Lab on a patch: a skin patch for rapid screening of malignant melanoma based on microneedles and structurally programmed microfluidic particle dam

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Background of research

Malignant melanoma is a highly aggressive skin cancer. The incidence rate is increasing rapidly year by year at a rate of 3% [1]. While it only accounts for 4% of skin tumors, it has caused 75% of its deaths [2,3]. Unfortunately, due to the rapid metastasis and poor prognosis, traditional radiotherapy and chemotherapy are ineffective. As such, preventive strategy by making early diagnosis of melanoma is of great importance.

Conventional diagnosis relies on dermoscopy and immunohistochemistry. Dermoscopy is currently used to observe microscopic substructures (**Figure 1a**). However, it is difficult to identify malignant melanoma of early stage because it shares many clinical features with benign moles. Alternatively, immunohistochemistry detection of S100A1 in the melanoma matrix has been commonly advised for further examination of melanoma (**Figure 1b**)[4,5]. However, it requires incisional biopsy, which is painful, invasive, and unsuitable for early and routine body check. Moreover, its quantification is not well developed yet.

On the other hand, the quantitative level of S100A1 in serum or plasma is correlated with the stages of melanoma [6], making it a good biomarker. However, serum or plasma detection requires expensive benchtop instruments (i.e. centrifugation or microplate reader) for sample pretreatment and post-analysis, which is impractical for local clinics. Furthermore, dilution of biomarker may occur when being released from melanoma tissue to the blood circulation[6], , which causes the detection less sensitive and error-prone. Most importantly, the high expression level of S100A1 in serum is also related to other diseases, which may lead to the false-positive diagnosis of melanoma [7-9]. Thus, instead of relying on serum biomarkers, it is urgent to develop a portable and ready-to-use platform that allows simple and painless sampling at the melanoma sites without dilution, and provide result quantification to determine the diseases progression.

Related works done by others

Near the end of COVID-19 pandemic, rapid antigen tests (RAT) based on lateral flow immunoassay (LFIA) have largely used to self-evaluate the infection status, demonstrating a new tool that relaxes and decentralizes the healthcare system to benefit both users and medical service providers. The success of COVID-19 RAT is aligned with the concept of "Lab on a Chip" which aim at providing precise fluidic control on a miniaturized microfluidic platform for automated sample collection, preparation, transportation, reaction, and result reporting[10,11]. If success, it will largely improve telemedicine for resource-limited sites such as home and private clinics [12]. However, unlike COVID-19 RAT that only reports yes/no through easily accessed specimen, "Lab on a Chip" is not yet populated to general use because a number of technical hurdles when applying to other diseases.

Appropriate specimen preparation is the first issue. Liquid biopsy such as blood plasma is the most essential specimen[13]. Carrying excessive amounts of circulating biomarkers, blood plasma can be used to diagnose diseases such as diabetes, blood disorders (anemia , hemophilia , leukemia), organ failure (liver , kidney , cardiovascular), and cancers[14]. Recent research has also broaden the diagnosis to Alzheimer's[15], and most recent viral outbreaks including COVID-19[16] and Zika viruses[17]. However, blood plasma needs to be first centrifuged to remove blood cells and clotting factors to prevent their interferences on the diagnosis results[18-21], which is exclusively a laboratory process. To achieve it on miniaturized platforms, on-chip blood separation was developed. However, it usually requires electricity to power up a separation using dielectrophoresis, inertial force, stiffness and weight through the acoustic separation[18,22-25]. As such, earlier attempts for device miniaturization still rely on separate, external devices such as centrifuge, power source, pump, which make them still laboratory exclusively (**Figure 2**). Moreover, even with successful serum preparation, the serum S100A1 is known to be unspecific to malignant melanoma, and presents at much lower level due to dilution, making the serum-based diagnosis difficult and unreliable.

The second issue for is the quantitative measurement on an easy-to-use platform. LFIA such as COVID-19 rapid tests only provide qualitative result (yes/no), which is insufficient because most disease diagnosis needs quantitative results such as abnormally elevated S100A1 in serum[6]. Attempts were made to miniaturize the conventional immunoassays into microfluidic platforms[12]. However, signal quantification still relies on complicated setups such as optical fiber, excitation light source, photodetectors, and amperemeter, making them still laboratory exclusively (**Figure 2**) [26-28].

