Honeycomb-nanowire field-effect transistors for bacterial activity determination in non-diluted growth media

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Abstract

The spread of antibiotic resistant bacteria is a threat for the effective prevention and treatment of infections, requiring immediate action in their detection and monitoring of their response against antibiotics and new drugs. The effect of antibiotics is measured by monitoring cell growth[1]. However, the absence of detectable growth does not necessarily mean cell death. It has been proposed that in adversity periods bacteria can adopt the viable but nonculturable phenotype (VBNC), conserving metabolic function but becoming unculturable[2,3]. pH measurements give complementary information here, indeed, cells are known to change pH as consequence of metabolism[4,5]. A miniaturized sensor capable of detecting this process would allow to minimize the needed culture volume, allowing at the same time parallelization and online measurements. Optical detection of pH changes due to cell metabolism has already been demonstrated [6], however, label-free methods would be preferable for simplification. In this context, ion-sensitive field-effect transistors have shown to be an important option to consider[7–9]. Generally, low concentration media have been used for this. Thus, development of a label-free sensor that monitors pH in standard microbiology environments is needed. In this work, we used highly sensitivite and reproducible honeycomb nanowire-based field-effect transistors (HC FET), fabricated in silicon following a top-down approach by electron beam lithography[10] (Figure 1a) to determine the metabolism of *Escherichia coli* (*E. coli*) in M9 and Luria Bertani (LB) media.

When a bacterial culture (10^8 cells/ml) was measured through time with the FET (Figure 1b), the pH changes affected the local carrier concentration of the semiconductor channel, bringing observable current changes at a gate voltage of 0.2 V. This was confirmed by measurements with a pH meter, as well as the growth of the microorganism population by optical density measurements at 600 nm with a spectrophotometer. After the addition of fresh medium during the exponential growth, supplemented with 0.1 mg/ml kanamycin, its effects on the three measurement techniques were observed. Kanamycin affects the function of the ribosomes, bringing a production of misread proteins[11], meaning that bacteria do not instantaneously die, as observed in the continuation of the growth for the first hour after antibiotic addition. During the next hours, the growth was strongly slowed down, reaching a saturation. On the contrary, the pH change, as well as the current from FET, did not stop, indicating that even if there was no observable growth, the bacteria were still metabolically active.

The used HC FET demonstrated to be successful in the label-free monitoring of bacterial metabolic activity using standard non-diluted culture media, M9 and LB, both of them frequently used in microbiology. The information provided followed the same trend observed using a pH meter but needing 500-fold lower volume. Additionally, this information is complementary to optical density measurements, which give information about population rather than insights on metabolism.

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Figures

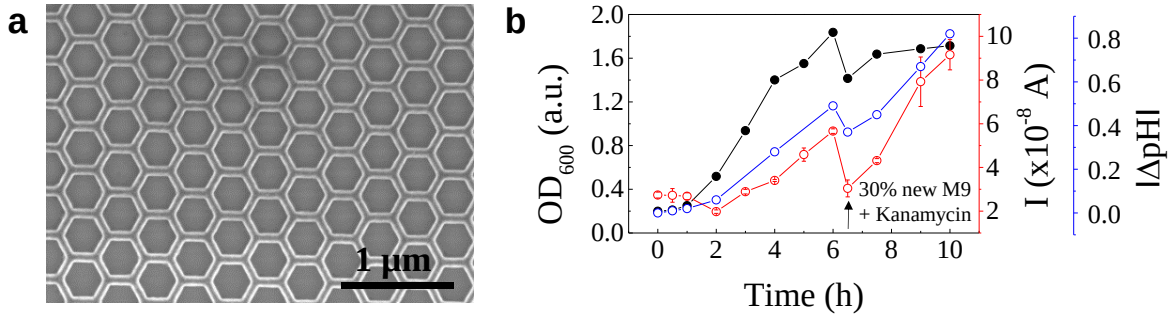

Figure 1. (a) Scanning electron microscopy of the honeycomb nanowires. (b) Triple parallel measurement of *E. coli* activity in M9 medium with kanamycin addition during exponential growth. Optical density confirms bacterial growth during initial hours and its stop after antibiotic addition. Monitoring of metabolic activity with HC FET and pH meter have coinciding trend. As they grow, there is a change in medium pH, which does not stop after antibiotic addition.