Replicate Nanopillar Arrays by Soft Lithography for Real-time Biosensing of C. Albicans Adhesion

Bin Zhou, Jingxuan Cai*, Jianhua Zhou*

School of Biomedical Engineering, Sun-Yat Sen University, Guangzhou, China

caijx27@mail.sysu.edu.cn; zhoujh33@mail.sysu.edu.cn

Whole-cell fungi detection is important in the evaluation of the anti-fungal property of material surface[1]. Conventional approaches for evaluating adhesion to material pathogens in aquatic environments are usually based on scanning electronic microscopy and fluorescence characterization. However, these techniques are usually slow, semi-quantitative, or not real-time monitoring. Recently, nanoplasmonics have been used for the microbes detection and monitoring the microbial biofilm formation of microbes on substrates.[2] However the formation of biofilms on the material is began from adhesion, while knowledge on this dynamic process is still limited.

In this research, we report the use of localized surface plasmon resonance (LSPR) sensor for real-time, label-free monitoring the adhering process of C. albicans onto the gold nanopillar array. Our LSPR sensor can capture the process of C. albicans' adhesion in real-time by measuring the shift of the plasmonic resonance with high temporal resolution. We also employ this sensor feature to elucidate how C. albicans' adhesion is affected by different electrical properties of the material surface.

The gold nanopillar array was prepared by soft lithography using a silicon mold fabricated by electron-beam lithography and reactive ion etching. After thermally evaporated with 50-nm thickness gold,[3] it was applied to sensing the adhesion of C. albicans, providing a simple and fast method for the direct monitoring of the antibacterial properties of the material surface. The schematic illustration of the detection of C. albicans adhesion is illustrated in Figure 1(a). The gold nanopillar array was vertically placed on the sidewall of a cuvette. After adding 1×10^6 CFU/mL of C. albicans, the LSPR peak at the ultraviolet-visible spectrum shifted, due to the adhesion of C. albicans onto the surface of the sensor. We obtained the adhesion dynamic curve of C. albicans by measuring the ultraviolet-visible spectra at different times. Figure 1(b) showed the cross-section of the gold nanopillar array. The height of the array column was 650 nm and covered with 50-nm Au. To carry different electric charges, the gold nanopillar array was modified with 3-mercaptopropionic acid (3-MPA) and cysteamine hydrochloride (HC), respectively, as described in figure 2(a). Figure 2(b) showed the kinetic binding curve for C. Albicans immobilized onto the negatively charged surface. Figure 2 (c) shows the kinetic binding curve for C. albicans immobilized onto the negatively charged surface. The results showed that the surface of the sensor with positive charge exhibited the obvious adsorption of C. albicans.

