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Microfluidic opportunities in the field of nutrition

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Nutrition has always been closely related to human health, which is a constant motivational force driving research in a variety of disciplines. Over the years, the rapidly emerging field of microfluidics has been pushing forward the healthcare industry with the development of microfluidic-based, point-of-care (POC) diagnostic devices. Though a great deal of work has been done in developing microfluidic platforms for disease diagnoses, potential microfluidic applications in the field of nutrition remain largely unexplored. In this Focus article, we would like to investigate the potential chances for microfluidics in the field of nutrition. We will first highlight some of the recent advances in microfluidic blood analysis systems that have the capacity to detect biomarkers of nutrition. Then we will examine existing examples of microfluidic devices for the detection of specific biomarkers of nutrition or nutrient content in food. Finally, we will discuss the challenges in this field and provide some insight into the future of applied microfluidics in nutrition.

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Introduction

Proper dietary nutrition is imperative to healthy child growth and maintenance of human wellbeing. Nutritional disorders, whether resulting from excessive (overnutrition) or inadequate (undernutrition) nutrient intake, are classified as malnutrition. Worldwide, malnutrition is directly or indirectly associated with various major causes of death.[1,2] In developed countries, overnutrition is a major health risk because it can lead to obesity and diseases such as diabetes and cardiovascular disease. Between 2009 and 2010, more than one-third of all adults in the United States were classified as obese.[3] As standards of living increase in developing countries this trend repeats itself, rendering obesity a global health challenge.[4]

While the world is faced with this increasing prevalence of overnutrition and obesity, undernutrition remains a major public health concern, affecting more than 900 million people worldwide.[5] Maternal and child undernutrition, highly prevalent in underdeveloped countries, is particularly malignant because it can substantially increase the mortality and disease burden of young children.[6] As shown in Fig. 1, the influence of child undernutrition is most drastic in south-central Asia and eastern Africa where stunting, underweight, and wasting resulting from severe macronutrient malnutrition contribute to the majority of child deaths.[7] Globally, stunting, severe wasting, and intrauterine growth restriction cause more than 2.2 million deaths each year and lead to 21% of disability-adjusted life-years (DALYs) for children under 5 years of age.[6] In addition to macronutrient malnutrition, micronutrient deficiencies are widespread, affecting about one third of the world's population and causing a variety of adverse effects on human health.[8]

Global action has already been taken to address this worldwide malnutrition challenge. International commitments to eliminate stunting, underweight and wasting of children, such as the United Nation's (UN) Zero Hunger Challenge, have been launched. In addition, food-based strategies, such as dietary diversification, food fortification, and micronutrient supplementation, have been widely adopted to tackle micronutrient deficiencies.[8] While global or regional nutrition reinforcement can be achieved through adequate food supply and micronutrient supplementation, effective approaches for accurately evaluating nutritional status and dietary nutrient content are still lacking.

For nutritional screening and malnutrition diagnosis, anthropometric indicators (body measurements), biochemical indicators (biomarkers) and clinical signs are often recommended.[9] Currently, the Malnutrition Universal Screening Tool (MUST) is still the major method of diagnosing malnutrition.[10] Relying on anthropometric indicators such as height, weight, body mass index (BMI), and unplanned weight loss, it lacks specificity and does not adequately include biomarkers for reliable assessment of micronutrient deficiency in early stages. In order to diagnose micronutrient

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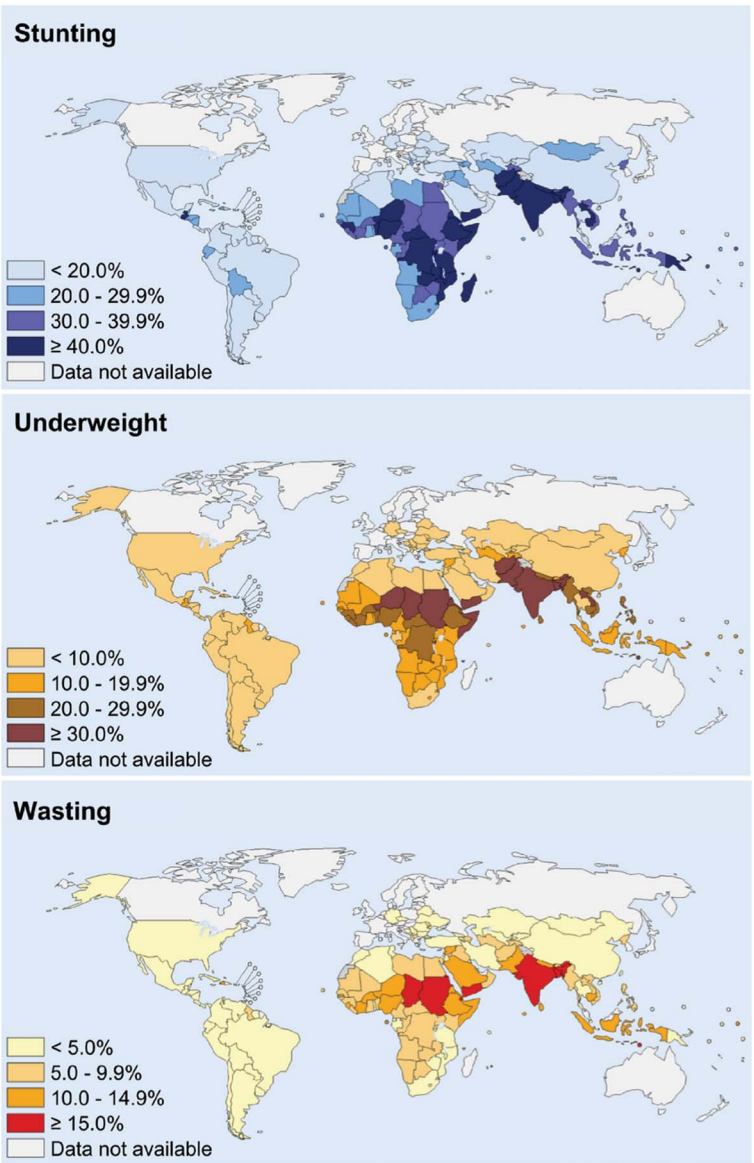

Fig. 1 Latest country prevalence estimates for stunting, underweight and wasting among children under-five years of age. Images reproduced from ref. 7 with permissions from WHO and UNICEF.

deficiencies before the effects are severe enough to cause perceptible symptoms, blood or urine tests are often utilized to measure specific micronutrient biomarkers. Unfortunately, conventional blood or urine tests must be carried out in centralized or regional laboratories capable of maintaining bulky, expensive equipment. The necessity of sample transport, along with long sample analysis times, makes real-time diagnoses in POC settings very difficult. This difficulty is compounded in poor and rural areas where the risk of malnutrition is highest and access to medical equipment and personnel is lowest. Additionally, high costs make it unlikely that conventional blood or urine tests can be scaled up for nutritional screening at the population level. Therefore, the development of portable, inexpensive devices that can efficiently detect biomarkers of nutrition will significantly benefit the global effort in fighting against malnutrition.

