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Research

Towards liquid biopsy on chip for Triple Negative Breast Cancer: preliminary results on monitoring circulating miRNA-21 using portable diagnostics

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Abstract
Liquid biopsy has emerged as a promising non-invasive, cost-effective and real-time approach for cancer diagnosis and monitoring, allowing for the detection of biomarkers in bodily fluids. Among these, microRNAs (miRNAs) are a valuable choice due to their stability and ability to reflect the tumor's heterogeneity. Concerning triple negative breast cancer (TNBC), an aggressive subtype, several studies have demonstrated consistent upregulation of miRNA-21. Its elevated levels, linked to poor prognosis, make it valuable for early detection, risk stratification, and targeted therapies. While traditional miRNA quantification methods are accurate, they often require complex procedures and skilled personnel, limiting their accessibility in low-resource environments. These challenges can be addressed by electrochemical point-of-care (POC) platforms, inspired by the glucose strip, offering a good alternative by reducing matrix effects, integrating cost-effective and eco-friendly substrates. In this work, miRNA-21 was selectively detected using a complementary DNA probe modified with methylene blue, as a redox mediator, immobilized onto an AuNPs-functionalized, paper-based screen-printed electrode. Significant experimental parameters and sensor's selectivity were carefully evaluated, allowing miRNA-21 detection in both standard solution and human serum with limit of detection (LOD) 1.2 nM and satisfactory repeatability of about 8%. The platform's performance improved tenfold with an external paper-based origami pre-concentration device, enabling pM-level miRNA detection and advancing its potential for real clinical practice applications. The platform is envisioned as a starting point for developing accessible, rapid, cost-effective POC testing, with significant implications for personalized medicine and early TNBC detection.

Keywords TNBC · miRNA · Liquid biopsy · Point-of-care · Sensors

1 Introduction

Liquid biopsy is a diagnostic method involving the analysis of various biological fluids, such as blood, rather than whole tissues for tumor-derived components like circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), and microRNAs (miRNAs). This approach is revolutionizing cancer diagnosis and monitoring by offering a cost-effective and less invasive

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method for analyzing blood circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), and microRNAs (miRNAs). It provides a real-time, minimally invasive method for cancer management [1]. In particular, it is aimed to replace traditional surgical tissue biopsies: even if these represent the gold standard for obtaining crucial diagnostic information, they are painful, risky, and may not be feasible for hard-to-reach tumors or patients with limited financial resources [2]. More specifically, several clinical risks and potential complications are associated with the standard procedures. These include the possibility of structural damage to surrounding tissues, bleeding, and infections. Additionally, tumors located in hard-to-reach anatomical areas increase the complexity of the procedure and the likelihood of further surgical complications, such as the spread of tumor cells, which could promote metastasis. These risks make tissue biopsy a delicate procedure, particularly in immunocompromised cancer patients, either due to the cancer itself or as a result of treatments such as chemotherapy and radiotherapy that weaken the immune system. Imaging-based diagnostic methods, such as computed tomography (CT), magnetic resonance imaging (MRI) and positron emission tomography (PET) scans, are valuable for detecting and tracking cancer progression, however they also have limitations, including high costs, radiation exposure, and difficulty in distinguishing between cancerous and non-cancerous tissues [3, 4]. Furthermore, these methods are not suitable for continuous monitoring due to the dynamic and heterogeneous nature of cancer.

All these features related to traditional analytical methodologies for cancer diagnosis and prognosis highlight the need for alternative and minimally invasive diagnostic methods, such as the emerging approach of liquid biopsy. Accessing the bloodstream might give important information regarding the early detection, treatment response assessment, and resistance mechanism identification, ultimately improving patient outcomes [5]. Among all the biomarkers that are currently under investigation, miRNAs represent a valuable choice due to their stability in biological fluids and their ability to dynamically reflect tumor biology. miRNAs are single-stranded non-coding RNAs, typically 17–27 nucleotides in length. Their remarkable stability has been widely demonstrated under conditions that would typically degrade most RNAs, including exposure to temperatures exceeding 100 °C, extreme pH levels, prolonged storage, and repeated freeze–thaw cycles. Furthermore, recent studies have demonstrated that miRNAs can be retained in long-term stored human serum samples for up to 10 years and in frozen plasma samples for even more than 14 years, remaining detectable [6, 7]. This stability is contributed to by their encapsulation within lipoprotein complexes, including small membrane vesicles of endocytic origin called exosomes. These vesicles, present in several types of body fluids, such as blood and saliva, help protect miRNAs from external damage and nucleotide degradation. MiRNAs play also an essential role in cell signaling pathways and regulate gene expression post-transcriptionally, by repressing translation or promoting mRNA degradation [8]. Therefore, altered levels of specific miRNAs have frequently been described as a major determinant for the initiation and progression of many diseases, including cancer [9].

In the context of triple-negative breast cancer (TNBC), an aggressive subtype of breast cancer lacking estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor 2 (HER2) amplification, miRNAs have shown significant promise as diagnostic, prognostic, and predictive biomarkers [10, 11]. Several clinical trials have investigated the role of miRNAs in TNBC, focusing on their potential as diagnostic, prognostic, and therapeutic biomarkers [12, 13]. For example, the WHEL trial [14] (n = 3008) correlated overall survival in breast cancer (BC) patients with altered plasma levels of miRNA-210, miRNA-29c, miRNA-187, miRNA-143, and miRNA-205, highlighting their potential as prognostic biomarkers and enhancing risk stratification within Prediction Analysis of Microarray 50 (PAM50)-defined groups; in the GeparSixto trial (n = 315), involving TNBC patients, increased miRNA-155 was linked to an increased likelihood of achieving pathologic complete response (pCR) after neoadjuvant therapy [15].

