Development of a Graphene-Based Microchip for Real-Time Label-Free Monitoring of Bacterial Conjugative Interactions

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Abstract: Every year, over 2 million people fall ill due to infections with antimicrobially-resistant (AMR) pathogens [1]. Conjugative horizontal gene transfer presents one of the major drivers of AMR and virulence evolution. Existing methods for studying conjugative gene transfer suffer from the need for genetic modification and labeling (fluorescence, luminescence, or antibiotic resistance labeling) which restrict the number of strains that can be studied and may introduce selective pressures and metabolic burdens [2]. Additional challenges with studying conjugation include limited temporal resolution of the existing characterization methods. To address these challenges, we propose the development of an electronic microchip based on the stress-dependent properties of graphene and demonstrate real-time label-free monitoring of conjugative interactions among *E. coli* cells.

During bacterial conjugation, shear forces are exerted on the 2D material channel due to the extension and retraction of protein-based pili between donor and recipient cells [3]. These forces cause strain in graphene, leading to changes in its electronic resistivity, which can be quantitatively measured on-chip [4]. We demonstrated the utility of the graphene-based chip by measuring conjugative interactions among engineered *E. coli* strains (donor and recipient cells). The resistance of the device was continuously monitored during bacterial immobilization and our results showed a significant increase in signal for the 'Donor + Recipient' device, while the 'Donor only' device exhibited no substantial change. This observation suggests that conjugative interactions generate shear forces that modulate graphene's resistivity, and this change is directly proportional to the frequency of conjugation events.

Our findings provide proof of concept for the use of the graphene-based microchip for label-free monitoring of bacteria conjugation in real time. Further research is needed to confirm these results through simultaneous electrical and optical imaging and to explore the broader implications of this technology for studying microbial interactions in heterogeneous cultures.

References:

1. CDC. Antibiotic resistance threats in the United States, 2013.
2. Getino, M. et al. Tanzawaic Acids, a Chemically Novel Set of Bacterial Conjugation Inhibitors. PLOS ONE 11, e0148098 (2016).
3. Clarke, M., Maddera, L., Harris, R. L. & Silverman, P. M. F-pili dynamics by live-cell imaging. Proc. Natl. Acad. Sci. 105, 17978–17981 (2008).
4. Smith, A. D. et al. Piezoresistive Properties of Suspended Graphene Membranes under Uniaxial and Biaxial Strain in Nanoelectromechanical Pressure Sensors. ACS Nano 10, 9879–9886 (2016).