1. Douglas, L. J. *Trends Microbiol.* **2003,** 11, (1), 30-36.
2. Funari, R.; Bhalla, N.; Chu, K. Y.; Soderstrom, B.; Shen, A. Q. *ACS Sensors* **2018,** 3, (8), 1499-1509.
3. Li, W. B.; Xue, J. C.; Jiang, X. Q.; Zhou, Z. K.; Ren, K. N.; Zhou, J. H. *RSC Adv.* **2015,** 5, (75), 61270-61276.
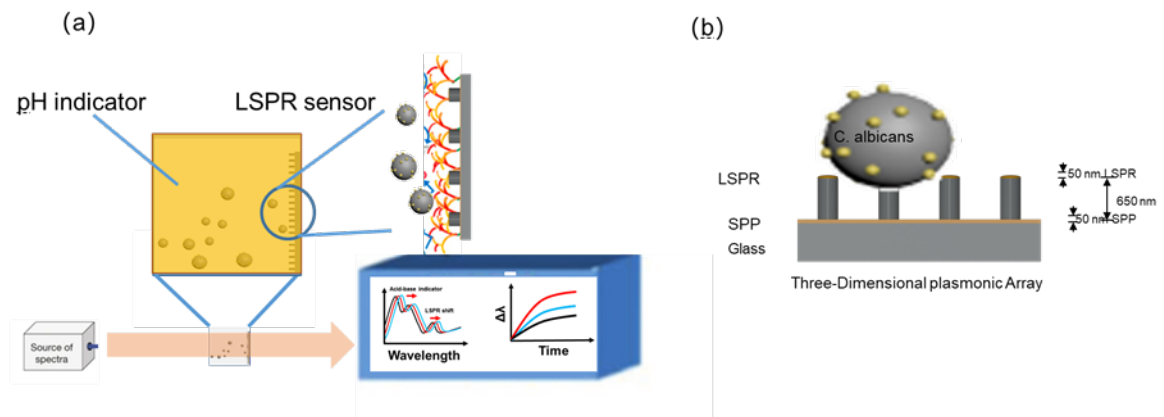
Figure 1. (a) Schematic illustration of the detection of C. albicans adhesion. (b) Cross-section of the Au nanopillar array, showing the assembly of C. albicans onto the surface of the Au nanopillar array.
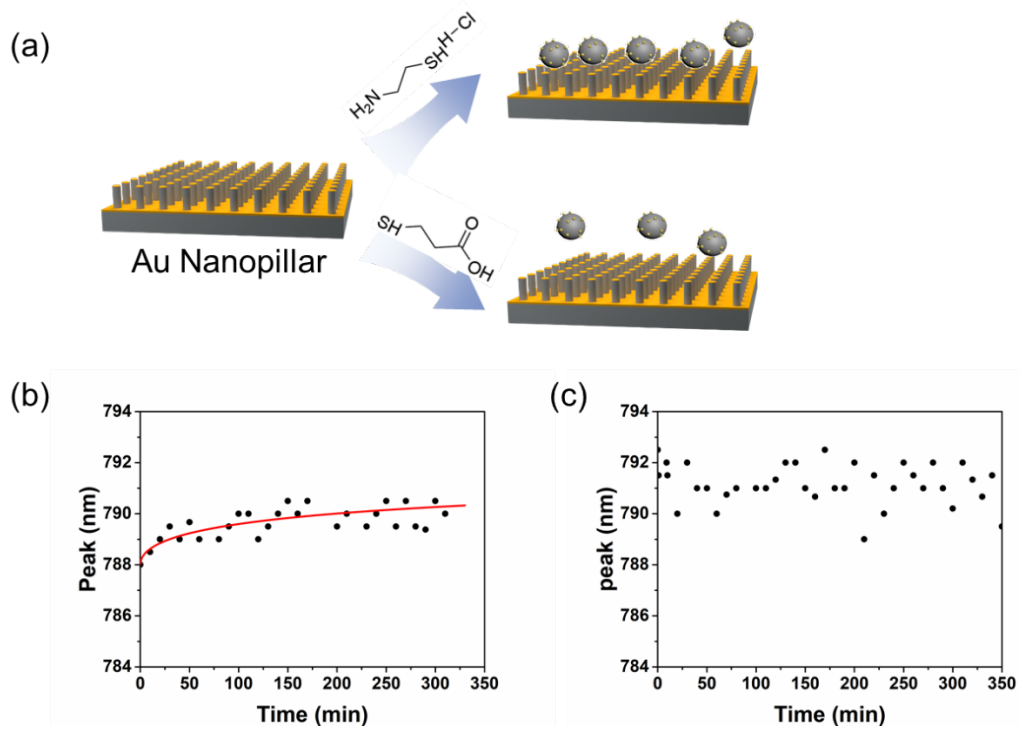

Figure 2. Displacement of spectral peaks during the adhesion of C. albicans onto the Au surface. (a) the gold nanopillar array was modified by 3-MPA and HC, and captured C. albicans, respectively. (b) the kinetic binding curve of C. albicans adhesion onto the positively charged surface. (c) the kinetic binding curve of C. albicans adhesion onto the a negatively charged surface. All the spectra were recorded when Au nanopillar were immersing in the solution.