Another well-known oncogenic miRNA, miRNA-21, has consistently shown elevated levels in TNBC patients and correlated with poor prognosis, making it an emerging early detection marker. For instance, in 2023, Kumar et al. [16] highlighted the diagnostic and prognostic significance of miRNA-21 using serum samples (n = 190), divided into three cohorts based on the disease stage: cohort-I (mixed stage), cohort-II (stage I–II), and cohort-III (stage III–IV). The study demonstrated that miRNA-21 exhibited significantly greater variations in advanced-stage (stage III–IV) patients compared to early-stage (stage I–II) and healthy individuals. Furthermore, in all three cohorts, the diagnostic evaluation showed higher sensitivity and specificity, demonstrating that miRNA-21 successfully distinguished TNBC cases across various stages to monitor disease progression.

Although traditional analytical methods, such as Northern blotting [17], RT-q-PCR [18], microarray [19], Next-generation sequencing [20] and NanoString nCounter [21] are widely used for miRNA quantification due to their accuracy. Nevertheless, they are characterized by high equipment costs and analysis, time-consuming steps and complex experimental setup together with the need of skilled personnel. These limitations often hinder their timely accessibility, especially in remote areas, and make them less practical for widespread or routine applications. On the other hand, Point-of-Care (POC) devices present a promising opportunity to overcome these limitations by offering portable, low-cost and user-friendly

tools that require minimal training, making them accessible to non-specialists. The simplicity associated with diabetes strips [22] and the recent COVID-19 tests [23], are good examples of ease-of-use and portable POC devices, further highlights their ability for rapid and accurate testing outside healthcare facilities, such as in low resource settings or at home. Similarly, the design and implementation of POC devices for miRNA detection could transform cancer management by providing timely and effective diagnostic solutions at a low cost, bridging the gap between the complexity of traditional methods and the need for accessible, real-time diagnostics. In the context of miRNA sequence detection, POC devices, have been widely reported in literature for miRNA detection: Raucci et al. recently reported on the application of a paper-based electrochemical POC device for the detection of miRNA-652 in serum samples [24]. In another work, Gao et al. [25] applied DNA-gold nanoparticles (DNA-AuNPs) based lateral flow nucleic acid biosensor for the analysis of miRNA-215 in biological samples. Lastly, the quantitative detection of miRNA-155 has been recently achieved using a novel label-free fluorescent biosensing technique based on AuNPs etching in normal human serum [26]. Out of these novel POC-based approaches, the electrochemical platforms (same technology as the glucose strip for diabetes) have demonstrated clear advantages being user-friendly, simple and rapid, without any interferences from complex matrices like serum and whole blood [27]. In addition, POC devices can be even more sustainable with the use of paper-based substrates for their manufacturing [28, 29]. Paper provides an inexpensive and biodegradable substrate, available all over the world which has led to wide interest for developing paper-based analytical devices for cancer biomarker detection [28]. This will also lessen the environmental impact arising from the mass-production and application of commercial screening kits [30, 31]. Therefore, the integration of paper in the POC devices development fulfills all the desired criteria [32] with the potential to be integrated with a wide range of sensing applications in oncology field.

Taking into consideration the societal and the medical sector needs, in the present study we designed and characterized an electrochemical device towards the analysis of miRNA-21 with application in biological fluids, i.e. human serum. To this aim, an anti-miRNA-21 probe, labeled with a redox mediator, namely methylene blue (MB), was rationally designed and immobilized onto gold nanoparticles (AuNPs). The ultimate device was capable to detect various levels of miRNA-21 in both buffer solutions and human serum, spiked in the pM-nM range. With regard to the fact that miRNA targets are found in traces in biofluids, the proposed POC tool was integrated with an external paper-based origami device. By exploiting the paper's porosity and the 3D-folding configuration, a decrease of the matrix effect and a pre-concentration of the mi-RNA-21 were achieved respectively. With this approach, diagnostically significant miRNA concentrations were detected, down to pM level, towards real clinical practice applications. This pioneering study opens up novel possibilities in the development of portable diagnostics for use by oncologists and patients, comparing it to existing POC miRNA detection tools that rely on amplification methods such as PCR, loop-mediated isothermal amplification, duplex nuclease amplification strategies, and CRISPR-based systems [33–36]. These methods, despite using alternative signal readouts and detection platforms, still require expensive equipment, lengthy protocols, and sample pretreatment. In contrast, our method offers faster assay times (30 min without amplification), cost-effective materials, and direct serum analysis. Finally, it also offers a widely extensible concept that can be adapted to various miRNAs, not only those relevant to TNBC, by appropriately selecting and modifying the corresponding detection probe.

2 Experimental section

2.1 Materials and apparatus

Sodium chloride (NaCl), chloroauric acid ($HAuCl_4$), sodium borohydride, sodium citrate, phosphate buffer solution (PBS) tablets (140 mM NaCl, 10 mM PBS, 3 mM KCl), 6-mercapto-1-hexanol (MCH, $C_6H_{14}OS$), tris (2-carboxyethyl) phosphine (TCEP; $C_9H_{15}O_6P$), and Hypo-Opticlear Human serum were purchased from Sigma-Aldrich (St. Louis, MO, USA). The anti-miR21 DNA probe tagged with MB (5′-Thiol-C6-TCA ACA TCA GTC TGA TAA GCT A-Atto-MB-3′) (MB-tagged DNA probe) selective to miRNA-21, the target miRNA-21 sequence (5′-uag cuu auc aga cug aug uug a-3′), and the control sequences used in the selectivity study were purchased from Metabion GmbH (Steinkirchen, Germany). Paper-based graphite screen-printed electrodes (SPEs) were in-house produced using office paper [37]. The three-electrode configuration was developed through manual screen-printing, following established procedures [27, 38]. The conductive silver ink was purchased from Loctite (Italy) and the carbon ink was purchased from Sun Chemical (USA). All the electrochemical measurements were carried out using a portable potentiostat PalmSens 4 (PalmSens, Netherlands) equipped with a multi-12 reader and interfaced to a laptop using PSTrace5.10.Filter paper Whatman Grade 1 (Merck KGaA, Darmstadt, Germany) was used as the substrate for the paper-based pre-concentration device.

