Corso di Dottorato di Ricerca in Scienze della Vita e dell'Ambiente - Ciclo XXXVII

Exploring the Potential of Graphene Field-Effect Transistors in Biosensing for Health and Environment

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INTRODUCTION

Graphene Field Effect Transistors (GFETs) have emerged promising for biosensing due to their unique properties, including high sensitivity, fast response times, and compatibility with different biological molecules.[1] The GFET's sensitivity to charge can be used as an indication of the presence of specifically bonded species such as proteins, nucleic acids, circulating tumor cells. By functionalizing the GFET surface with specific biomolecules (*i.e.*, antibodies, proteins or aptamers), these biosensors can selectively capture and detect specific markers related to diseases diagnosis or environmental monitoring. This allows for the early detection of diseases such as cancer, infectious diseases, and neurological disorders, enabling timely interventions and improved patient outcomes.

We herein have employed a GFET-based biosensor that we recently developed for SARS-CoV-2 detection and extended its application to the detection of exosomes from liquid biopsy and environmental pollutants.[2] Analyzing exosomes in liquid biopsy can provide valuable insights into disease while detecting emerging environmental pollutants holds promise for environmental monitoring. GFET biosensors exhibit selective detection capabilities and rapid response times, potentially revolutionizing disease diagnosis, monitoring, and environmental surveillance.

AIM OF THE STUDY

The aim of this study was to investigate the potential of GFET as a biosensor for the detection of exosomes and environmental pollutants. For exosomes detection, small extracellular vesicles with endogenous origin, we targeted the membrane protein CD63 using both anti-CD63 antibody and TIMP1 protein, an interactor of CD63. Furthermore, we utilized the GFET biosensor to detect environmental pollutants, using Peroxisome Proliferator-Activated Receptor gamma (PPAR-γ) protein as a probe. PPAR-γ, a nuclear receptor involved in regulating gene expression and cellular processes, can be activated by environmental pollutants leading to metabolic dysregulation.

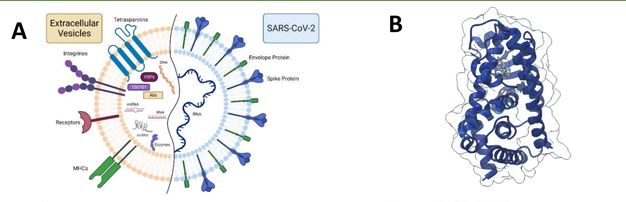


Figure 1. *(A) Picture comparing the biological composition of SARS-CoV-2 and exosomes. (B) Illustration of PPAR-γ protein PDB ID 7WGL*

RESULTS

ELECTRICAL MEASUREMENTS

To assess the functionality of the GFET, we conducted tests to determine its capability to detect exosome markers, specifically CD63. At the same time GFET was used to detect the PPAR-γ agonist Rosiglitazone, an antidiabetic drug which selectively binds this protein. Transfer curves were used to detect the capture of the samples by sweeping the gate voltage V_g while maintaining a fixed bias V_{ds} between the source and drain electrodes. (Figure 3). The resulting current I_{ds} were plotted as a function of the gate bias.

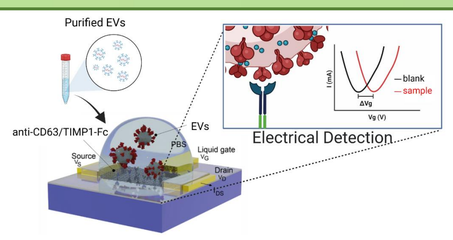


Figure 2. *Picture showing the working principle of GFET*

EXOSOMES

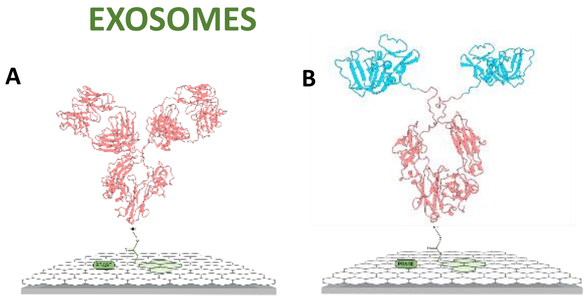
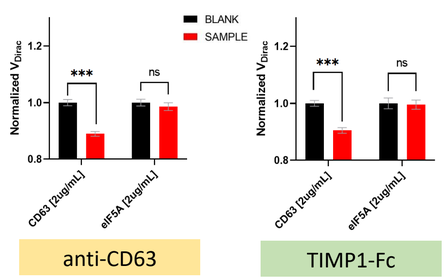


Figure 3. *Illustration of the graphene layer functionalied with (A) antibody anti-CD63 and (B) TIMP1-Fc protein.*

The GFET was functionalized with both anti-CD63 antibody (left) and TIMP1-Fc (right) to enable the detection of exosomes.



To confirm the interaction between the probes used for graphene functionalization and CD63, measurements were conducted using recombinant CD63 protein.