In addition to the detection of nutritional biomarkers, microfluidic platforms can be used to monitor the nutrient content in food and micronutrient supplements. Conventional nutrient analysis techniques, utilized by the food industry, are ill-suited for use when food supplies and micronutrient supplements are being distributed in resource-limited regions, as is the case in the UN's Zero Hunger Challenge. This also calls for the development of small-size, low-cost, easy-to-use devices that can sensitively analyze multiple types of nutrients.

Given these constraints, microfluidic technologies seem to have great potential for applicability. With its capacity to precisely handle small volumes of liquid, microfluidics has transformed the way biomedical and chemical assays are

designed and executed.[11] Despite the extensive and fruitful work that has been done over the past two decades, the field of nutrition is still largely unexplored by the microfluidics community. Thus, the purpose of this Focus article is to invoke interest among microfluidic researchers in the field of nutrition. In the following sections we will highlight some of the recent advances in microfluidic blood analysis systems that have the capacity to detect biomarkers of nutrition. Then we will examine existing examples of microfluidic devices for the detection of specific biomarkers of nutrition or nutrient content in food. Finally, we will discuss the challenges in this field and provide some insight into the future of applied microfluidics in nutrition.

Microfluidic blood analysis platforms

With the advantages of small size, low cost, ease of integration and automation, microfluidics technology is expected to revolutionize the healthcare industry with portable, inexpensive, and high-performance medical devices for POC diagnostics.[12–16] Because human blood contains massive amounts of diagnostic information, miniaturization of blood analysis is an area of intense interest for the microfluidics community. Many researchers have devoted themselves to developing microfluidic platforms for the purpose of tackling specific human health problems through blood analysis. The integrated blood barcode chip (IBBC) developed by the Heath group is one such example.[17] Through the integration of a densely patterned antibody microarray based on the DNA-encoded antibody library (DEAL) techniques,[18] this IBBC device is capable of multiplexed detection of serum proteins in minutes from only a finger prick of blood. Furthermore, the Sia group recently miniaturized the standard enzyme-linked immunosorbent assay (ELISA) in a microfluidic platform to develop a portable device specifically for HIV and syphilis diagnosis in developing countries.[19] In a field-testing in Rwanda, this device was used to analyze hundreds of locally collected human samples, demonstrating performance comparable to its benchtop counterparts.

Despite recent innovations and significant potential, microfluidics has yet to live up to lofty expectations. Oftentimes microfluidic devices are designed and tested in laboratory settings where external connections (tubing, optical fiber, *etc.*) and components (power supply, compressed air, pumping systems, *etc.*) are taken for granted. This can be an obstacle when the devices are to be applied in remote settings. Learning from the widely used home pregnancy test kits, we believe that connection-free, self-powered, and highly integrated microfluidic blood analysis devices will be better-suited for POC diagnostics.

Several groups have taken steps towards this goal by developing self-powered, self-contained microfluidic blood analysis devices.[20–23] As shown in Fig. 2(a), the Lee group developed a stand-alone, self-powered integrated microfluidic blood analysis system (SIMBAS).[23] This connection-free, vacuum-powered device can achieve fluid propulsion, plasma separation, and biomarker detection on a single chip. To demonstrate the capabilities of the device, the authors analyzed whole blood samples spiked with various concentrations of biotin (vitamin B7). Their results show that 5 μL of whole blood can be analyzed in 10 min, with a limit of detection of approximately 1.5 pM.

In addition to merely modifying conventional PDMS microfluidic chips, researchers have endeavored to introduce innovative microfluidic formats to achieve connection-free, automated, and integrated microfluidic devices. Centrifugal microfluidics, or a lab-on-a-disc (LOD) system, is one particularly promising, alternative microfluidic format. Although the concept was first brought to the public as early as 1969,[24] it was not until Abaxis introduced its Piccolo system in the early 1990s that the diagnostic potential of LOD systems became well recognized. While efforts to commercialize centrifugal microfluidic platforms have persisted, research has pushed forward, continuing to expand the functions and applications of LOD blood analysis systems since the 2000s.[25–36]

Though the significant advances in LOD technologies have been reviewed extensively in other works,[37,38] we will briefly highlight one example of a recently developed platform to illustrate that a connection-free, integrated blood analysis system can be accomplished using the LOD technology. Fig. 2(b) presents the disc design of a fully automated LOD ELISA system developed by the Cho group for the diagnosis of infectious diseases from whole blood.[34] Like other LOD platforms, the centrifugal forces generated during disc spinning drive sample flow. This centrifugal pumping requires only an integrated motor to generate spin, minimizing the amount of necessary external components. In addition, preloading of reagents into the microfabricated channel network eliminates the need for external tubing or sample pretreatment. In this work, the authors utilized novel laser irradiated ferrowax microvalves (LIFMs)[35,36] in the LOD system for fluid control. By exposing the iron oxide nanoparticle-incorporated paraffin wax to low-intensity laser light, one can precisely melt the wax to control fluid transfer. The LIFM makes it possible to execute the high-speed spinning necessary for plasma separation while maintaining the ability to execute further operations through the confinement of the sample during each individual step. This is also the basis for fully automating the immunoassay with the preset spin program. To demonstrate the functionality of this device, the authors conducted microbead-based suspension ELISA assays to detect the antigen and antibody of Hepatitis B Virus (HBV). The total assay time was reduced from the 2 h necessary for conventional ELISA to less than 30 min while maintaining a comparable limit of detection. In their later work, the Cho group achieved simultaneous biochemical assay and immunoassay[33] or multiplexed immunoassays[28] in a single integrated LOD device, thereby further demonstrating the great application potential of LOD devices in POC blood analysis.

