Novel point of care strategies for biomarker detection

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***Abstract*— Stress biomarkers produced by the body are briefly reviewed and our recent research is presented. Quantitative detection using aptamer-LFA demonstrated against cortisol, dopamine, and endotoxin. Microfluidic label-free optical method presented simultaneous detection of multiple biomarkers in bodily fluids, promising real-time detection. Lastly, future biomarker detection is discussed**

Keywords—biomarker, lateral flow assay, aptamer, optical detection, microfluidics

I. Introduction

Physical and mental conditions of human beings are strongly affected by various hormones and neurotransmitters generated in the body. The study of these biomarkers, representing molecular signatures for certain biological conditions, has become increasingly important. Evaluation of the level of biomarkers provides an important clue not only to diagnose physical diseases but also to evaluate mental conditions from stress, depression, etc. Historically, biomarker detection was accomplished after proper sampling biological fluids, such as whole blood, plasma, sweat, urine, and saliva. Low cost and highly sensitive diagnostic devices, such as our aptamer-based lateral flow assay, are becoming a powerful option for this purpose. Although the conventional laboratory-based detection and diagnosis approach is still the routine procedure, demand is increasing for rapid and/or continuous monitoring of biomarkers, especially for mental health issues. Another increasing demand is for the option of evaluating multiple biomarkers in order to provide a more global understanding of related symptoms. Our versatile label-free optical detection using simple microfluidic devices provides simultaneous detection of multiple biomarkers and can be utilized to monitor the biomarker levels in real-time. Furthermore, biomarker detection strategy can be strengthened in combination with active or passive treatments upon detecting targeted biomarkers. This approach requires more sophisticated system design and novel methods, such as bioelectronics, wireless signaling, electrically/biologically controlled "on-demand" actuations.

II. Stress Biomarkers

More people are suffering from mental health issues caused by severe or chronic stress, especially for those dealing with extreme conditions, such as soldiers, firefighters, police, emergency medical personnel and athletes. Proper level of stress can improve the cognitive level and even improve the resilience of personnel, but severe or chronic stress often results in the reduction of cognitive level, leading to misjudgments in life-threatening situations. Routine monitoring of the stress level is very important to maintain healthy mental and physical conditions.

A. Biomarker concentrations in bodily fluids

Most biomarkers can be detected from various bodily fluids. However, their concentrations and abundancies are very different in different type of bodily fluids. Molecular weight and size of biomarkers are important considerations to determine the detection methodology. Interestingly, biomarkers with higher molecular weight tend to have a lower concentration in bodily fluids. Although blood testing is considered the "gold standard" for detecting biomarker varieties and determining concentrations, some biomarkers present higher concentration in other bodily fluids. For example, dopamine concentration is highest in urine and BDNF and NPY concentrations in saliva is higher than that in blood plasma. Concentration range and equivalent number of molecules per mL of key stress biomarkers in blood, urine, sweat and saliva are shown in Fig. 1. Considering selected biomarkers and their abundance, specific bodily fluids can be selected for targeted applications. For example, sweat is a good candidate to develop wearable sensor to detect cortisol, while urine will be beneficial for Parkinson's patients to monitor the dopamine level.[1]

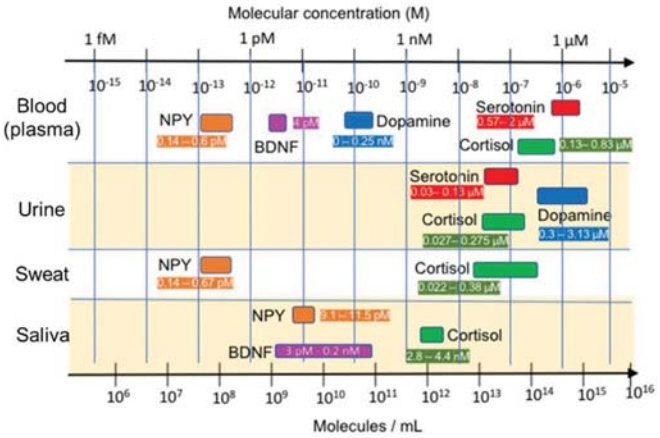

Fig. 1 Molecular concentration ranges of stress biomarkers in various bodily fluids. Reprinted with permission from ref [1]. Copyright 2018 Amer. Chem. Soc.

