



Semiconductor technology for early detection of DNA methylation for cancer: From concept to practice

Melpomeni Kalofonou*, Chris Toumazou

Department of Electrical and Electronic Engineering, Centre for Bio-Inspired Technology, Institute of Biomedical Engineering, Imperial College London, SW7 2AZ, UK

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ABSTRACT

The electrical detection of DNA methylation based biomarkers using semiconductor technology shows great promise for early cancer screening. Presented is the very first proof-of-concept example of using CMOS-based technology for real-time DNA methylation detection using Ion-Sensitive Field-Effect Transistors (ISFETs). An electrochemical label-free approach was applied in two gene assays, each one of which incorporated the sequences of *DAPK1* and *CDKN2A/p16-INK4 (p16)* gene promoters at a both methylated and unmethylated state, performing isothermal methylation-specific amplification and detection both in-tube and on-chip (real-time). Good discrimination was shown between the two states, achieving a very good average pH signal change for the methylated state of 1.91 for *DAPK1* assay and of 1.58 for *p16* assay in the tube test. The real-time on-chip test showed similarly good real-time differential signal change in favour of methylated DNA, reaching 37 mV for *DAPK1* assay and 23 mV for *p16* assay, validating the results from the proof-of-concept test of pH-LAMP in-tube while confirming the sensitivity of real-time methylation-specific pH-LAMP on-chip.

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1. Introduction

Cancer is exponentially increasing nowadays in numbers, an observation that is not only correlated with the *genetic* predisposition of the individual towards the disease but also with the *epi-genomic* or *epigenetic*, as commonly referred, inheritance passed by our ancestors in combination with our environmental influences. Epigenetic events have been deeply recognised as mechanisms contributing in processes of tumorigenesis, being greatly involved in novel approaches towards the definition of predictive and prognostic factors when dealing with the complexity of cancer as a disease.

By *epigenetics* we refer to inheritable changes that do not affect the genetic sequences but the way the genes are expressed, forming a driving force in the initiation and progression of various cancer types through their molecular pathology [1]. Even more interestingly, epigenetic events occur more frequently than genetic events and are highly reversible, causing significant dysregulation of gene functions, a key feature observed in cancer development. One of the key epigenetic factors is *DNA methylation*, a chemical modification that plays a critical role in the mechanisms behind genetic functions and gene expression [2]. Disruptions in key areas of genes such as their promoter regions, which are responsible for their

normal expression, may lead in their inhibition by turning those genes from on to off as can be seen in Fig. 1. These certain disruptions that are the consequence of aberrancies in the methylation levels of such areas may significantly influence the mechanisms behind the initiation as well as the progression or recurrence of cancer or they may affect whether or not certain drugs are effective on an individual patient [3,4].

If cancer cannot be entirely prevented then the earlier it is detected the more the chances for an effective and well-personalised treatment to the patient, resulting to the prolongation of the patient's life expectancy. For certain cancer types, the patient's survival rate may drop dramatically once it is diagnosed with the current diagnostic methods, leading to higher risks taken by the oncologists for a targeted treatment affecting the patient's quality of life as well. Therefore, cancer early detection and treatment needs to be reshaped using well-studied epigenetic biomarkers, such as DNA methylation, for closed-loop cancer detection and therapy, Fig. 2. Since mass population early screening of cancer may still be a challenging topic for the healthcare sector, these early detection closed-loop systems based on semiconductor technology could be part of a scheme whereby the epigenetic description of a tumour could provide the clinician with the initial information about the organ of origin or the stage of cancer progression through a deeper screening analysis, dependent on the epigenetic analysis result. This will ultimately contribute in the monitoring process of cancer and the clinical implications behind cancer management by helping individuals keep track of their health progression regarding any malignancies that may

* Corresponding author. Tel.: +44 2075940742.

E-mail addresses: m.kalofonou@imperial.ac.uk (M. Kalofonou), c.toumazou@imperial.ac.uk (C. Toumazou).

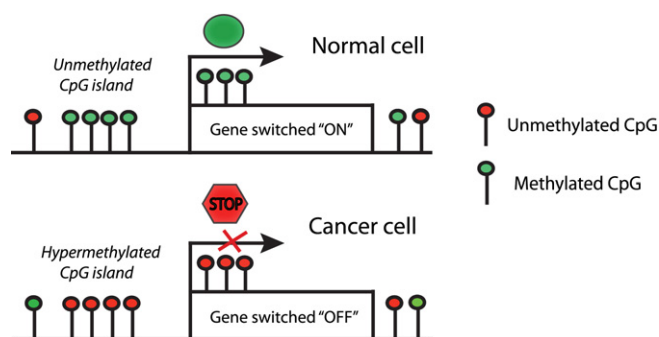


Fig. 1. DNA methylation disruptions in promoter genetic regions in the cases of a normal and a cancer cell.

unexpectedly arise, providing a better prognosis. For that goal, developing assays that would serve that need would form an attractive territory for the new generation of early detection systems, putting semiconductor technology at the centre of attention.