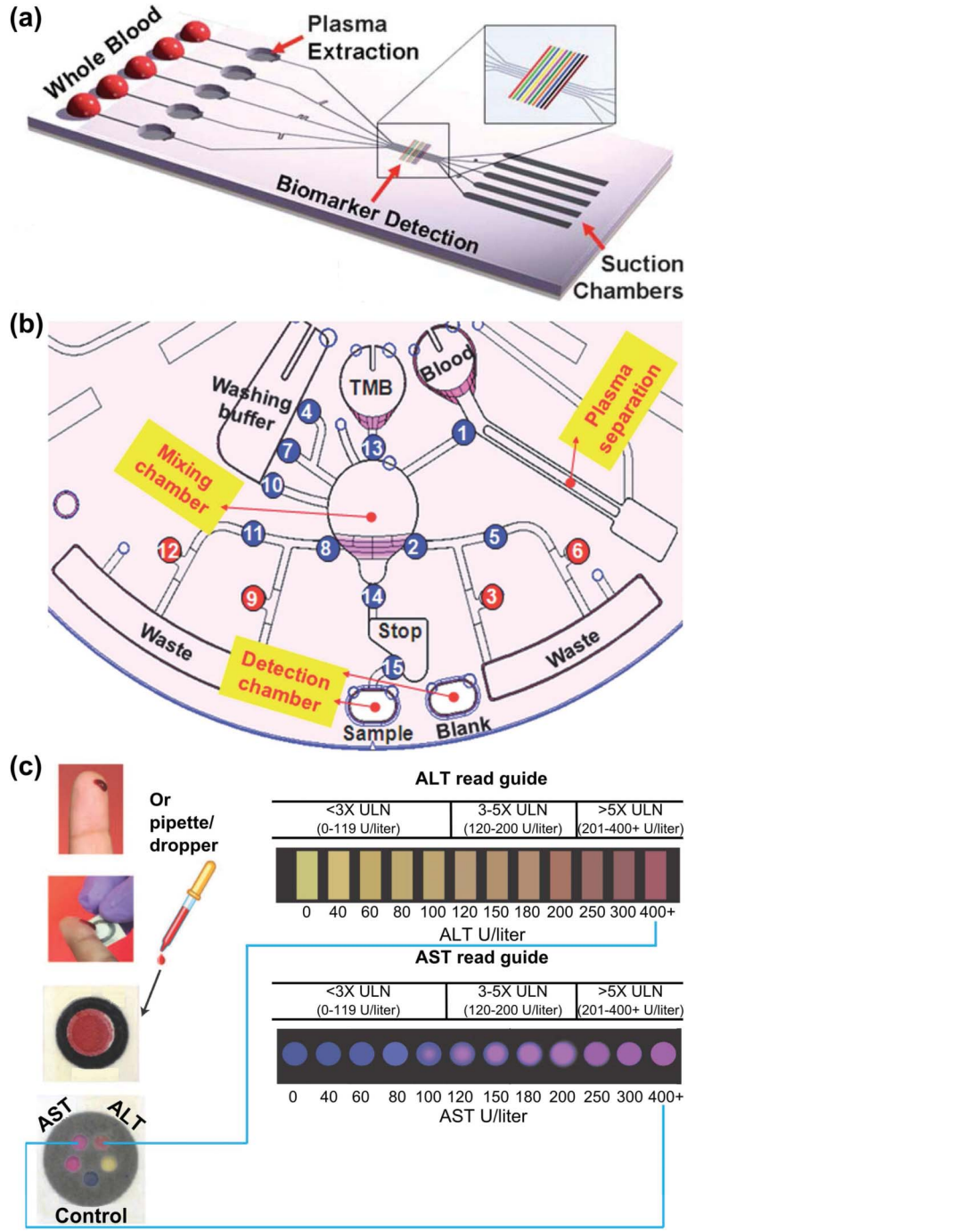

Fig. 2 (a) Schematics of the stand-alone, self-powered integrated microfluidic blood analysis system (SIMBAS); (b) Disc design of the fully automated LOD ELISA system; (c) The brief workflow of the μPAD used for multiplexed transaminase test. Images reproduced from ref. 23, 34 and 55 with permissions from the Royal Society of Chemistry and the American Association for the Advancement of Science.

Paper microfluidics, or microfluidic paper analytical devices (μPAD), provides another innovative format for connection-free microfluidic platforms. Pioneered by the Whitesides group, paper microfluidics has made significant strides despite the technology's very recent introduction. The potential of paper microfluidics in developing low-cost, POC diagnostic tools has been well recognized by the microfluidics community, demonstrated by the rapid research advancement seen in recent years.[39–55]

In-depth analysis of paper microfluidics is available in other review papers[56–58] and is not our focus in this review. Instead, we will elucidate the potential of paper microfluidics in realizing low-cost, connection-free, easy-to-use blood analytical platforms with the use of one recent example.

Shown in Fig. 2(c), the μPAD newly developed by the Whitesides group is capable of multiplexed detection of two major liver function indicators, aspartate aminotransferase (AST) and alanine aminotransferase (ALT).[55] Requiring only a finger prick of whole blood, the device is capable of performing simultaneous assays in 15 min. Capillary forces are utilized to drive the fluid flow, rendering the platform pump- and connection-free. By patterning hydrophobic barriers onto the layered paper device, the authors are able to create three-dimensional hydrophilic microchannels to realize more complex fluid manipulations such as splitting, mixing, and filtration. Moreover, this three-dimensional fluid network also enables the μPAD to run multiplexed assays with unique conditions and no risk of cross-reactivity between assays. The clinical test results show that the accuracy of the μPAD is comparable to the current gold standard automated methods. In addition to simplicity and accuracy, the device is extremely low-cost, light-weight, and disposable, making it perfect for POC settings.

Microfluidic detection of nutritional biomarkers

Although biomarker detections have not been as popular in the field of nutrition as in disease diagnoses, the importance of proper biomarkers in nutritional screening and malnutrition diagnosis has evoked attention recently.[59,60] Direct measurement of biomarkers of nutrition in bodily samples such as blood and urine provides more specific and less biased diagnostic information than anthropometric indicators (body measurements). Moreover, seemingly healthy individuals can unknowingly lack vital nutrients in their diets, eventually leading to disease. In these cases, the detection of biomarkers of nutrition can help diagnose malnutrition in early stages. Portable devices that can specifically and sensitively detect biomarkers of nutrition will greatly enhance nutritional monitoring targeting real-time and home-based evaluation. In this section, we will discuss the work that has already been done in microfluidics regarding the detection of nutritional biomarkers.

