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

gFET for biomarkers detection: an important tool for cancer diagnosis

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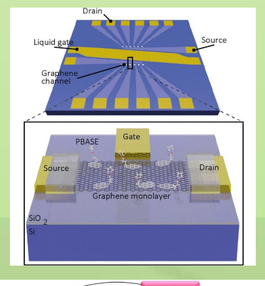
Introduction

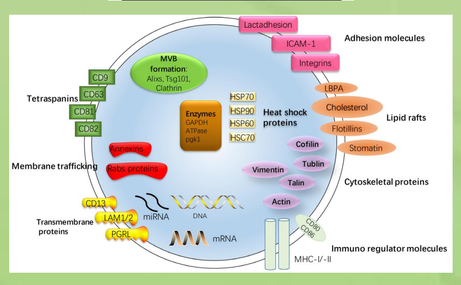
Graphene Field-Effect Transistors (**gFET**), represent a rapidly expanding technology due to their high sensitivity and rapid detection. In biosensor applications, the gFET's sensitivity to charge can be used as an indication of the presence of specifically bonded species such as proteins and cells. At NY-MaSBiC, a gFET biosensor able to specifically **detect all the SARS-CoV-2 variants** known to date, has been developed. For this biosensor, a recombinant version of ACE2 was used. The binding event of the capsid viral Spike protein with ACE2, is transduced into an electrical signal which can give informations about the presence or not of the SARS-CoV-2. Thanks to all the advantages that gFET give, these are attractive in point-of-care (POC) diagnosis due to their miniaturization, potential for large-scale manufacture and no need for specialized personnel.

Such a system can be routinely implemented in the design of biosensors, which properly functionalized, could be prominent for the detection of essential biomarkers such as proteins, circulating tumour cells, nucleic acids, and a new class of biomarkers called **exosomes**, which are gaining prominence. The interest on exosomes research is growing as their composition represents a 'mirror' of the physiological as well as the pathological state of the donor cells. Furthermore, those vesicles are 50-150 nm in size, thus similar to SARS-CoV-2. Accordingly, a gFET for exosomes detection will be implemented to obtain a device able to achieve early-stage diagnosis and therefore better prognosis of cancer and neurodegenerative disease from liquid biopsy. This would be advantageous not only for the earlier detection of the pathology, but also for avoiding the time consuming techniques known to date for exosomes analysis and isolation.

Background



gFET (size 10 mm × 10 mm) includes two electrodes, called source and drain which make direct contact with the graphene and enable the flow of electrical current in the graphene through the application of a difference of electrical potential between them. This system, which exploits protein to protein interaction, can be implemented for different biomarkers detection.



Exosomes are a subset of extravescicles which contain various lipids, proteins, and nucleic acids, including DNAs and RNAs. These contents can offer vital information associated with pathological and physiological status. From the aspect of cancer diagnosis, exosomes are ideal resources of biomarkers because molecular features of tumor cells are transcribed on them.

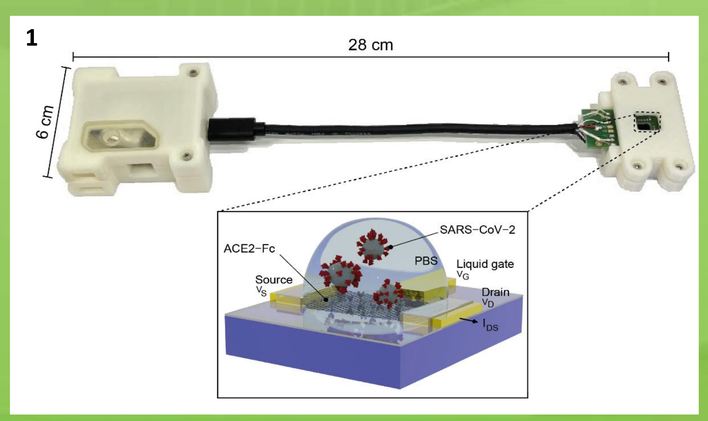
Aim

- Selection of different exosomes biomarkers related to different type of cancer
- Functionalization of gFET with different biomarkers
- Detection of exosomes
- Early cancer diagnosis

From the technology used for ViruSensing…

- Functonalization with **ACE2-Fc**
- **Detection of all SARS-CoV-2 variants** known to date directly from nasopharingeal swab samples
- **LOD** of **20 pg/mL**