Related works done by the principal investigator's (PI) group

To provide a better platform for disease diagnosis, the PI's team has developed a microfluidic particle dam that enables visual and quantitative results without relying on any external instrumentation. Magnetic microparticles (MMPs) and polystyrene microparticles (PMPs) are designed to connect to a target simultaneously. An on-chip magnetic separator is first used to remove the MMPs-target-PMPs and trap the freely flowing PMPs at a particle dam, resulting in PMP accumulation. Like the ordinary thermometer, users can easily read the length of PMP accumulation by the naked eye. Such an instrument-free and power-free platform enables a limit of detection (LOD) of DNA oligonucleotide at 13 fmol (***Lab on a Chip***; IF: 6.045, Rank 6/78 in Biochemical Research Methods) [29] and is applied to determine lead intoxication (***ACS Sensors***, IF: 7.333, 3/86 in Chemistry, Analytical) [30,31]. Similar principle has been applied to other metal ions for detection of cadmium [32] copper [33,34] and silver ions [35] in water. In addition, integrating a structurally programmed capillary flow to achieve automated sample collection, on-chip reaction, and microfluidic particle dam, we have achieved simultaneous detections of multiple ions on an all-in-one device (***Analytical Chemistry***, IF: 7.4, Rank 7/86, 8.1% in Chemistry, Analytical, JCR 2022) [36] (**Figure 3**). Most importantly, we have also demonstrated ability for detecting macromolecules in complex biofluid such as serum alpha-fetoprotein levels [37], flap endonuclease 1 activity in cell lysate (***Biosensors and Bioelectronics***, IF: 12.6, Rank 2/86, 2.3% in Chemistry, Analytical, JCR 2022), and serum IgG antibodies against SARS-CoV-2 (***Science Advances,*** IF: 13.6, Rank 7/73, 9.6% in Multidisciplinary Sciences, JCR 2022)[16] (**Figure 4**). Most recently, we further demonstrated the application for detecting S100A1 in interstitial fluid (ISF) collected by hydrogel-fabricated swellable microneedle. (***Advanced Science***, IF: 15.1, Rank 24/344, 7% Materials Science, Multidisciplinary, JCR 2022).[38]

Research Question

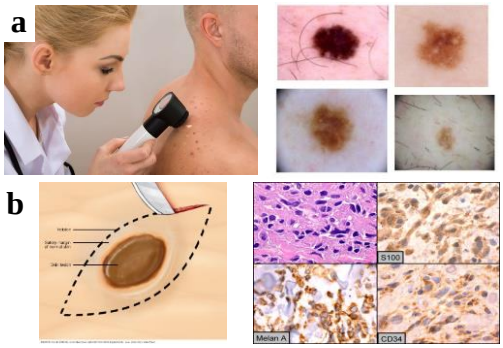

Figure 1. Traditional diagnosis of melanoma based on (a) dermoscopy and (b) incisional biopsy and histochemistry.

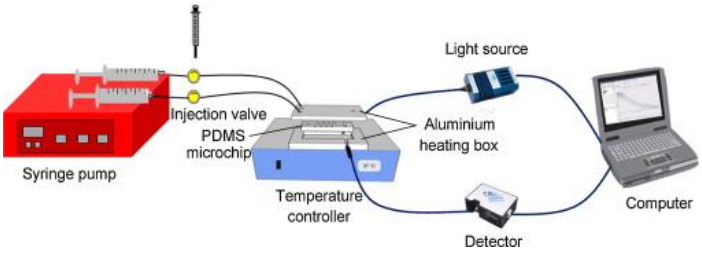

Figure 2. Research question. However, disease diagnosis mostly requires blood specimen and quantitative results, which is hardly achieved on at-home tests. So far, many miniaturized devices still rely on external devices such as pump, valves, light source, and optical detectors for sample preparation and result quantification, making them still exclusively laboratory based and unsuitable for home use. Miniaturization does not necessarily lead to simplicity.