Figure 4. *Comparative bar charts of gFET before (black bars) and after (red bars) the addition of samples. *** $p < 0.001$, error bars represent standard error of the mean (s.e.m.).*

Exosomes positive for CD63 marker were isolated from THP1 cell line through multiple centrifugation steps. Nanoparticle Tracking Analysis (NTA) measurements were performed to determine the concentration and size of the particles; Bicinchoninic Acid assay (BCA) was used to obtain the total protein concentration and the positivity to CD63 marker was determined with the cytofluorimeter.

Additionally, exosomes negative for the CD63 marker were isolated from the plant *S.sclarea*, and their characterization was carried out through ultracentrifugation, NTA, BCA, and proteomic analysis.[3]

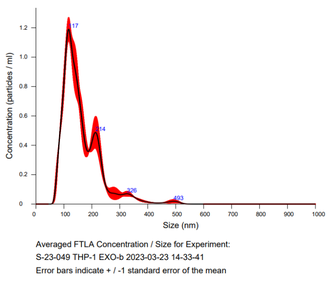


Figure 5. *Graph showing average size and concentration of exosomes isolated from THP1 cell line.*

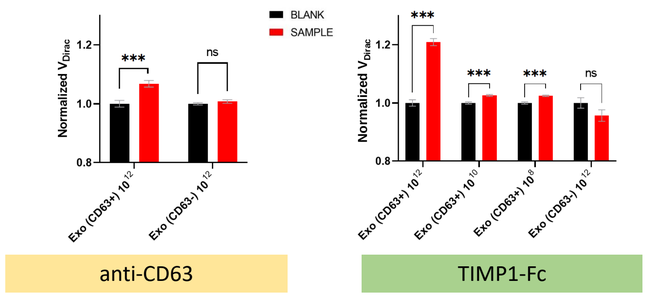


Figure 6. *Comparative bar charts of gFET before (black bars) and after (red bars) the addition of samples. *** $p < 0.001$, error bars represent standard error of the mean (s.e.m.).*

Exosomes were successfully detected with both GFETs.

PPAR-γ

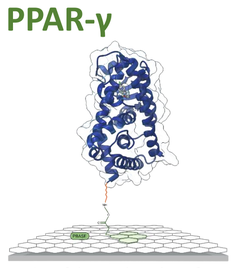


Figure 7. *Illustration of the graphene layer functionalied with PPAR-γ.*

The GFET was functionalized with PPAR-γ recombinant protein to detect its agonist rosiglitazone (RSGLT).

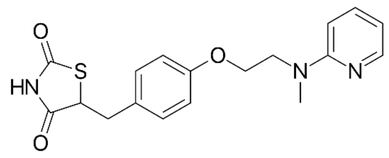
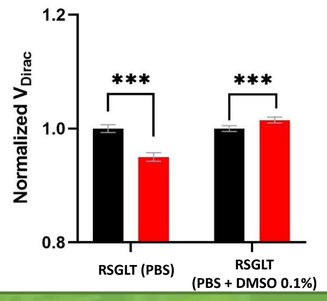


Figure 8. *Picture showing the chemical structure of rosiglitazone, an agonist of PPAR-γ.*



The GFET showed an high sensitivity in the detection of rosiglitazone [1µg/mL], both when dissolved in PBS and when dissolved in PBS and DMSO 0.1%.

Figure 9. *Comparative bar charts of gFET before (black bars) and after (red bars) the addition of samples. *** $p < 0.001$, error bars represent standard error of the mean (s.e.m.)*

CONCLUSIONS

Through our experimental evaluations, we successfully detected particles of different size and composition such as exosomes and rosiglitazone. This highlights the sensitivity and selectivity of the GFET in capturing and detecting specific biomarkers related to diseases or environmental monitoring. Overall, our GFET biosensor represents a significant advancement in biosensing technology, and its ability to detect exosomes and pollutants highlights its potential for advancing healthcare diagnostics and environmental monitoring. GFET-based biosensors hold great promise in revolutionizing disease detection and monitoring, leading to more effective and targeted interventions.

REFERENCES

[1] A. Béraud, M. Sauvage, C. M. Bazán, M. Tie, A. Bencherif, D. Bouilly, *Analyst* (**2021**), *146*, DOI 10.1039/D0AN01661F.

[2] A. Romagnoli, M. D'Agostino, E. Pavoni, C. Ardiccioni, S. Motta, P. Crippa, G. Biagetti, V. Notarstefano, J. Rexha, N. Perta, S. Barocci, B. K. Costabile, G. Colasurdo, S. Caucci, D. Mencarelli, C. Turchetti, M. Farina, L. Pierantoni, A. La Teana, R. Al Hadi, F. Cicconardi, M. Chinappi, E. Trucchi, F. Mancia, S. Menzo, B. Morozzo della Rocca, I. D'Annessa, D. Di Marino, *Nano Today* (**2023**), *48*, DOI 10.1016/j.nantod.2022.101729.

[3] Boccia, E., Alfieri, M., Belvedere, R. *et al.* Plant hairy roots for the production of extracellular vesicles with antitumor bioactivity. *Commun Biol* **5**, 848 (**2022**). https://doi.org/10.1038/s42003-022-03781-3