So far, for the past few decades, several DNA methylation assays have been developed for the examination and analysis of gene-specific or genome-wide levels of DNA methylation. As has been summarised by [5–7], the combination of different types of pre-treatment of sample DNA followed by different analytical steps has resulted in a plethora of techniques for determining DNA methylation patterns and profiles. The methods for DNA methylation analysis involved *methylation sensitive restriction enzymes* for DNA methylation analysis by combining their use with experimental approaches (HPLC, RLGS, DMH, etc.) for global methylation analysis, applied to any genome without any prior knowledge of the DNA sequence. Although these methods are highly reliable for analysis of large amounts of methylated sites, the use of restriction enzymes is a limiting factor. On the other hand, *immunoprecipitation* based methods are useful for the identification of differentially methylated regions in tumours through the precipitation of a protein antigen out of a solution by using an antibody directed against the protein. Despite several advantages, protein based methods are limited in detecting methylated groups in defined sites, with limitations on the data obtained by the frequency of the restriction enzyme recognition sequence, becoming complex when extra amplification is needed after the antibodies are attached. Finally,

the *sodium bisulfite* based methods, through the use of bisulfite conversion [8], opened a new era for gene based DNA methylation analysis, allowing methylated DNA to be treated and then amplified and detected using PCR based approaches in a single base-pair resolution, in a label-free approach. Not until the early 2000s, did the introduction of microarrays and next-generation sequencing technologies bring a breakthrough in the way DNA methylation is analysed at a single base-pair resolution. However, the computational mapping challenges of million reads, the accuracy of detection as well as the cost of sequencing technologies are challenges that should deeply be considered.

However, if we focus on the bigger picture of the requirements that the current DNA methylation detection methods are limited by, we will acknowledge that the use of optical methods including fluorescent labels [9] and/or magnetic beads [10] or the use of other fluorescent dyes [11] and nanoparticles [12] increase the levels of complexity when the need for large scale technologies stays paramount. Further attempts have been also made on performing on-chip DNA methylation analysis by microfluidic droplet arrays combined with amplification of DNA without avoiding the use of dyes however [13]. Moreover, synthetic nanopores have also been used to test the electromechanical properties of methylated DNA, correlating the methylation level with the threshold voltage observed through the permeation of the DNA through the pores [14]. Finally, a nanowire transistor can provide DNA methylation detection [15] using monoclonal antibodies immobilised on the sensing surface of the device and magnetic tags capturing the DNA molecules. However, the use of affinity based technologies as well as tags for detecting the target sequence increase the complexity by adding post-processing steps with a secondary limitation of incompatibility with semiconductor based methods.

Hence, what is required to accommodate these needs is first of all a technology that would overcome the limitations of current methods for DNA methylation detection and enhance the applicability through large scale integration and a method that would be applied to easily accessible blood based biomarkers capable to give a clear sign on the status and aggressiveness of the disease, avoiding the inherent complexity that cancer itself has by nature, a task which may be realised using semiconductor based microelectronic platforms. Thus, there is an urgent need for the development of DNA methylation detection assays using well-established CMOS technologies that meet the current demands of healthcare such as

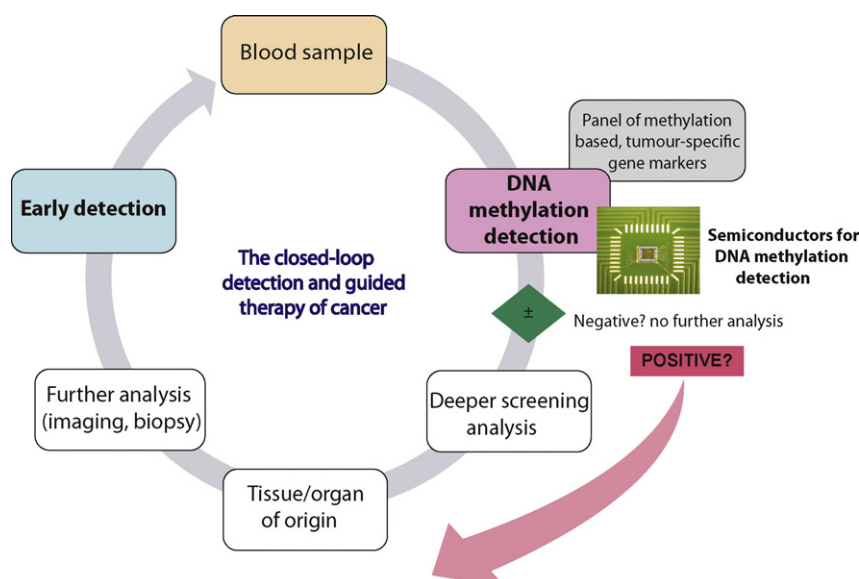


Fig. 2. The future scene of a closed-loop strategy towards cancer management using semiconductors for DNA methylation detection.

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