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The future of microfluidics for biomarker detection

“Explosive progression in biochemical detection technologies in recent times has raised the interest in searching for new biomarkers for various disease states.”

Keywords: biomarker ■ cancer ■ microfluidic device ■ miRNA ■ point-of-care testing

A biomarker is a measurable characteristic that reflects the severity or presence of a disease state. This broad definition of biomarkers includes traditional physiological parameters such as body temperature, blood pressure and heart rate. These days, however, the meaning of the term ‘biomarkers’ is practically being limited to molecules in body fluids, typically proteins in blood. Explosive progression in biochemical detection technologies in recent times has raised interest in searching for new biomarkers for various disease states. Among them, cancers are the most intensively studied targets, not only because they are a major cause of mortality in developed countries (top in Japan), but also because early-stage diagnosis of a cancer usually decreases the likelihood of mortality. For such early-stage diagnosis, biomarker detection *in vitro* is ideal because of its minimal invasiveness and low cost. However, there have been very few biomarkers accepted for this purpose. In fact, the prostate-specific antigen (PSA) in serum is the only protein biomarker currently recommended by the American Cancer Society as an early cancer screening tool [1]. The PSA test is now a common feature in annual medical checkup for males at ages over 50. However, even the PSA test is not perfect in terms of specificity: approximately 70% people falling in the danger zone (>4.0 ng/ml PSA) are later diagnosed as false positive [2]. To compensate for this uncertainty of single cancer biomarkers, measurement of multiple biomarkers is likely to be more effective [1].

Recently, a new class of biomarker molecules has been reported – miRNAs. miRNAs are small non-coding RNAs with lengths of 19–24 bases. miRNAs were first discovered in the early 1990s, and have been recognized as quite common throughout eukaryotic cells. A major role of miRNAs is gene regulation, especially negative

translational regulation by binding to their target messenger RNAs. To date, more than 1400 miRNAs have been identified in humans, and they are estimated to regulate more than 60% of protein-coding genes [3]. Considering the importance of miRNAs in regulating cellular processes including proliferation, differentiation, and apoptosis, it is not surprising that genetic or epigenetic aberration of key miRNAs leads to genesis and progression of cancers. What is surprising is that those key miRNAs are found not only in cancerous tissues, but also in remote body fluids such as blood. Because RNAs are generally prone to decomposition by nucleases which ubiquitously exist, not many had imagined that miRNAs are present in blood in such a stable form, until those ‘circulating’ miRNAs were actually discovered in human serum in 2008 [4]. In that report, the amount of miR-141 in serum was well-correlated to prostate cancer. This observation opened a new possibility of miRNAs as biomarkers for early-stage cancer detection. The stability of the circulating miRNAs is explained by several hypothetical mechanisms: some are encapsulated in vesicles and others are bound to specific proteins [5].

While biomarker discovery, including miRNA, has been of great research interest, biomarker detection is also becoming a hot target of technological development. In a sense, there has already been a ‘gold standard’ detection technology for every biomarker species: immunoassays including ELISA for proteins [6] and quantitative PCR for nucleic acids [7]. Although these technologies are best in most situations at present, this does not necessarily mean that they are perfect. Common drawbacks of these technologies are long assay time and expensive equipment. These technologies are suited for centralized testing laboratories, and can be likened to classical mainframe computers. In contrast,

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personal computer-like (or smartphone-like) technologies are the center of interest in modern engineering research, namely, point-of-care testing (POCT) technologies. More specifically, POCT is defined as diagnostic testing at, or near, the point of patient care. Successful examples of POCT devices include blood glucose sensors and pregnancy test strips. They return the answers very quickly (within a few minutes) and require minimal equipment (the latter can be read by naked eye). However, they cannot be easily applied to other biomarkers, mainly because of their low sensitivities.