Macronutrients

Carbohydrate, protein, and fat are classified as macronutrients. The excess consumption of macronutrients is a major cause of obesity, while chronic, insufficient intake of macronutrients leads to severe protein-energy malnutrition. The most successful example of POC detection of macronutrient levels is blood glucose detection. Low-cost, pocket-sized glucose meters are easily accessible in stores. However, because blood glucose levels are more closely tied to diabetes than to nutritional status, we will not discuss microfluidic glucose detection due to the limited length of this Focus article. Instead, we will focus on the detection of biomarkers related to protein and fat status.

Historically, the measurement of serum hepatic proteins (albumin, prealbumin, transferrin, *etc.*) has been used to assess protein nutritional status. Although controversy has arisen recently about the accuracy of using serum hepatic proteins in evaluating nutritional status, the detections of serum hepatic proteins together with indicators of inflammation such as C-reactive protein (CRP) can still provide useful information about nutritional status.[61–64]

Miniaturization of immunoassay is a common strategy for microfluidic protein detection. Laiwattanapaisal *et al.* has developed an on-chip immunoassay for detecting urinary albumin with performance comparable to conventional spectrophotometric methods.[65] However, the immunoassay-based protein detection often means a tedious procedure and high reagent costs. In order to overcome these limitations, the Lee group developed non-immunological urinary albumin sensors, either based on electrochemical detection[66] or specific dye binding.[67] By modifying the device design and introducing magnetic bead-based chemiluminescence detection, they also detected serum CRP in microfludic systems.[68,69] Besides the Lee group, several other groups have also demonstrated the detection of serum CRP in microfluidic devices.[28,70–74] Fig. 3(a) shows the capillary-driven immunodiagnostic device developed by Gervais and Delamarche for detecting CRP from human serum.[74] With the integration of all the functional elements and reagents on-chip, this device achieves one-step immunoassays, demonstrating the potential advantage of microfluidics in simplifying the detection procedure. Their results show that CRP with a concentration of 1 ng mL^{-1} can be detected from only 5 μL of serum within 14 min.

In addition to albumin and CRP, microfluidic platforms have been developed to detect cortisol,[75–77] a steroid hormone reported to be an indicator of protein-energy malnutrition.[78] The utilization of portable microfluidic devices can help realize real-time cortisol monitoring to establish diurnal cortisol concentration behavior.[75] Moreover, the integration of nanotechnology into microfluidic platforms significantly increases the sensitivity of cortisol detection, enabling ultrasensitive cortisol sensors.[76,77]

Cholesterol is a lipid with crucial roles in maintaining the integrity of cell membrane, as well as in biosynthesis of steroid hormones and vitamin D. The blood cholesterol level, as an important indicator of fat nutritional status, is also closely related to diseases: increased cholesterol level is believed to increase the risk of cardiovascular diseases while low cholesterol level may increase the risk of cancer, depression, or respiratory diseases. Microfluidic detections of blood cholesterol levels have recently been realized in centrifugal microfluidics[33] or paper microfluidics[40]. Additionally, there is also a recent trend of integrating nanostructured materials into microfluidic devices to realize cholesterol detection.[79–84] Fig. 3(b) illustrates one recent example of such microfluidic devices for detecting total serum cholesterol.[84] In this work, Ali *et al.* fabricated a microfluidic nano chip utilizing nickel oxide nanorods (NRs-NiO) immobilized with cholesterol esterase (ChEt) and cholesterol oxidase (ChOx). The electrons generated during the enzymatic reactions were transferred to NRs-NiO, resulting in an electrochemical signal indicating total serum cholesterol concentration. Their results show that the

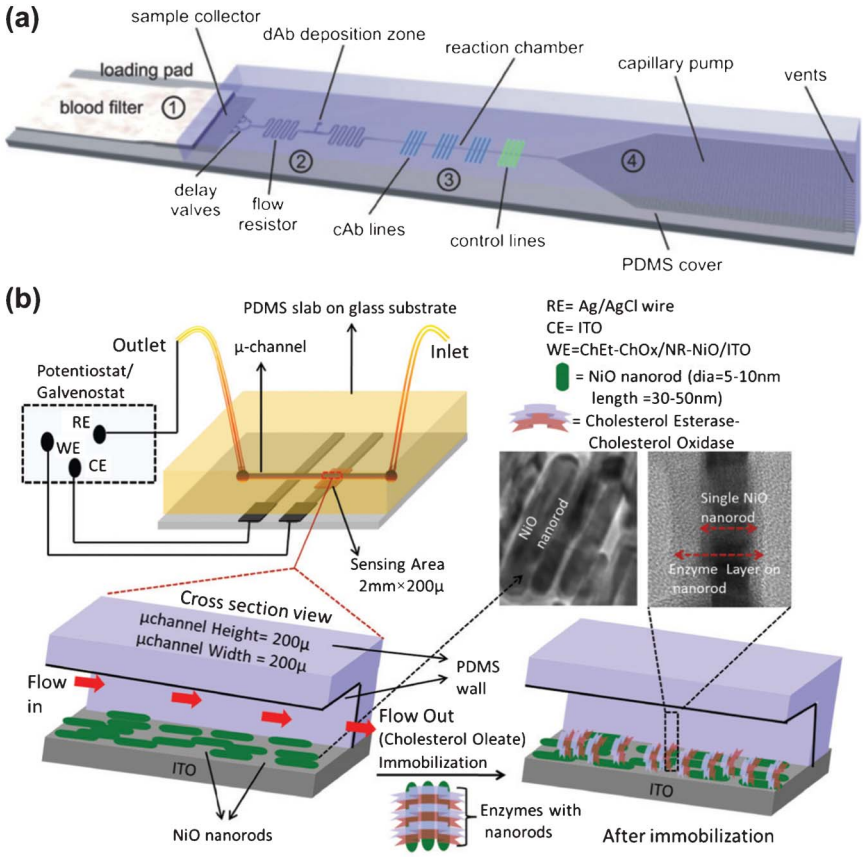

Fig. 3 (a) The schematic of the one-step, capillary-driven, POC CRP detection device; (b) The microfluidic chip based on nickel oxide nanorods (NRs-NiO) used for the detection of total cholesterol. Images reproduced from ref. 74 and 84 with permissions from the Royal Society of Chemistry.

use of NRs-NiO gives the device a wide detection range and high sensitivity.