2.2 Paper-based screen-printed electrodes fabrication

Fabriano (80 g/m^2) office paper was used as the substrate for fabricating electrochemical biosensors. A hydrophobic wax pattern was printed onto the paper using a well-defined model created with Adobe Illustrator software and a Xerox ColorQube 8580 office printer (Xerox, USA). The resulting sheet was then heat-treated at 100 °C for approximately 1 min to melt the wax and obtain a hydrophobic layer to enable liquid handling. The electrodes were printed on the wax layer, using screen-printing technology. The conductive inks and frames used for the printing process, enabled the production of SPE in the characteristic three-electrode configuration: a first layer of silver ink was used to print the connections and the reference electrode, a second layer of graphite ink was printed with a dedicated frame to create the working (4 mm in diameter) and counter electrodes. After each printing step, the sheet was heat-treated for 30 min at 60 °C to remove the solvent and induce polymerization of the ink's components. The obtained electrochemical strips were approximately 2.5 cm in height and 1 cm in width.

2.3 AuNPs synthesis

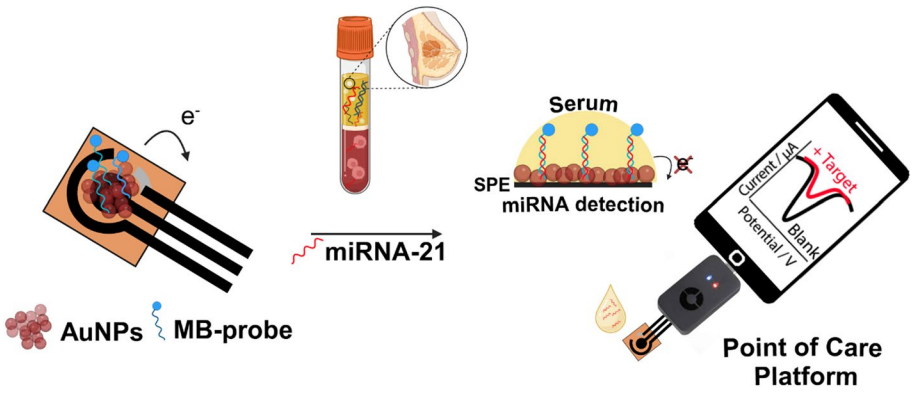
The synthesis of AuNPs was carried out following a previously reported method [39]. First, the glassware and magnetic rod used in this synthesis were first cleaned with aqua regia (HCl/HNO_3 3:1 (v/v)), rinsed with distilled water, and then treated with piranha solution (H_2SO_4/H_2O_2 7:3 (v/v)), followed by another rinse with distilled water before use. Next, AuNPs were synthesized in a round flask at room temperature (RT). This involved mixing 9 mL of distilled water with 1 mL of $HAuCl_4$ at a concentration of 0.01 g/mL and 2 mL of sodium citrate at a concentration of 0.01 g/mL. Subsequently, 0.5 mL of 20 mM sodium borohydride were added dropwise to the mixture. The solution was left to stir in dark overnight. The resulting dispersion of AuNPs was then stored at 4 °C.

2.4 Preparation of the office paper-based strip for miRNA-21 detection

To tailor the biosensor for optimal performance, the surface of the working electrode was initially modified with 4 µL of AuNPs via drop casting, enabling mass-scale production and allowing the simultaneous modification of multiple electrodes without compromising performance. The AuNPs provide a stable platform for the immobilization of the DNA probe, which was functionalized with a thiol group at the 5′ end to facilitate covalent binding through Au–S bonds, and with MB at the 3′ end to enable electron transfer at the electrode surface. The Au–S immobilization strategy was chosen over other common approaches (such as biotin-avidin interactions and carbodiimide chemistry) due to its high stability, resistance to desorption, excellent reproducibility. Moreover, the superior conductivity of gold enhances electrochemical performance by enabling efficient electron transfer, making it the ideal choice for highly reliable electrochemical sensors. Prior to immobilization, the 1 µM anti-miRNA-21 DNA probe underwent a reduction step in the presence of 10 mM TCEP for 1 h at RT. TCEP is essential for reducing disulfide bonds, ensuring that the probe is in its active form for covalent attachment to the AuNPs [40]. The resulting solution was then diluted to the desired concentration (in the nanomolar range) for immobilization onto the AuNPs-SPE. Subsequently, 20 µL of the probe was applied to the working electrode area and incubated for 1 h at RT in a humid chamber. The SPEs were gently washed with distilled water and incubated in the humid chamber with 20 µL of 2 mM 6-mercapto-1-hexanol for 1.5 h to passivate unoccupied areas of the working electrode. All modification steps were carried out in a humid chamber to prevent SPEs drying during incubation steps. Finally, the electrodes were thoroughly washed with double-distilled water to remove any residue and complete the preparation process.

2.5 Measurement of miRNA-21 target

The proposed platform is based on "signal off" detection strategy. In this approach, conformational changes on the electrode surface have an impact on electron transfer rates. Briefly, in the absence of hybridization the electroactive labels remain close to the surface by preserving the hairpin structure of the probe, which maintains rapid electron transfer between MB and the electrode. However, when the DNA probe hybridizes with the target miRNA, the hairpin structure of the probe undergoes a conformational shift, which impedes the electron transfer between MB and the electrode [41] (Scheme 1).