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III. Aptamer-based Lateral Flow Assay

LFAs are known as robust, cost-effective, and user-friendly POC systems. Sample solution dispensed on the LFA flows through a porous membrane without the need for any external accessories. Biorecognition molecules printed on the nitrocellulose membrane capture target biomarkers during flow. Colorimetric or fluorescent test lines formed on the membrane indicate the presence of the biomarkers. Recently, aptamers have emerged as attractive biorecognition elements. Aptamers are single strand DNA or RNA oligomers synthesized by an *in vitro* selection process named systematic evolution of ligands exponential enrichment (SELEX). Compared to conventional antibody/antigen immunoassays, aptamers provide several attractive features, including easy chemical synthesis, adaptive modification, small size, high stability, lack of immunogenicity and cell-free evolution. We have developed aptamer-based LFA devices to detect various biomarkers, such as dopamine (in urine), cortisol (in sweat or saliva), and endotoxins (in saliva).[2] The importance of endotoxins has increased because of the recent discovery related with neurodegeneration and cardiovascular diseases, in addition to oral health problems. Fig. 2 illustrate the aptamer-LFA design (Fig. 2a) and interactions between aptamers and the targeted biomarker dopamine (Fig. 2b). Two different complementary aptamers for test and control lines are printed on the nitrocellulose membrane. In the presence of dopamine, sensor probe DNA_2 undergoes conformational changes leading to its dissociation from the capture probe DNA_3. When the dehybridized AuNP-DNA_1-DNA_3 is captured by complementary $cDNA_3$ printed on the membrane, a positive test line is formed. In the absence of dopamine, because DNA_3 is already hybridized with DNA_2, $cDNA_3$ cannot bind with AuNP-aptamer duplex, forming no signal.

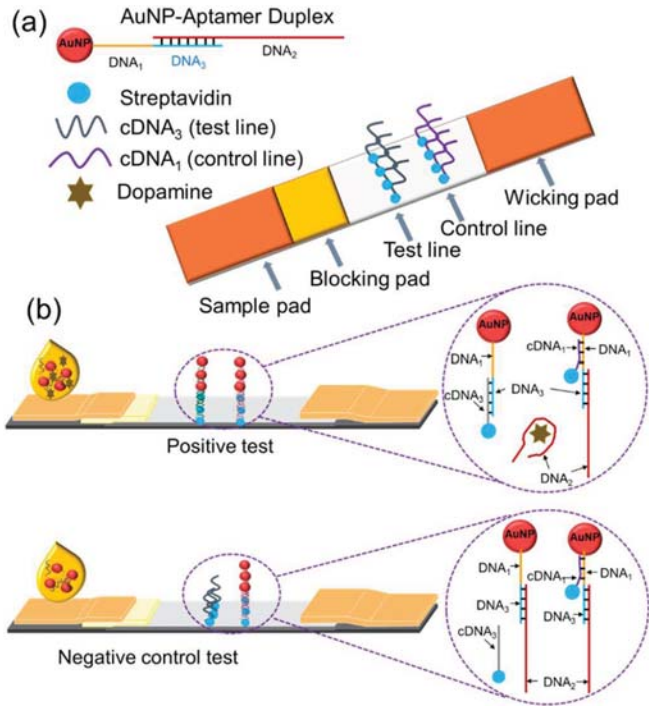

Fig. 2 Illustration of (a) aptamer-based LFA device and (b) interactions in the presence or absence of dopamine. Modified with permission from ref [2]. Copyright 2020 Elsevier.

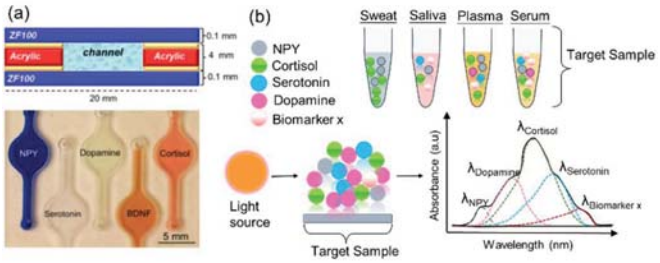

Fig. 3 Label-free optical detection: (a) device design, (b) concept illustration. Reprinted with permission from ref [3]. Copyright 2019 Amer. Chem. Soc.

IV. Label-free Optical Detection

We have also developed a novel method for label-free quantitative detection of multiple stress biomarkers in different bodily fluids.[3] As shown in Fig. 3b, biomarkers such as cortisol, serotonin, dopamine, and neuropeptide Y have different absorption peaks of 247, 220, 204, 190 nm, respectively. Absorption peak intensity is proportional to the concentration. In the presence of multiple biomarkers, the obtained optical spectrum is deconvolved into individual peaks corresponding to specific biomarkers. An optical microfluidic device has been designed (Fig. 3a) and the limit of detection of 200 ng/mL was obtained for cortisol in sweat, which is within a normal physiological range. UV light and photodiodes are also integrated for optoelectronic detection and measured photocurrent was proportional to concentrations of biomarkers (serotonin and dopamine). This label-free optical detection provides a potential for simultaneous monitoring of multiple biomarkers.

V. Future Prospect

Both routine testing using cost-effective and highly sensitive devices, and continuous monitoring of a specific or multiple biomarkers have their own merits. Biomarker sensors can be combined with actuators and control electronics to provide timely treatments corresponding to detected biomarkers in an active or passive manner. For example, different stages of wound recovery can be recognized by detecting related biomarker levels, triggering the release of the proper drug or stimulation of the wound to accelerate the recovery process. Novel sensor concepts, sophisticated bioelectronic circuits, and actuator design will be integrated. Interdisciplinary research will bring a significant impact on motivations of biomarker research.

Acknowledgment

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