Micronutrients

Compared with macronutrient malnutrition, micronutrient deficiencies are not easily diagnosable only based on anthropometric indicators, unless they are severe enough to cause obvious illness symptoms. Thus the detection of biomarkers of micronutrient status should significantly enhance the diagnosis of micronutrient deficiency. In industry, the potential of microfluidic technology in realizing real-time, POC monitoring of micronutrient status has already been recognized. NanoSpeed Diagnostics Inc. has introduced a series of Test4^{TM} products, which are basically Lateral Flow Immunoassay (LIFA)-based portable devices. Among the products, the Test4D^{TM}, Test4Ca^{TM} and Test4Fe^{TM} are capable of measuring vitamin D, calcium, and iron levels in human blood, respectively. Meanwhile, recent progress has also been made in academia in developing microfluidic systems for measuring micronutrient levels in blood.

Iron deficiency is one of the most common micronutrient deficiencies around the world and causes iron-deficiency anemia. Besides serum iron level, hemoglobin and serum ferritin are widely used indicators of iron status. Steigert *et al.* developed a LOD platform based on colorimetric assay that can directly measure the concentration of hemoglobin from only 2 μL of whole blood within 100 s.[85] Microfluidic detections of serum ferritin have also been reported by several groups using on-chip immunoassays.[86–88] Through the integration of pneumatic microvalves, Kartalov *et al.* realized a high-throughput, multi-antigen microfluidic fluorescence immunoassay system, which can simultaneously measure serum ferritin, CRP and several other serum proteins.[88]

Recently, the first monolithically fabricated microfluidic electrochemical sensor capable of measuring zinc in serum was reported.[89] In this work, microfabricated bismuth electrodes interact with the zinc in the sample to generate quantitative information about zinc levels in blood serum treated with HCl. This reported microfluidic sensor requires only 200 μL of blood sample and shows good sensitivity in the 5 mM to 50 mM range in pH 6 sodium acetate buffer.

In addition to detection of trace elements, microfluidics has been successful in the monitoring of vitamin A status.[90] Vitamin A levels are conventionally monitored through characterization of retinol levels in the blood. Due to the 1 : 1 ratio between retinol binding protein (RBP) and retinol, RBP is a suitable surrogate for vitamin A monitoring. Based on this, the on-chip measurement of RBP instead of retinol was employed in this work, speeding up the process and eliminating the need for extensive lab work. To test the functionality of this microfluidic RBP enzyme immunoassay

(EIA), the authors executed a field evaluation in a population at risk of vitamin A deficiency using both the microfluidic RBP EIA and the HPLC retinol measurement. The results showed that the microfluidic platform is as reliable as the conventional HPLC method in estimating vitamin A deficiency, while with obvious advantages in terms of portability, low cost, and rapid detection.

Microfluidic monitoring of nutrient content

In the fight against global malnutrition, food-based strategies are the most straightforward approaches. Providing adequate food supply, micronutrient supplementation, dietary diversification, and food fortification are the simplest and most direct ways to combat malnutrition.[8] Therefore, the monitoring of nutrient content in dietary and micronutrient supplementation is a necessity for ensuring the efficiency of these strategies.

Conventional methods used in food analysis include mass spectroscopy (MS),[91] gas chromatography (GC),[92] high-performance liquid chromatography (HPLC),[93] capillary electrophoresis (CE),[94] and enzyme-linked immunosorbent assay (ELISA).[95] Although these analytical methods are widely used in the food industry for quality control and safety inspection, they do not meet the requirements of field use necessary for application in resource-limited areas because they necessitate sophisticated equipment, are relatively time-consuming, and require high reagent costs when conducted at large scales. Therefore, for large-scale, routine monitoring of nutrient content in dietary and micronutrient supplementation where malnutrition is most severe, portable, fast, and low-cost analytical platforms are desirable. Portability, miniaturization, and the capability to manipulate small amounts of fluid make microfluidic technology an ideal match for this type of application. Recently, the potential for microfluidics in food analysis has started to be realized.[96–98] In this section, we will review existing applications of microfluidics in monitoring nutrient content.

Microchip capillary electrophoresis

The introduction of microfluidics technology enabled the miniaturization of capillary electrophoresis (CE) and the realization of microchip CE.[99] Compared with conventional counterparts, microchip CE has the potential to simultaneously assay multiple small samples very rapidly, thereby significantly increasing throughput, reducing reagent consumption, and decreasing assay time.[100] With these advantages, microchip CE is emerging as a useful tool in analysis of carbohydrates, proteins, amino acids, and water-soluble vitamins in food.[101,102] The first application of microchip CE in nutrient analysis was protein separation and quantification in fish muscle.[103] In this work, protein extract from both farmed and wild-caught fish was analyzed with microchip CE to evaluate the differences in relative protein concentration to assess the impact of aquaculture on fish quality.[103] With the integration of light emitting diode-induced fluorescence (LIF) detection, Ueno *et al.* were able to quantify amino acids in functional foods such as sport beverages, jelly-form beverages, and tablet-form functional foods.[104] In another work, Crevillén *et al.* demonstrated fast analysis of water-soluble vitamins (vitamin B and C) in tablets within 350 s, clearly showcasing the fast-analysis capacity of microchip CE.[105] Continuous advances in research in microchip CE have resulted in commercialization by the analytical industry. The Agilent 2100 Bioanalyzer is the first commercially available microchip CE device for DNA, RNA and protein analysis. To compare the performance of this lab-on-a-chip device with conventional CE, Blazek and Caldwell analyzed the protein content of twenty different soybean cultivars with both conventional CE and the Agilent 2100 Bioanalyzer.[106] In addition to increased time-efficiency, the lab-on-a-chip device demonstrated better performance in terms of the repeatability of migration times and peak areas.

Nanomaterial-enabled microfluidic sensors

Since microfluidic devices usually handle very small sample volumes, ultra-sensitive sensing schemes are necessary to detect limited amounts of analytes. The integration of nanomaterial-based sensors[107–109] is a promising solution. Due to their high sensitivity and specificity, nanomaterial-based sensors have already attracted growing attention in food analysis.[110] However, the introduction of nanotechnology into microfluidics to analyze low-concentration nutrients in foods is still in its infancy.[111–113] One successful example is the use of carbon nanotubes (CNT) in microchip CE to detect water-soluble vitamins in foods.[111] In this work, CNT-based electrochemical detection (ED) demonstrated decreased overpotential, enhanced sensitivity, and improved resolution due to the large surface-to-volume ratio, enhanced electronic transfer, and strong sorption capacity of CNT.