Scheme 1 Schematic representation for the POC platform for detection of miRNA 21 in TNBC

Measurements were performed simultaneously on twelve separate SPEs by inserting them into the 12-channel multiplexer connected to the portable potentiostat. For each miRNA analysis, the SPEs were initially assessed in the presence of 70 µL of a blank solution (PBS pH 7.4 or untreated serum), allowing the probe to stabilize for 30 min before measurement. Following this, the miRNA target was added to the working solution, and the sample was analyzed again after a 30-min incubation to promote interaction between the miRNA and the anti-miRNA probe. The signal was recorded using square wave voltammetry (SWV) as the electrochemical technique with the following parameters: equilibrium time = 5 s, E_{start} = 0 V, E_{end} = −0.6 V, E_{step} = 0.001 V, amplitude = 0.01 V, frequency = 50 Hz. All the measurements were carried out in both standard PBS and human serum samples. The percentage signal change was calculated using the following equation:

$$\text{Signal change}\ (\%) = 100 \times \left(I_0 - I_t\right) / I_0$$

Here, I_0 represents the current recorded in the absence of miRNA, while I_t corresponds to the current measured after the addition of the target miRNA.

2.6 Pre-concentration on paper-based origami device

The paper-based origami pre-concentration device was prepared as previously reported [42]. The circles pattern was designed with Adobe Illustrator and wax-printed on chromatographic paper (Whatman No. 1) through the solid ink printer (ColorQube 8580). The wax-printed paper was heat-treated for approximately 1 min at 100 °C to obtain a hydrophobic barrier for liquid sample handling. The device consisted of nine layers of 3-mm diameter discs and a final layer with a 9-mm diameter disc. Briefly, A 10-µL aliquot of each sample was applied to every layer of the pre-concentration device and left to dry at RT. Following the drying step, the origami device was folded, and the pre-concentrated target was obtained in the final layer by adding 10 µL of buffer solution. The last layer was then dried and trimmed, and the pre-concentrated sample was reconstituted in 50 µL of PBS buffer. The resulting 50-µL sample was subsequently measured using the proposed electrochemical sensor by SWV, as described above.

3 Results and discussion

3.1 Optimization of the experimental parameters

Several experimental parameters were optimized to assess the sensor's performance. All the optimization studies were performed using the SWV technique. Measurements were performed in triplicate (n = 3) in PBS solution (pH 7.4), with a target concentration of 40 nM. Firstly, we optimized AuNPs volume on the working electrode area. Three different volumes were tested: 4, 8, and 12 µL as shown in Fig. 1a.

The observed signal variation was plotted in histogram for each AuNPs volume, following the hybridization of the DNA probe with the target miRNA. The most significant signal change was observed in the electrodes modified with 4 µL of AuNPs, which provided a sufficiently functionalized surface area with optimal electrochemical signal resolution. Subsequently, the effect of DNA probe density was investigated at different concentrations of 50, 100, 200, 400 nM as shown in Fig. 1b. Selecting the appropriate DNA probe density minimizes the signal-to-noise ratio and achieves higher

affinity between the probe and the target. By performing this study, we observed that, with increasing the probe density, the recorded signal also was enhanced due to the higher number of redox molecules i.e. MB. However, this increases also introduced limitations: densely packed probes not only restrict the diffusion of target molecules, but also lead to steric hindrance, where the proximity of probes reduces hybridization efficiency. To this, we decided to continue our study with the immobilization of 50 nM of probe, considering the right balance between effective signal variation and probe-target affinity, as shown in Fig. 1b. Subsequently, we optimized the SWV frequency by testing values within the range of 50, 100, and 200 Hz. As illustrated in Fig. 1c, 50 Hz emerged as the best compromise, offering a balance between signal variation and repeatability. In contrary, higher frequencies resulted in a greater average signal change, producing also voltammograms with higher noise levels.

3.2 Selectivity studies

We evaluated the selectivity of the developed platform using three potential interfering (non-complementary RNA strands): miRNA-125b (5′-ucc cug aga ccc uaa cuu gug a -3′), miRNA-4676 (5′-cac ugu uuc acc acu ggc ucu u -3′) and miRNA-107 (5′-agc agc auu gua cag ggc uau ca-3′).

Measurements were performed both in PBS and in human blood serum in presence of 40 nM of each interferent. As shown in Fig. 2, the signal change associated with the interferents examined was negligible compared to the observed for our target of interest. This highlights the selectivity of the developed platform. Furthermore, its ability to maintain high specificity, even in complex matrices such as human serum, underscores its reliability and robustness.

3.3 Analytical characterization in standard and human serum

After optimizing the key experimental parameters and selectivity of the proposed platform, its analytical performance was evaluated in PBS pH 7.4 and in human serum. For all the experiments, SWV was used, and the carefully optimized settings were adopted. The analytical characterization was conducted at increasing concentrations of miRNA-21, ranging from 0.1 to 1000 nM, as shown in Fig. 3.

As illustrated in Fig. 3a, b, both the buffer solution and human serum measurements exhibited a characteristic semi-logarithmic sigmoidal correlation between the signal change percentage and the logarithmic scale of the target concentration, expressed in nanomolar. The correlations were satisfactory, calculated as the R^2, equal to 0.985 and 0.987 for buffer and human serum solutions, respectively. The dynamic range of detection was observed to span from approximately 1.7 nM to 1000 nM, demonstrating the platform's capability to reliably detect miRNA-21 over a wide range of concentrations. In addition, the limit of detection (LOD) was evaluated for both the buffer solution and human serum. For the buffer solution, the LOD was approximated to be around 1.7 nM, calculated as the concentration corresponding to a 10% signal change. In serum, the LOD was similarly determined, resulting equal to 1.2 nM. A good repeatability of ca. 8% was obtained (calculated on five replicates in the presence of a target concentration of 40 nM). These results underscore the potential of the proposed electrochemical platform as a rapid, sensitive, and cost-effective solution for the clinical detection of miRNA-21.

