A MAGNETORESISTIVE BIOSENSOR FOR CIRCULATING EXOSOME DETECTION

Simona Di Giulio[1], Manuel Antonio González Gomez[2], Yolanda Piñeiro[2], Silvia Rizzato[1], Valentin Dediu[3*], José Rivas[2*], Giuseppe Maruccio[1*]

[1] Omnics Research Group, Department of Mathematics and Physics "Ennio De Giorgi", University of Salento and INFN Sezione di Lecce, Via per Monteroni, 73100 Lecce, Italy.
[2] Nanotechnology and Magnetism Lab — NANOMAG; Materials Institute – iMATUS Health Research Institute – IDIS; Department of Applied Physics: Universidade de Santiago de Compostela: E-15782 Santiago de Compostela, Spain.
[3] CNR ISMN, Via Gobetti 101, 40129 Bologna, Italy.
* Corresponding authors

Contact e-mail: simona.digiulio@unisalento.it

Abstract

Extracellular vesicles (EVs) are small, membrane-bound particles released by cells into the extracellular environment. These vesicles play a crucial role in cell-to-cell communication by transporting bioactive molecules, such as proteins, lipids, and nucleic acids (Figure 1) [1].
EVs are classified into different subtypes based on their biogenesis and size, with the main categories being exosomes, microvesicles, and apoptotic bodies. Exosomes, originating from endosomal compartments are released upon fusion of multivesicular bodies (MVBs) with the cell membrane and typically range from 30 to 150 nm in diameter. Microvesicles, formed by the outward budding or shedding from the plasma membrane, exhibit a broader size range (100 nm to 1 µm). Apoptotic bodies, released during programmed cell death, are larger vesicles (1 to 5 µm) containing cellular organelles and fragments from the dying cell [2].
EVs are a promising source of diagnostic biomarkers and have gained a wide interest in the biomedical and biosensing field [3]. Their presence in body fluids, such as blood, urine, saliva, and cerebrospinal fluid has opened new avenues for understanding physiological processes and exploring diagnostic and therapeutic applications [4]. Therefore, EVs have been proposed as novel diagnostic biomarkers for several pathologies such as cardiovascular diseases, autoimmune diseases, and cancer. Tumor-derived exosomes have been found to accumulate in human fluids and to be enriched in a set of membrane and soluble molecules reflecting the status of cells [5]. Thus, targeting exosomes could provide a promising tool for tumor biology and early disease detection, without invasive biopsy [6]. However, the implementation of exosomes as a diagnostic and prognostic tool has been hampered by the lack of highly reliable, sensitive isolation, characterization, and quantification techniques. Standard ultracentrifugation isolation is time-consuming and yields low recovery and low purity. In addition, in a clinical setting, fast results are also required [7]. Biosensing platforms, designed to detect and analyze EVs, offer several advantages, including non-invasiveness, the ability to access valuable biological information, and potential applications in diagnostics and therapeutic monitoring. The aim is to translate their importance in cell communication into measurable signals in point-of-care (POC) setups [8]. Biosensors offer significant advantages compared to traditional methods like ELISA, including the potential for miniaturization, automation capabilities, rapid and sensitive analysis, leading to cost-efficient designs [9].
This project aims to develop a portable magnetoresistive (MR) biochip platform to detect, characterize, and quantify exosomes in biological samples, order of magnitudes better than the state-of-the-art diagnostic devices. The core concept is to detect very low concentrations of biomarkers by coupling a magnetic sensor technology, easily integrable into microfluidic device, with iron oxide magnetic nanoparticles (MNPs) functionalized for the efficient capture of circulating exosomes. The detection scheme is based on an immune-sandwich assay, where exosomes from biological samples will be captured by MNPs, previously coated with antibodies against the tetraspanin protein CD63 [10], an enriched surface marker in exosomes (Figure 1). Streptavidin-coated MNPs are intended to be used, due to their consistent and reproducible properties. They will be incubated with biotinylated anti-CD63 antibodies, exploiting biotin-streptavidin strong interaction for MNPs functionalization. The biosensor will be functionalized with detection antibodies for the tetraspanin protein CD9, another common exosome marker, also reported as breast, ovarian, melanoma, and pancreatic cancer exosomal marker (Figure 2) [11]. Anti-CD9 antibodies will be immobilized on the surface of the sensor by amine-coupling according to standard protocols, in order to recognize and affinity-capture the MNPs labeled exosomes, by applying a magnetic field for separation of the MNPs from the solution.
The binding of the exosomes on the sensor brings the MNPs close to the surface of the sensor, leading to a measurable voltage change. The difference in voltage before and after the bio-recognition event indicates the amount of EVs in the sample. This detection strategy could allow the enrichment of the EVs from complex samples such as serum.
The biosensor will be feasible not only for the quantification of total exosomes but could be also implemented to detect multiple exosome markers simultaneously, providing a more comprehensive understanding of their profile in each sample. Importantly, the development of miniaturized and portable MR biosensors could