Surface plasmon resonance (SPR)[114–118] biosensors are also versatile tools in various fields. Commercialized SPR biosensors have been available since 1990, when the first SPR instrument was developed by Biacore AB. Recently, SPR biosensors have been applied successfully in food analysis.[119] Using a Biacore Q® instrument, Gao *et al.* successfully quantified several water-soluble vitamins (B2, B12, folic acid, biotin, and pantothenic acid) in infant formula samples.[113] In another work, Fernández *et al.* integrated the gold diffraction grating surface into a six-channel microfluidic device to achieve on-site, label-free, multiplexed antibiotics analysis in milk samples.[112]

Microfluidic bioassays and chemical assays

With its extraordinary capacity for handling small volumes of liquid, microfluidics provides an attractive solution for miniaturizing conventional bioassays and chemical assays.[120] By immobilizing L-glutamate dehydrogenase and D-phenylglycine aminotransferase onto the surface of the microchannel or microparticles, Laiwattanapaisal *et al.* developed two miniature platforms capable of detecting L-glutamate in food samples with a limit of detection of 3 μM.[121] Recently, microfluidic chemiluminescence systems for measurements of vitamin B1 and B12 levels in tablets or eggs have also been reported, with very high sensitivity compared to reported methods.[122–124] Besides system miniaturization, another dis-

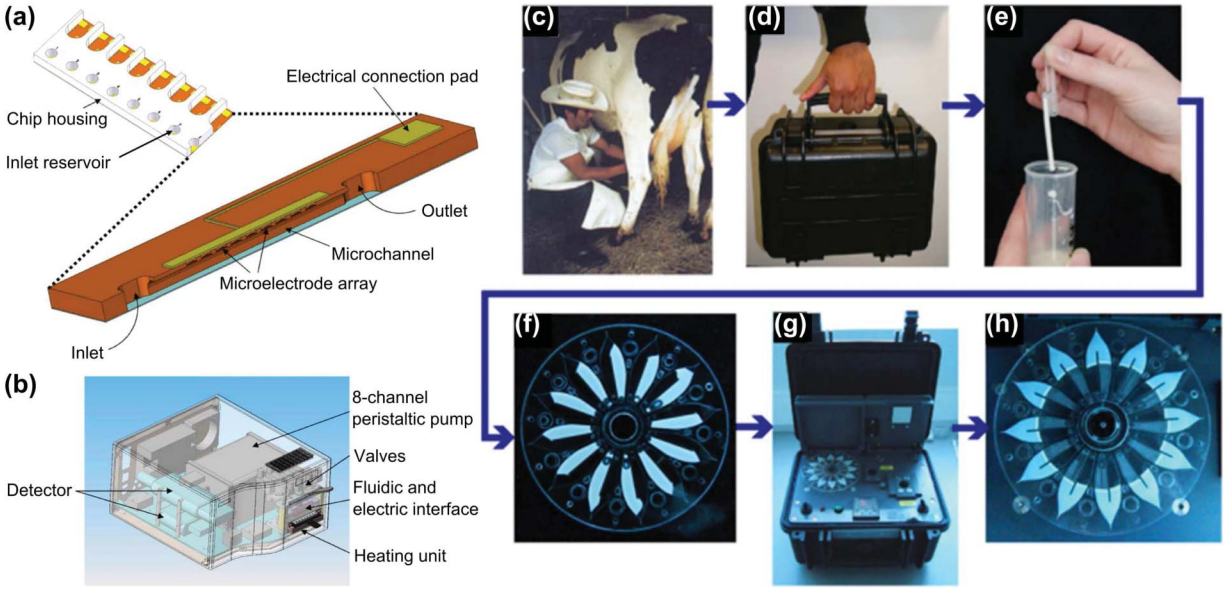

Fig. 4 (a) and (b) show the schematic of the microfluidic ELISA device for the quantification of folic acid in infant formula samples. (c) to (h) show the fully integrated, portable microfluidic sedimentation cytometer (SeCy) used for milk analysis. Images reproduced from ref. 125 and 127 with permission from Springer.

tinct merit of microfluidics technology is the ease of parallelization, which can be realized simply by introducing multiple channels. This can increase throughput or enable multiplexed analysis. Hoegger *et al.* developed a disposable microfluidic ELISA device for the quantification of folic acid in infant formula samples within 5 min, shown in Fig. 4 (a) and (b).[125] The detection of folic acid is based on an indirect competitive immunoassay, in which free folic acids in the samples compete with pre-coated folic acids on the channel surface for the binding sites of the antibody-enzyme conjugates. Thus the concentration of folic acids in the food samples is inversely proportional to the amount of antibody-enzyme conjugate bound to the surface-immobilized folic acids, which is detected electrochemically. The integration of eight parallel microchannels allows calibration and analysis in one step.

In addition to conventional microfluidic devices, newly developed paper-based microfluidics and centrifugal microfluidics technology have been applied in nutrient analysis.[126–128] Fig. 4 (c) to (h) show an integrated, low-cost, portable microfluidic sedimentation cytometer (SeCy) based on centrifugal microfluidics.[127] This device consists of 12 independent flattened funnel-like units fabricated on a single plastic compact disc (CD) for loading 150 μl milk samples. When the CD is rotating, somatic cells and fat globules can be separated for analysis under centrifugal forces, with detection ranges of 2.0–6.5% fat content and 5×10^4 to 5×10^5 cells ml^{-1} cell counts. This sample-to-answer device can be used for on-site, rapid quality control of milk product.

Conclusions and perspectives

In the previous sections, we highlighted some of the recently developed microfluidic blood analysis platforms and examined the current status of applied microfluidics in the detection of nutritional biomarkers or monitoring of nutrient content. Despite great achievements in disease diagnostics, the application of microfluidics in the field of nutrition is still in its infancy. One reason for this is the limited popularity of biomarkers in nutritional screening and malnutrition diagnosis. It is understandable that before blood or urine tests became affordable and easily accessible, people relied on anthropometric indicators to evaluate the likelihood of existing malnutrition or the risk of developing future malnutrition. However, these anthropometric indicators are subject to influences other than nutritional status and are ill-suited for diagnosing early micronutrient deficiencies. Therefore, biomarkers of nutrition will be useful for overcoming the limitations of anthropometric indicators and facilitating early diagnosis of micronutrient deficiency. The development of portable and affordable diagnostic devices capable of detecting biomarkers of nutrition will thus be necessary and helpful. This unmet need has been realized by the healthcare industry with efforts devoted to this endeavor. For example, Diagnostics For All, a non-profit enterprise saving lives through the creation of low-cost, easy-to-use, POC diagnostic devices specifically for the developing world, is working with MC10 Inc. to combine the low-cost patterned paper-based diagnostics with the flexible electronics platform in order to develop innovative diagnostic devices capable of quantitatively and accurately monitoring micronutrient status in POC settings and at low cost. These affordable and portable microfluidic devices, once realized, will essentially bring nutritional evaluation off bench and enable on-site, real-time assessment of nutritional status especially in remote areas.