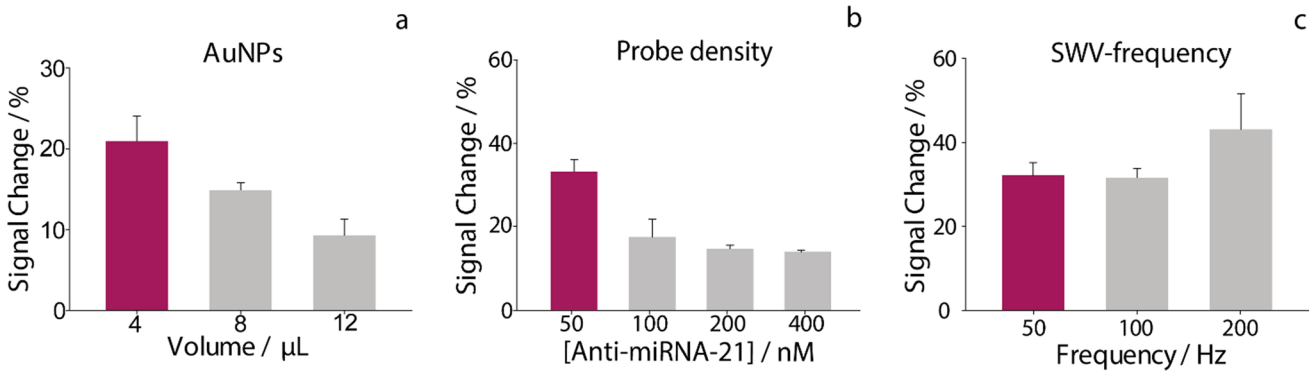


Fig. 1 **a** Evaluation of AuNPs volume (4,8, 12 µL); **b** Study of anti-miRNA-21 specific probe density (50, 100, 200, 400 nM); and c) Optimization of the frequency of the square wave (50, 100,200 Hz). Measurements were performed in triplicate (n = 3) in a standard PBS pH 7.4, with a target concentration of 40 nM

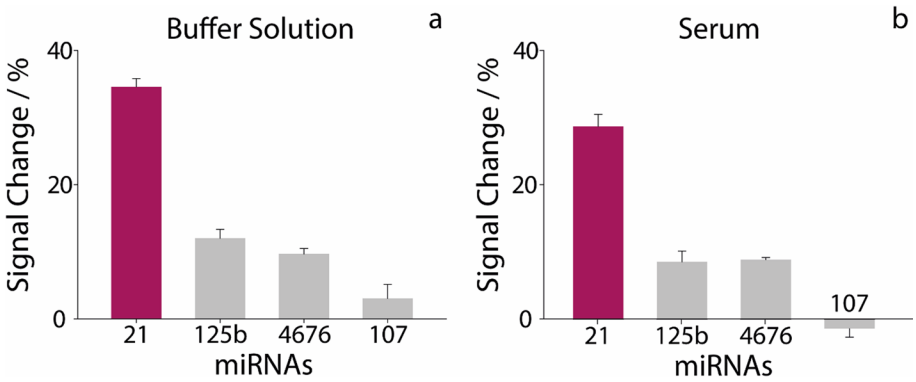


Fig. 2 Selectivity studies comparing signal change percentage obtained in the presence of 40 nM miRNA-21 and in the presence of three potential interferents. **a** Buffer solution; **b** human serum. Measurements were performed in triplicate (n = 3)

3.4 Pre-concentration with paper-based origami device

To tackle the limitations of detecting trace miRNA concentrations in complex liquid biopsy samples, a paper-based pre-concentration device was employed. This innovative pre-concentration tool addresses the limitations of existing conventional methods, providing increased sensitivity without the use of expensive and time-consuming procedures. The paper's porosity, which enables capillary action and facilitates the retention of analyte molecules, along with the 3D folding configuration, were leveraged to enhance detection sensitivity. The 3D origami design optimizes this process by creating multiple layers that progressively pre-concentrate the target analyte as the sample flows through the paper's porous structure. As the liquid moves vertically, target molecules are enriched while components of the sample, here serum, are filtered through the device, reducing matrix effect. This significantly amplifies the signal response and thereby increasing detection sensitivity [42]. Three miRNA-21 concentrations-0.1 nM, 1 nM, and 10 nM- were pre-concentrated using the device, resulting in a signal improvement of up to 270% across all samples, as shown in Fig. 4. The pre-concentration protocol was applied in PBS solution and in untreated human serum samples.

Notably, the pre-concetration device amplified the sensor's signal response corresponding to an order of magnitude in target's concentration, enabling the detection of even the lowest concentration (0.1 nM). In particular, the signal obtained from the pre-concentrated 0.1 nM sample was equivalent to that of the non-preconcentrated 1 nM sample, demonstrating the device's capacity to enhance detection ability. This significant improvement in signal response enables the integration of the proposed electrochemical sensor into real-world clinical workflows and its application in routine diagnostics. By providing highly sensitive, real-time detection of miRNA biomarkers, this approach has the potential to support oncologist in making timely clinical decisions for disease diagnosis and therapy monitoring.