facilitate POC applications, allowing for exosome detection in diverse settings, including clinical environments and resource-limited areas.

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Figures

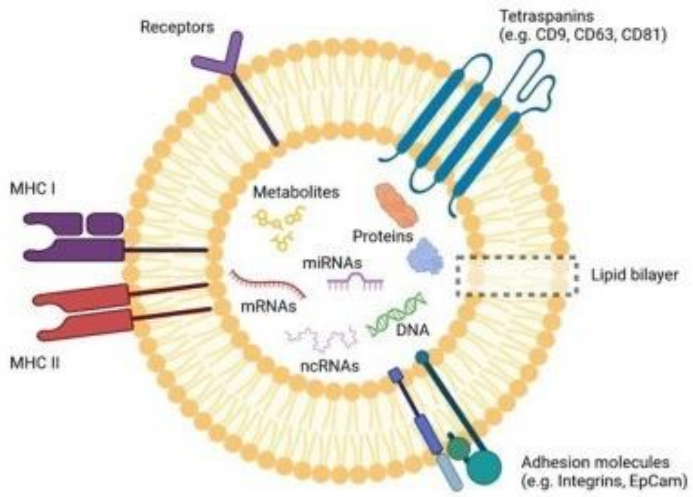

Figure 1. Schematic representation of the structure of EVs

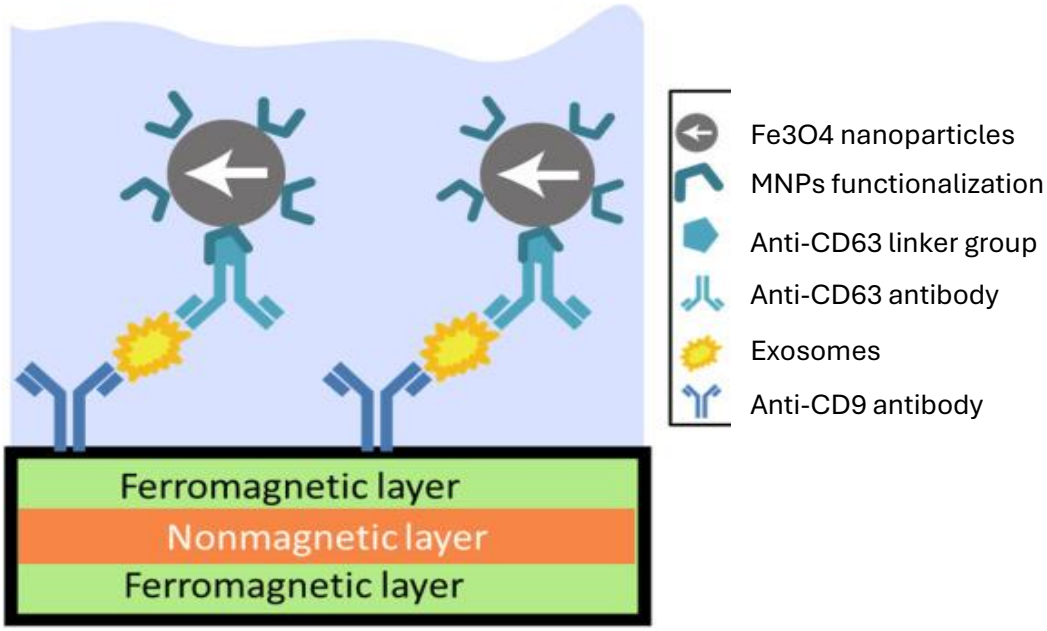

Figure 2. Schematic representation of the magnetic biosensor for exosome selective capture