Bioelectroanalytical technologies for determining mirnas

Brief description

The technologies developed allow the reliable, selective and sensitive determination of microRNAs (miRNAs or miRs), biomarkers for the early diagnosis of cancer, in total RNA (tRNA) extracted from complex clinical samples in a fast, simple way and without the need to carry out additional steps of amplification or retrotranscription to complementary DNA.

How does it work?

The technologies are based on the development of different affinity formats on the surface of commercial magnetic particles (MBs), enzymatic labeling with the enzyme horseradish peroxidase (HRP) and signal transeduction by amperometry using disposable screen-printed electrodes.

The developed methodologies consist of selective hybridization of the target miRNA with a biotin-modified synthetic complementary probe, in solution or previously immobilized on the surface of properly functionalized commercial MBs (Figure 1). Hybrids formed in solution are captured by highly selective bioreceptors (viral p19 protein, zinc finger protein or selective antibody to DNA/RNA heterohybrids), previously effectively immobilized on functionalized commercial MBs and enzymatically labeled with a streptavidin-HRP polymer in a last step via biotin-streptavidin interaction, while hybrids formed on the surface of conveniently functionalized commercial MBs are recognized and enzymatically labeled by a mixture of specific bioreceptors (viral p19 protein or selective antibody to DNA/RNA heterohybrids and HRP-labeled secondary antibodies). Regardless of the assay strategy employed, the modified MBs are magnetically trapped on the surface of the disposable transducers used to monitor the amperometric response using the H_2O_2/HRP/hydroquinone (HQ) system, which allows establishing the relationship between the magnitude of the current intensity obtained and the concentration of the target miRNA present in the sample.

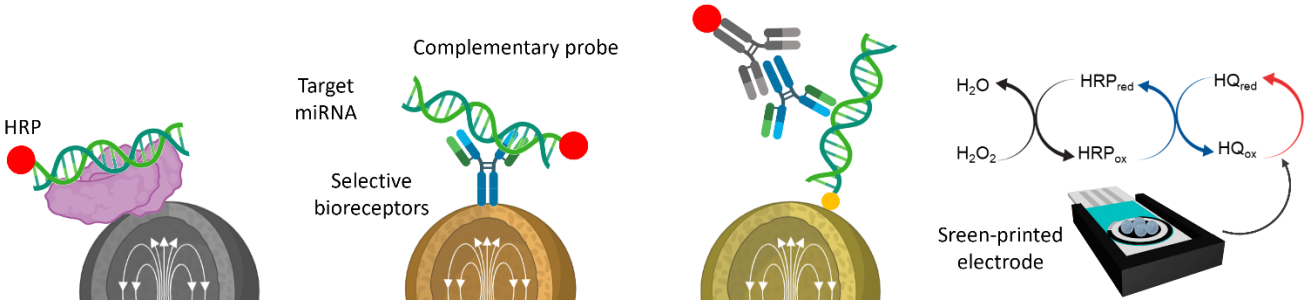

Figure 1. *Representative examples of bioelectroanalytical technologies available to determine miRNAs and schematic diagram of the amperometric detection process.*

What problem does it solve?

The developed technologies have been successfully applied to the determination of several mature miRNAs relevant for the diagnosis and prognosis of breast and colorectal cancer in tRNA extracted from cell lines, fresh or paraffin-embedded tissues and breast cytologies, using significantly lower sample amounts (100-1000 ng) than those required by other more conventional methodologies, and in assay times between 75-120 minutes, which can be reduced to as little as 15 minutes, without significantly compromising sensitivity.

Medicine Faculty. Entrepabellones building 7 y 8.
C/ Doctor Severo Ochoa, 7. 28040 Madrid.
comercia@ucm.es

The high selectivity that characterizes the developed biotechnologies, evaluated against miRNAs with non-complementary sequences and against synthetic miRNAs with a single missing base, ensures the obtaining of reliable and accurate results, an essential requirement to carry out the analysis of this type of biomarkers considering the high homology existing between sequences of different miRNAs that can coexist in the same sample. In addition, the versatility of the proposed technologies allows their transfer to the determination of any miRNA as well as the multiplexed analysis of miRNA panels that allow the identification of unique expression profiles of these biomarkers, at tissue and cellular level, inherent to each type of cancer and stage of the disease, allowing their molecular classification and characterization and the selection/application of the most appropriate treatment in each case.

What future products will it develop?

The integration of these bioelectroanalytical technologies, which are affordable, fast, simple and reliable and compatible with portability, into clinical and hospital practice promises substantial benefits for improving the reliability of early diagnosis and prognosis and the efficiency of therapeutic programs applied to patients with chronic diseases of high global prevalence, such as cancer, which will positively impact their survival and quality of life.

Competitive advantages

Among the advantages offered by these technologies, the following stand out:

- Easy application to the detection of any miRNA and other RNAs of interest in tRNA extracted from any type of biological sample or directly in liquid biopsy samples.
- Easy implementation in point-of-care diagnostic devices using simple and low-cost instrumentation.
- Adequate sensitivity for direct detection of miRNAs in the absence of additional prior steps of reverse transcription, amplification and purification.
- Multiplexing capability and possibility of automation.
- Lower time and cost per assay than conventional qRT-PCR miRNA analysis strategy.
- Reliable quantitative results.
- Comparable efficiency in the analysis of fresh and paraffin-embedded tissue samples.

Where has it been developed?

These bioplatforms have been developed at the Electroanalysis and Electrochemical (Bio)Sensors Group of the Faculty of Chemical Sciences of the Complutense University of Madrid, in collaboration with the Molecular Pharmacology Group of the CIB-CSIC, ProteoFun of the ISCIII, and the company CANNAN RESEARCH & INVESTMENT S.L.

And moreover…

Website with additional information: https://gebeucm.wordpress.com/.

Researcher in charge

Susana Campuzano Ruiz, susanacr@quim.ucm.es; José M. Pingarrón Carrazón, pingarro@quim.ucm.es; Rebeca M. Torrente-Rodríguez, rebecamt@ucm.es
Department: **Analytical Chemistry**
Faculty: **Chemical Sciences**