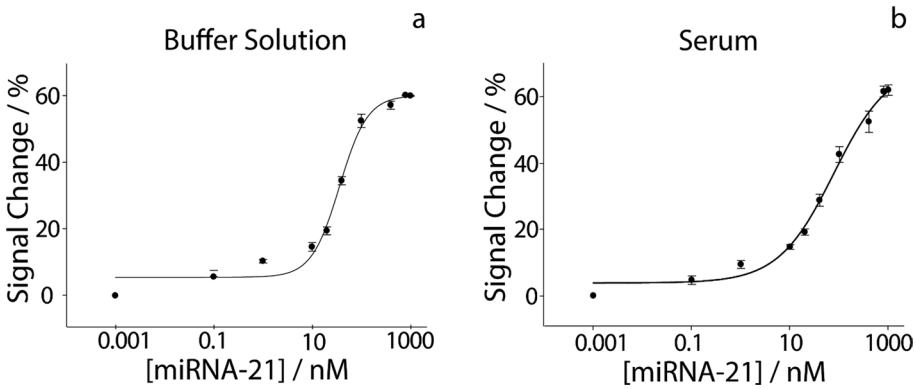


Fig. 3 Calibration curve obtained in PBS (**a**) and human serum (**b**) in different concentrations of miRNA-652 target from 0.1 to 1000 nM. Measurements were performed in triplicate (n = 3). SWV parameters: $t_{equilibration}$ = 5 s, E_{start} = 0.0 V, E_{end} = − 0.5 V, E_{step} = 0.001 V, Amplitude = 0.01 V, Frequency = 50.0 Hz

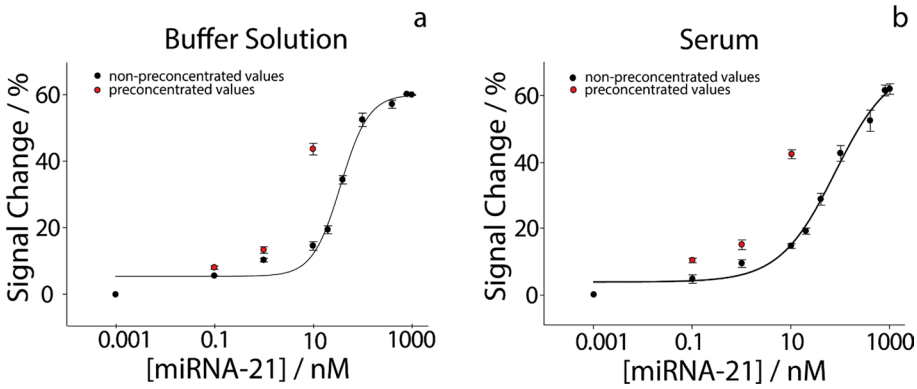


Fig. 4 Calibration curves showcasing the effect of target miRNA pre-concentration on platform's signal response. **a** Calibration curve in buffer solution. **b** Calibration curve in human serum. Measurements were performed in triplicate (n = 3). SWV parameters: $t_{equilibration}$ = 5 s, E_{start} = 0.0 V, E_{end} = − 0.5 V, E_{step} = 0.001 V, Amplitude = 0.01 V, Frequency = 50.0 Hz

4 Conclusions

In this study, a paper-based POCT-platform is proposed to be a starting point for the development of a sensitive and selective detection method for miRNA-21 for TNBCs patients. In particular, liquid biopsy provides a real-time snapshot of circulating biomarkers, such as miRNAs, facilitating TNBC monitoring. Our electrochemical biosensor enables non-invasive miRNA-21 detection, allowing for longitudinal tracking of treatment response and disease progression. The platform aims to provide an accessible, rapid, and cost-effective POCT, paving the way for advancements in personalized medicine and early cancer detection strategies. Office paper was used as the substrate for fabricating the device through a screen-printing technique. Subsequently, the miRNA detection is achieved via a redox mediator labelled DNA specific probe, that is immobilized on the sensing area by modifying it with AuNPs. The sensor was successfully characterized analytically, by performing calibration curves both in buffer solution and human serum resulting in a range of 0.1 nM to 1000 nM miRNA. Selectivity studies demonstrate the sensor's ability to discriminate miRNA-21 in presence of other potential interferent miRNAs showing also good reproducibility (RSD = 8%). The LOD value was calculated as low as 1.2 nM in serum, proving the feasibility of the sensor's application in complex biofluids. To further improve the POCT performance, it integrates an office paper-based SPE for miRNA detection with an external paper-based origami device for sample pre-concentration. MiRNA abundance varies significantly across patients due to factors like age, sex, cancer stage, and therapy type, making continuous monitoring essential for correlating miRNA levels with disease progression and treatment response [43]. While miRNAs are typically found at lower concentrations in body fluids compared to tissue biopsies [44], studies show they can be detected at low nanomolar levels [45], and sequencing studies have observed thresholds at the micromolar (μM) level after amplification [46], while in liver biopsy samples in the low fmol range per μg of total RNA [47]. Our method, which requires no amplification, achieves enhanced sensitivity through a pre-concentration device, offering sensitivity comparable to amplification methods without the need for sample pre-treatment. This all-in-one system significantly enhances the sensor's signal response, enabling the detection of even low miRNA concentrations in serum samples. Such precision is crucial for clinical applications, as it allows the identification of miRNA levels that are significant for the diagnosis and monitoring of TNBC.

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Data availability Data will be available up on request.

Declarations

Ethics approval and consent to participate Not applicable.

Consent for publication Not applicable.