It is essential to recognize that there are several challenges to applying microfluidics in the field of nutrition. To design

POC microfluidic devices for the detection of biomarkers of nutrition, the first decision is what biomarkers should be targeted. Unlike disease diagnoses in which one or a few biomarkers can provide useful information, the evaluation of nutritional status based on biomarkers is more complicated with a continued debate as to which biomarkers are to be used for specific purposes.[59,60] Although accumulated knowledge about biomarkers of nutrition will certainly help us make wiser decisions in the future, currently, it seems reasonable to measure multiple biomarkers when no single biomarker is available. In addition, for diagnosing micronutrient deficiency, it is also beneficial to be able to detect multiple micronutrient levels at one time. To fulfill these demands, multiplexed detection seems a reasonable solution. As we have discussed in previous sections, multiplexed detection has been recognized by the community as a characteristic of microfluidic systems. With proper assay design and parallelization, it is possible to develop POC microfluidic devices capable of detecting multiple biomarkers of nutrition. The benefit of such devices is the ability to provide more comprehensive information regarding the overall nutritional status.

As for microfluidic monitoring of nutrient content, the major challenge is the complexity of the food matrix. Multiple steps of sample preparation are often required for the analysis of food samples, often hindering the development of fully integrated systems. Although enormous efforts have been devoted to developing effective food sample preparation techniques, a really satisfying approach is still yet to come. In the meantime, the emerging trend of integrating nanomaterial-based sensors into microfluidics sheds light on tackling this sample preparation problem. The miniaturization nature of microfluidics means that only a very small amount of food sample is required. The large surface area to volume ratio and excellent electron transfer rate of nanomaterials help realize ultrasensitive and ultrafast sensors for monitoring nutrient content from only minute amounts of food samples. Combined together, this may reduce the burden of pretreatment of large amounts of food samples before analysis and lead to a practical microfluidic nutrient content analyzer.

In conclusion, microfluidic technology holds enormous potential in terms of realizing POC, multi-parameter nutritional evaluation and integrated nutrient content analysis devices. Despite this potential, the field of nutrition is still a relatively unexplored area for the microfluidics community. We hope that this article can draw more attention to this field so that we can together push nutrition towards POC settings with microfluidics technology.

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References

1 World Health Organization, *Global nutrition policy review: what does it take to scale up nutrition action?*, 2013.
2 U. E. Schaible and S. H. E. Kaufmann, *PLoS Med.*, 2007, **4**, e115.
3 K. M. Flegal, M. D. Carroll, B. K. Kit and C. L. Ogden, *JAMA, J. Am. Med. Assoc.*, 2012, **307**, 491.
4 M. Chopra, S. Galbraith and I. Darnton-Hill, *Bulletin of the World Health Organization*, 2002, **80**, 952.
5 V. J. B. Martins, T. M. M. Toledo Florêncio, L. P. Grillo, M. do Carmo P. Franco, P. A. Martins, A. P. G. Clemente, C. D. L. Santos, M. de Fatima A. Vieira and A. L. Sawaya, *Int. J. Environ. Res. Public Health*, 2011, **8**, 1817.
6 R. E. Black, L. H. Allen, Z. A. Bhutta, L. E. Caulfield, M. de Onis, M. Ezzati, C. Mathers and J. Rivera, *Lancet*, 2008, **371**, 243.
7 M. de Onis, D. Brown, M. Blössner and E. Borghi, *Levels & Trends in Child Malnutrition*, 2012.
8 L. Allen, B. de Benoist and O. Dary, *Guidelines on food fortification with micronutrients*, 2006.
9 M. Blössner and M. de Onis, *Malnutrition: quantifying the health impact at national and local levels*, 2005.
10 J. Kondrup, S. P. Allison, M. Elia, B. Vellas and M. Plauth, *Clin. Nutr.*, 2003, **22**, 415.
11 G. M. Whitesides, *Nature*, 2006, **442**, 368.
12 X. Mao and T. J. Huang, *Lab Chip*, 2012, **12**, 1412.
13 P. Neuži, S. Giselbrecht, K. Länge, T. J. Huang and A. Manz, *Nat. Rev. Drug Discovery*, 2012, **11**, 620.
14 Y. Zhao, Z. S. Stratton, F. Guo, M. I. Lapsley, C. Y. Chan, S.-C. S. Lin and T. J. Huang, *Lab Chip*, 2013, **13**, 17.
15 X. Mao and T. J. Huang, *Lab Chip*, 2012, **12**, 4006.
16 P. Li, Z. S. Stratton, M. Dao, J. Ritz and T. J. Huang, *Lab Chip*, 2013, **13**, 602.
17 R. Fan, O. Vermesh, A. Srivastava, B. K. H. Yen, L. Qin, H. Ahmad, G. A. Kwong, C.-C. Liu, J. Gould, L. Hood and J. R. Heath, *Nat. Biotechnol.*, 2008, **26**, 1373.
18 R. C. Bailey, G. A. Kwong, C. G. Radu, O. N. Witte and J. R. Heath, *J. Am. Chem. Soc.*, 2007, **129**, 1959.
19 C. D. Chin, T. Laksanasopin, Y. K. Cheung, D. Steinmiller, V. Linder, H. Parsa, J. Wang, H. Moore, R. Rouse, G. Umviligihozo, E. Karita, L. Mwambarangwe, S. L. Braunstein, J. van de Wijgert, R. Sahabo, J. E. Justman, W. El-Sadr and S. K. Sia, *Nat. Med.*, 2011, **17**, 1015.
20 K. Hosokawa, M. Omata, K. Sato and M. Maeda, *Lab Chip*, 2006, **6**, 236.
21 K. Hosokawa, K. Sato, N. Ichikawa and M. Maeda, *Lab Chip*, 2004, **4**, 181.
22 L. Qin, O. Vermesh, Q. Shi and J. R. Heath, *Lab Chip*, 2009, **9**, 2016.
23 I. K. Dimov, L. Basabe-Desmonts, J. L. Garcia-Cordero, B. M. Ross, Y. Park, A. J. Ricco and L. P. Lee, *Lab Chip*, 2011, **11**, 845.
24 R. M. Rocco, *Landmark Papers in Clinical Chemistry.*, Elsevier, Amsterdam, 2006, vol. 52.