As a new platform for high-performance POCT, microfluidic devices (microchips) have been attracting considerable interest. A microchip is a small plate containing microchannels for guidance of fluids. Typically, microchannels have dimensions of tens to hundreds of micrometers and are fabricated with semiconductor process technologies. The downsizing has an acceleration effect on certain chemical reactions. For example, solid-phase immunoassays, such as ELISA, take several hours in a conventional scale because of the slow, diffusion-limited mass transport. This process can be dramatically accelerated by downsizing, because the simple scaling law tells that the time for diffusion is proportional to the square of the dimension (100-fold downsizing leads to 10,000-fold acceleration) [8]. On a microchip, diffusion is no longer the rate-limiting process of an immunoassay, which is typically completed in 20–30 min without spoiling the sensitivity. A similar principle to those of immunoassays can be applied to nucleic acid detection, that is, hybridization with surface-immobilized probe DNA molecules. This strategy is widely used for DNA microarrays and can also be accelerated by the use of microchips. Some research groups call this combination ‘microfluidic microarray’ [9]. In the past decade, immunoassay microchips and microfluidic microarrays have shown great promise for POCT applications in terms of assay time, which has been shortened from several hours to less than 30 min. However, reduction of peripheral equipment does not seem as successful as reduction of assay time. Most microchips still depend on external pumps, valves, fluorescence microscopes and computers. These conventional pieces of equipment are bulky and expensive. Mechanical equipments, such as the pumps and valves, are especially difficult to reduce in terms of size and cost, when compared with the optical and electrical equipments.

Our research group has been developing microchip-based POCT devices that need no mechanical equipment, since 2004 [10]. We call them ‘power-free’ microchips, meaning that these microchips are free from external power sources for fluid pumping. The energy for pumping is pre-stored in the microchip itself in a very unique form: degassed state of poly(dimethylsiloxane) (PDMS). PDMS is a kind of silicone elastomer, which is widely used as a microchip material because of its easy fabrication [11]. At the same time, PDMS has a unique property that is rarely exploited, namely, a high gas solubility. Solidified PDMS contains large amount of gas. Under atmospheric conditions, unit volume of PDMS contains approximately 1/10 volume of air in the standard gas state. This amount is proportional to the pressure outside the PDMS (Henry’s law). Therefore, a PDMS microchip can be degassed in a vacuum. When the microchip is brought back to the atmosphere, it absorbs air again. This air absorption action creates a vacuum environment inside the power-free microchip, which pulls in the liquid aliquots dispensed by the user. The degassed microchip can be preserved in a gas-tight package [12], therefore the degassing task is not required at the point-of-care. Using the power-free microchips, we have succeeded in implementing immunoassays of PSA [13] and hybridization assay of miRNAs [14]. With assay time of approximately 20 min, we detected these substances at concentrations of picomoles per liter. This sensitivity is comparable to those of state-of-the-art ELISA.

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This article outlines the present status of microfluidics for biomarker detection, with an emphasis on our own efforts. As a conclusion, I would like to make a few comments on the future of this research field. I agree with the opinion that commercialization of microchips has seen only limited success compared with the expectation in the late 1990s [15]. This is partly due to the high expectations, which are generally needed to launch a new research field. Another reason is the lack of a ‘killer application’, in that, a biomarker which is best suited for a microchip. Most of us have been developing individual, fundamental technologies (i.e., the

power-free microchip) for general purposes. As a result, this research field is full of fragmented technologies awaiting integration. Although this may be the natural course of evolution of a new research field, I feel that the current phase – making a library of technologies – is coming to an end for biomarker detection microchips. Instead, finding a killer application and integrating appropriate technologies toward that application would be key to the future success of this research field. I hope that microchips will establish their position somewhere between the pregnancy test strips and the fully equipped test laboratories in the near future. To achieve this

goal, collaboration between a variety of researchers including those in medical and industrial sectors will be important.

Financial & competing interests disclosure

The author has no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties.

No writing assistance was utilized in the production of this manuscript.

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