Competing interest The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

1. Lone SN, Nisar S, Masoodi T, Singh M, Rizwan A, Hashem S, et al. Liquid biopsy: a step closer to transform diagnosis, prognosis and future of cancer treatments. Mol Cancer. 2022;21:79. https://doi.org/10.1186/s12943-022-01543-7.
2. Pandey S, Yadav P. Liquid biopsy in cancer management: Integrating diagnostics and clinical applications. Pract Lab Med. 2025;43: e00446. https://doi.org/10.1016/j.plabm.2024.e00446.
3. Weissleder R, Pittet MJ. Imaging in the era of molecular oncology. Nature. 2008;452:580–9. https://doi.org/10.1038/nature06917.
4. Pantaleo MA, Nannini M, Maleddu A, Fanti S, Ambrosini V, Nanni C, et al. Conventional and novel PET tracers for imaging in oncology in the era of molecular therapy. Cancer Treat Rev. 2008;34:103–21. https://doi.org/10.1016/j.ctrv.2007.10.001.
5. Feng J, Li B, Ying J, Pan W, Liu C, Luo T, et al. Liquid biopsy: application in early diagnosis and monitoring of cancer. Small Struct. 2020;1:2000063. https://doi.org/10.1002/sstr.202000063.
6. Cortez MA, Bueso-Ramos C, Ferdin J, Lopez-Berestein G, Sood AK, Calin GA. MicroRNAs in body fluids—the mix of hormones and biomarkers. Nat Rev Clin Oncol. 2011;8:467–77. https://doi.org/10.1038/nrclinonc.2011.76.
7. Balzano F, Deiana M, Dei Giudici S, Oggiano A, Baralla A, Pasella S, et al. miRNA stability in frozen plasma samples. Molecules. 2015;20:19030–40. https://doi.org/10.3390/molecules201019030.
8. O'Brien J, Hayder H, Zayed Y, Peng C. Overview of microRNA biogenesis, mechanisms of actions, and circulation. Front Endocrinol. 2018. https://doi.org/10.3389/fendo.2018.00402.
9. Moro G, Fratte CD, Normanno N, Polo F, Cinti S. Point-of-care testing for the detection of microRNAs: towards liquid biopsy on a chip. Angew Chem Int Ed. 2023;62: e202309135. https://doi.org/10.1002/anie.202309135.
10. Madhavan D, Cuk K, Burwinkel B, Yang R. Cancer diagnosis and prognosis decoded by blood-based circulating microRNA signatures. Front Genet. 2013. https://doi.org/10.3389/fgene.2013.00116.
11. Bhat SA, Majid S, Hassan T. MicroRNAs and its emerging role as breast cancer diagnostic marker—a review. Adv Biomark Sci Technol. 2019;1:1–8. https://doi.org/10.1016/j.abst.2019.05.001.
12. Kleivi Sahlberg K, Bottai G, Naume B, Burwinkel B, Calin GA, Børresen-Dale A-L, et al. A serum microRNA signature predicts tumor relapse and survival in triple-negative breast cancer patients. Clin Cancer Res. 2015;21:1207–14. https://doi.org/10.1158/1078-0432.CCR-14-2011.
13. de Freitas AJA, Nunes CR, Causin RL, Calfa S, de Oliveira MA, de Souza CP, et al. 49P Circulating miRNA signatures to predict recurrence in patients with pathological complete response of triple-negative breast cancer. Ann Oncol. 2023;34:S202. https://doi.org/10.1016/j.annonc.2023.09.1551.
14. Natarajan L, Pu M, Davies SR, Vickery TL, Nelson SH, Pittman E, et al. MiRNAs and long-term breast cancer survival: evidence from the WHEL Study. Cancer Epidemiol Biomark Prev Publ Am Assoc Cancer Res Cosponsored Am Soc Prev Oncol. 2019;28:1525–33. https://doi.org/10.1158/1055-9965.EPI-18-1322.
15. Stevic I, Müller V, Weber K, Fasching PA, Karn T, Marmé F, et al. Specific microRNA signatures in exosomes of triple-negative and HER2-positive breast cancer patients undergoing neoadjuvant therapy within the GeparSixto trial. BMC Med. 2018;16:179. https://doi.org/10.1186/s12916-018-1163-y.
16. Kumar V, Gautam M, Chaudhary A, Chaurasia B. Impact of three miRNA signature as potential diagnostic marker for triple negative breast cancer patients. Sci Rep. 2023;13:21643. https://doi.org/10.1038/s41598-023-48896-7.
17. Koscianska E, Starega-Roslan J, Sznajder LJ, Olejniczak M, Galka-Marciniak P, Krzyzosiak WJ. Northern blotting analysis of microRNAs, their precursors and RNA interference triggers. BMC Mol Biol. 2011;12:14. https://doi.org/10.1186/1471-2199-12-14.
18. Kroh EM, Parkin RK, Mitchell PS, Tewari M. Analysis of circulating microRNA biomarkers in plasma and serum using quantitative reverse transcription-PCR (qRT-PCR). Methods San Diego Calif. 2010;50:298–301. https://doi.org/10.1016/j.ymeth.2010.01.032.
19. Hamam R, Ali AM, Alsaleh KA, Kassem M, Alfayez M, Aldahmash A, et al. microRNA expression profiling on individual breast cancer patients identifies novel panel of circulating microRNA for early detection. Sci Rep. 2016;6:25997. https://doi.org/10.1038/srep25997.
20. Wu X, Somlo G, Yu Y, Palomares MR, Li AX, Zhou W, et al. De novo sequencing of circulating miRNAs identifies novel markers predicting clinical outcome of locally advanced breast cancer. J Transl Med. 2012;10:42. https://doi.org/10.1186/1479-5876-10-42.
21. Oikonomopoulos A, Polytarchou C, Joshi S, Hommes DW, Iliopoulos D. Identification of circulating microRNA signatures in Crohn's disease using the nanostring ncounter technology. Inflamm Bowel Dis. 2016;22:2063–9. https://doi.org/10.1097/MIB.0000000000000883.
22. Luppa PB, Müller C, Schlichtiger A, Schlebusch H. Point-of-care testing (POCT): current techniques and future perspectives. TrAC Trends Anal Chem. 2011;30:887–98. https://doi.org/10.1016/j.trac.2011.01.019.