Relevant Research Experience of the PI

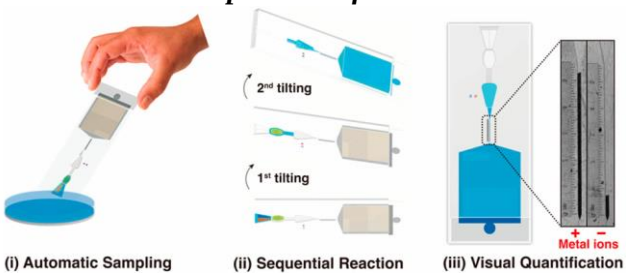

Figure 3. A structurally programmed capillary flow to achieve automated sample collection, on-chip reaction, and microfluidic particle dam for simultaneous detections of multiple ions in an integrated device (***Analytical Chemistry,*** **2022**).

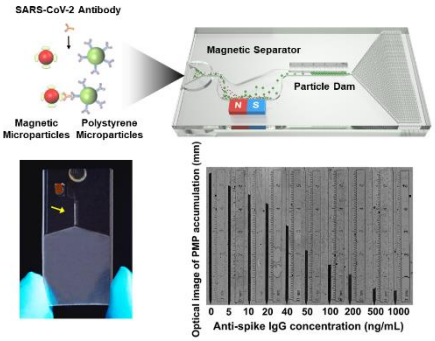

Figure 4. Microfluidic particle dam for detection of serum IgG antibodies against SARS-CoV-2 (***Science Advances,*** **2022**).

Research Overview

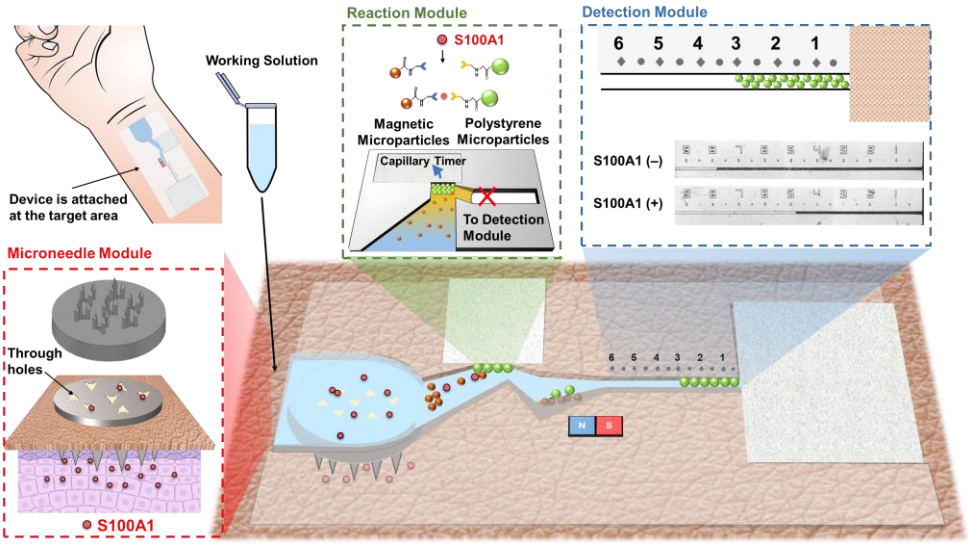

Figure 5. The schematic of "Lab on a Patch" as a skin patch integrating microneedles and structurally programmed microfluidic particle dam. This microfluidic device contains microneedle module, reactor module, and detection module. After attaching to skin and deposit a droplet of working solution into a manifold channel in the microneedle module, the interstitial fluid will be extracted by the microneedles surrounding by through holes and subsequently resuspend magnetic microparticles (MMPs) and polystyrene microparticles (PMPs). MMPs and PMPs will be surface functionalized so S100A1 binds to MMPs and PMPs simultaneously. By forcing the MMPs to flow passing the immobile PMPs at the reactor module, the reaction is maximized and timed through a capillary timer until it is fully filled. When completed, the particle solution is redirected to the detection module containing a magnetic separator that removes the MMPs and MMPs-S100A1-PMPs, leaving free PMPs to keep on flowing until they are trapped at a particle dam. Therefore, the quantity of S100A1 proportionally reduces free PMPs escaping from magnetic separation and shorten PMP accumulation length at the particle dam, achieving the visual quantification of S100A1.

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