1



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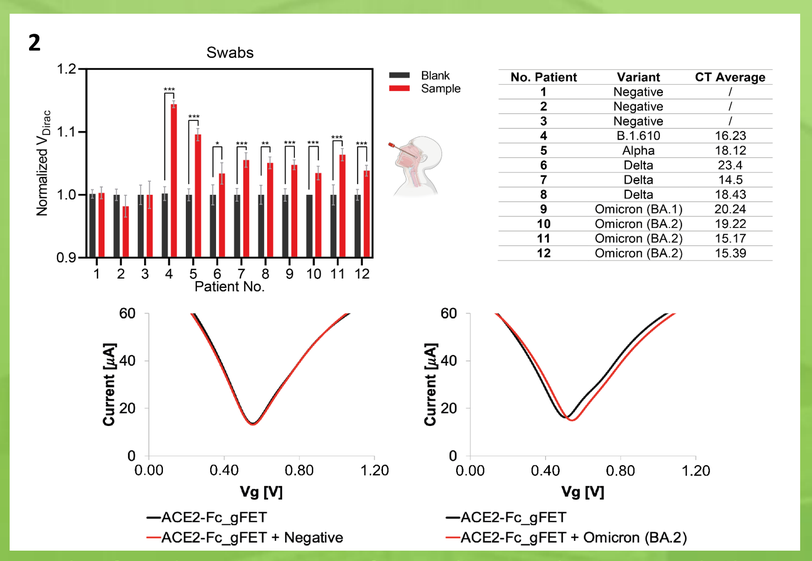
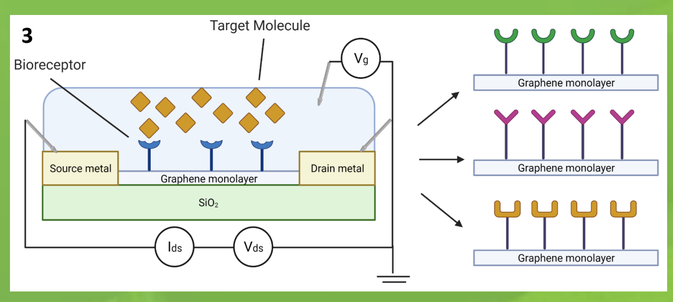


No. Patient	Variant	CT Average
1	Negative	/
2	Negative	/
3	Negative	/
4	B.1.610	16.23
5	Alpha	18.12
6	Delta	23.4
7	Delta	14.5
8	Delta	18.43
9	Omicron (BA.1)	20.24
10	Omicron (BA.2)	19.22
11	Omicron (BA.2)	15.17
12	Omicron (BA.2)	15.39

(1) Photograph of the gFET Cartridge Unit and the Signal acquisition modules connected to form the entire POC. A reference dimension bar is reported. Also, a schematic representation of gFET modified with ACE2-Fc tested with different SARS-CoV-2 samples is shown *(Romagnoli et al, 2022, submitted)*
(2) Bar graph reporting ACE2-Fc_gFET signal before (black) and after the addition of nasopharyngeal swab samples from patients (red). Different SARS-CoV-2 variants and relative Ct values in positive clinical samples are listed in the table. Also transfer curves (below) of ACE2-Fc_gFET in response to patient samples are reported. *(Romagnoli et al, 2022, submitted)*

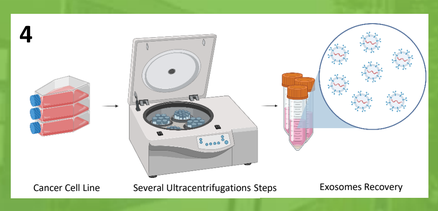
…To future perspectives

- Exosomes detection for early cancer diagnosis
- Use of liquid biopsy instead of the invasive techniques used up to now for cancer detection
- Detection of different type of viruses and pathologies

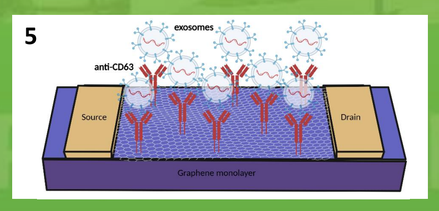


(3) Schematic representation of the graphene monolayer functionalized with differents bioreceptor for the detection of different targets

Methods



- Cancer cell culture
- Supernatant collection
- Several centrifugations at increasing speed (from 300xg to 100000xg)
- Resuspention of the final pellet with PBS1X
- Lysis of exosomes and detection with Western Blot



- Immobilization of the linker PBASE at the graphene surface with π-π interactions
- Covalent immobilization of the bioreceptor (anti-CD63) expoiting NHS ester of the PBASE linker
- Addition of exosomes previously purified

(4) Schematic representation of the exosomes isolation protocol
(5) Schematic representation of the gFET functionalized with anti-CD63 is shown

References

- Romagnoli et al, **2022**, *submitted*
- H. Bu, D. He, X. He, K. Wang, Exosomes: Isolation, Analysis, and Applications in Cancer Detection and Therapy. *ChemBioChem* **2019**, *20*, 451
- Jia Y., Chen Y., Wang Q., Jayasinghe U., Luo X., Wei Q., Wang J., Xiong H., Chen C., Xu B., Hu W., Wang L., Zhao W., et al Exosome: emerging biomarker in breast cancer. *Oncotarget*. **2017**; 8: 41717-41733.