. Five-microlitre of the mixture was loaded onto the gold surface and incubated for 5 min. The immobilization procedure was completed after wash with HBS–Ca^{2+} buffer.

2.3. Liposome preparation

Liposomes for the experiment were prepared as follows. L-α-phosphatidyl-L-serine sodium (PS), L-α-phosphatidylethanolamine (PE), L-α-phosphatidylcholine (PC) were purchased as in powder (Sigma-Aldrich) and dissolved in chloroform. Each phospholipid solution was mixed with specific molar ratio (PS/PE/PC, 35:50:15). The mixture was dried under a gentle nitrogen gas and re-suspended in 2.6 mL of HBS buffer (20 mM HEPES, 100 mM NaCl, pH 7.4) by vigorous vortexing. After the liposome preparation the concentration of liposomes were determined. The number of liposomes in 10 μl of prepared liposome