23. Valera E, Jankelow A, Lim J, Kindratenko V, Ganguli A, White K, et al. COVID-19 point-of-care diagnostics: present and future. ACS Nano. 2021;15:7899–906. https://doi.org/10.1021/acsnano.1c02981.
24. Raucci A, Cimmino W, Grosso SP, Normanno N, Giordano A, Cinti S. Paper-based screen-printed electrode to detect miRNA-652 associated to triple-negative breast cancer. Electrochim Acta. 2024;487: 144205. https://doi.org/10.1016/j.electacta.2024.144205.
25. Gao X, Xu H, Baloda M, Gurung AS, Xu L-P, Wang T, et al. Visual detection of microRNA with lateral flow nucleic acid biosensor. Biosens Bioelectron. 2014;54:578–84. https://doi.org/10.1016/j.bios.2013.10.055.
26. Liu X, Wang Q, Diao Z, Huo D, Hou C. Label-free fluorescent biosensor based on AuNPs etching releasing signal for miRNA-155 detection. Talanta. 2024;278: 126481. https://doi.org/10.1016/j.talanta.2024.126481.
27. Singh S, Wang J, Cinti S. Review—an overview on recent progress in screen-printed electroanalytical (Bio)sensors. ECS Sens Plus. 2022;1: 023401. https://doi.org/10.1149/2754-2726/ac70e2.
28. Singh S, Raucci A, Cimmino W, Cinti S. Paper-based analytical devices for cancer liquid biopsy. Anal Chem. 2024;96:3698–706. https://doi.org/10.1021/acs.analchem.3c04478.
29. Kalligosfyri PM, Cinti S. Paper-based materials for diagnostics. ACS Mater Lett. 2024;6:1184–98. https://doi.org/10.1021/acsmaterialslett.3c01474.
30. Shalaby AA, Tsao C-W, Ishida A, Maeki M, Tokeshi M. Microfluidic paper-based analytical devices for cancer diagnosis. Sens Actuators B Chem. 2023;379: 133243. https://doi.org/10.1016/j.snb.2022.133243.
31. Dukle A, Nathanael AJ, Panchapakesan B, Oh T-H. Role of paper-based sensors in fight against cancer for the developing world. Biosensors. 2022;12:737. https://doi.org/10.3390/bios12090737.
32. Land KJ, Boeras DI, Chen X-S, Ramsay AR, Peeling RW. REASSURED diagnostics to inform disease control strategies, strengthen health systems and improve patient outcomes. Nat Microbiol. 2018;4:46–54. https://doi.org/10.1038/s41564-018-0295-3.
33. Wang Z-Y, Sun M-H, Zhang Q, Li P-F, Wang K, Li X-M. Advances in point-of-care testing of microRNAs based on portable instruments and visual detection. Biosensors. 2023;13:747. https://doi.org/10.3390/bios13070747.
34. Kimura Y, Ikeuchi M, Inoue Y, Ikuta K. 3D microdevices that perform sample purification and multiplex qRT-PCR for early cancer detection with confirmation of specific RNAs. Sci Rep. 2018;8:17480. https://doi.org/10.1038/s41598-018-35772-y.
35. Wu D, Yi X, Xia N. Electrochemical biosensors for microRNA detection using duplex-specific nuclease based signal amplification strategies. Int J Electrochem Sci. 2020;15:12136–48. https://doi.org/10.20964/2020.12.67.
36. Wang K, Yin H, Li S, Wan Y, Xiao M, Yuan X, et al. Quantitative detection of circular RNA and microRNA at point-of-care using droplet digital CRISPR/Cas13a platform. Biosens Bioelectron. 2025;267: 116825. https://doi.org/10.1016/j.bios.2024.116825.
37. Glovi A, Miglione A, Maresca DC, Somma F, Romano B, Ianaro A, et al. Paper-based electrochemical device for the determination of H_2S in murine lysates for liquid biopsy application. Anal Chim Acta. 2025;1342: 343669. https://doi.org/10.1016/j.aca.2025.343669.
38. Preparation of paper-based devices for reagentless electrochemical (bio)sensor strips | Nature Protocols n.d. https://www.nature.com/articles/s41596-019-0186-y. Accessed 27 Jan 2025.
39. Miglione A, Raucci A, Amato J, Marzano S, Pagano B, Raia T, et al. Printed electrochemical strip for the detection of miRNA-29a: a possible biomarker related to Alzheimer's disease. Anal Chem. 2022;94:15558–63. https://doi.org/10.1021/acs.analchem.2c03542.
40. Oberhaus FV, Frense D, Beckmann D. Immobilization techniques for aptamers on gold electrodes for the electrochemical detection of proteins: a review. Biosensors. 2020;10:45. https://doi.org/10.3390/bios10050045.
41. Kivrak E, Kara P. Simultaneous detection of ovarian cancer related miRNA biomarkers with carboxylated graphene oxide modified electrochemical biosensor platform. Bioelectrochemistry. 2025;161: 108806. https://doi.org/10.1016/j.bioelechem.2024.108806.
42. Kalligosfyri PM, Cinti S. 3D paper-based origami device for programmable multifold analyte preconcentration. Anal Chem. 2024;96:9773–9. https://doi.org/10.1021/acs.analchem.4c02032.
43. Alfardus H, De Los Angeles Estevez-Cebrero M, Rowlinson J, Aboalmaaly A, Lourdusamy A, Abdelrazig S, et al. Intratumour heterogeneity in microRNAs expression regulates glioblastoma metabolism. Sci Rep. 2021;11:15908. https://doi.org/10.1038/s41598-021-95289-9.
44. Li Y, Kowdley KV. Method for microRNA isolation from clinical serum samples. Anal Biochem. 2012;431:69–75. https://doi.org/10.1016/j.ab.2012.09.007.
45. Bosson AD, Zamudio JR, Sharp PA. Endogenous miRNA and target concentrations determine susceptibility to potential ceRNA competition. Mol Cell. 2014;56:347–59. https://doi.org/10.1016/j.molcel.2014.09.018.
46. Rodgers O, Watson C, Waterfield T. miRNA library preparation optimisation for low-concentration and low-volume paediatric plasma samples. Non-Coding RNA. 2025;11:11. https://doi.org/10.3390/ncrna11010011.
47. Bissels U, Wild S, Tomiuk S, Holste A, Hafner M, Tuschl T, et al. Absolute quantification of microRNAs by using a universal reference. RNA. 2009;15:2375–84. https://doi.org/10.1261/rna.1754109.