25 U. Y. Schaff and G. J. Sommer, *Clin. Chem.*, 2011, **57**, 753.
26 M. Amasia and M. Madou, *Bioanalysis*, 2010, **2**, 1701.
27 M. Grumann, J. Steigert, L. Riegger, I. Moser, B. Enderle, K. Riebeseel, G. Urban, R. Zengerle and J. Ducrée, *Biomed. Microdevices*, 2006, **8**, 209.
28 J. Park, V. Sunkara, T.-H. Kim, H. Hwang and Y.-K. Cho, *Anal. Chem.*, 2012, **84**, 2133.
29 J. Steigert, T. Brenner, M. Grumann, L. Riegger, S. Lutz, R. Zengerle and J. Ducrée, *Biomed. Microdevices*, 2007, **9**, 675.
30 S. Haeberle, T. Brenner, R. Zengerle and J. Ducrée, *Lab Chip*, 2006, **6**, 776.
31 J. Steigert, M. Grumann, T. Brenner, L. Riegger, J. Harter, R. Zengerle and J. Ducrée, *Lab Chip*, 2006, **6**, 1040.
32 J. Steigert, M. Grumann, T. Brenner, K. Mittenbuhler, T. Nann, J. Ruhe, I. Moser, S. Haeberle, L. Riegger and J. Riegler, *J. Assoc. Lab. Autom.*, 2005, **10**, 331.
33 B. S. Lee, Y. U. Lee, H.-S. Kim, T.-H. Kim, J. Park, J.-G. Lee, J. Kim, H. Kim, W. G. Lee and Y.-K. Cho, *Lab Chip*, 2011, **11**, 70.
34 B. S. Lee, J.-N. Lee, J.-M. Park, J.-G. Lee, S. Kim, Y.-K. Cho and C. Ko, *Lab Chip*, 2009, **9**, 1548.
35 J.-M. Park, Y.-K. Cho, B.-S. Lee, J.-G. Lee and C. Ko, *Lab Chip*, 2007, **7**, 557.
36 Y.-K. Cho, J.-G. Lee, J.-M. Park, B.-S. Lee, Y. Lee and C. Ko, *Lab Chip*, 2007, **7**, 565.
37 R. Gorkin, J. Park, J. Siegrist, M. Amasia, B. S. Lee, J.-M. Park, J. Kim, H. Kim, M. Madou and Y.-K. Cho, *Lab Chip*, 2010, **10**, 1758.
38 M. Madou, J. Zoval, G. Jia, H. Kido, J. Kim and N. Kim, *Annu. Rev. Biomed. Eng.*, 2006, **8**, 601.
39 C.-M. Cheng, A. W. Martinez, J. Gong, C. R. Mace, S. T. Phillips, E. Carrilho, K. A. Mirica and G. M. Whitesides, *Angew. Chem., Int. Ed.*, 2010, **49**, 4771.
40 Z. Nie, F. Deiss, X. Liu, O. Akbulut and G. M. Whitesides, *Lab Chip*, 2010, **10**, 3163.
41 E. Fu, B. Lutz, P. Kauffman and P. Yager, *Lab Chip*, 2010, **10**, 918.
42 B. R. Lutz, P. Trinh, C. Ball, E. Fu and P. Yager, *Lab Chip*, 2011, **11**, 4274.
43 E. Fu, P. Kauffman, B. Lutz and P. Yager, *Sens. Actuators, B*, 2010, **149**, 325.
44 M. S. Khan, G. Thouas, W. Shen, G. Whyte and G. Garnier, *Anal. Chem.*, 2010, **82**, 4158.
45 W. Dungchai, O. Chailapakul and C. S. Henry, *Anal. Chim. Acta*, 2010, **674**, 227.
46 E. M. Fenton, M. R. Mascarenas, G. P. López and S. S. Sibbett, *ACS Appl. Mater. Interfaces*, 2009, **1**, 124.
47 A. W. Martinez, S. T. Phillips, M. J. Butte and G. M. Whitesides, *Angew. Chem., Int. Ed.*, 2007, **46**, 1318.
48 A. W. Martinez, S. T. Phillips, E. Carrilho, S. W. Thomas, H. Sindi and G. M. Whitesides, *Anal. Chem.*, 2008, **80**, 3699.
49 A. K. Ellerbee, S. T. Phillips, A. C. Siegel, K. A. Mirica, A. W. Martinez, P. Striehl, N. Jain, M. Prentiss and G. M. Whitesides, *Anal. Chem.*, 2009, **81**, 8447.
50 X. Li, J. Tian and W. Shen, *Anal. Bioanal. Chem.*, 2010, **396**, 495.
51 J. L. Osborn, B. Lutz, E. Fu, P. Kauffman, D. Y. Stevens and P. Yager, *Lab Chip*, 2010, **10**, 2659.
52 X. Yang, O. Forouzan, T. P. Brown and S. S. Shevkoplyas, *Lab Chip*, 2012, **12**, 274.
53 S. J. Vella, P. Beattie, R. Cademartiri, A. Laromaine, A. W. Martinez, S. T. Phillips, K. A. Mirica and G. M. Whitesides, *Anal. Chem.*, 2012, **84**, 2883.
54 A. W. Martinez, S. T. Phillips and G. M. Whitesides, *Proc. Natl. Acad. Sci. U. S. A.*, 2008, **105**, 19606.
55 N. R. Pollock, J. P. Rolland, S. Kumar, P. D. Beattie, S. Jain, F. Noubary, V. L. Wong, R. A. Pohlmann, U. S. Ryan and G. M. Whitesides, *Sci. Transl. Med.*, 2012, **4**, 152ra129.
56 A. K. Yetisen, M. S. Akram and C. R. Lowe, *Lab Chip*, 2013, **13**, 2210.
57 A. W. Martinez, S. T. Phillips, G. M. Whitesides and E. Carrilho, *Anal. Chem.*, 2010, **82**, 3.
58 W. Zhao and A. van der Berg, *Lab Chip*, 2008, **8**, 1988.
59 D. J. Raiten, S. Namasté, B. Brabin, J. Combs, Gerald, M. R. L'Abbe, E. Wasantwisut and I. Darnton-Hill, *Am. J. Clin. Nutr.*, 2011, **94**, 633S.
60 V. E. Hedrick, A. M. Dietrich, P. A. Estabrooks, J. Savla, E. Serrano and B. M. Davy, *Nutr. J.*, 2012, **11**, 109.
61 C. J. Davis, D. Sowa, K. S. Keim, K. Kinnare and S. Peterson, *JPEN, J. Parenter. Enteral Nutr.*, 2012, **36**, 197.
62 M. P. Fuhrman, P. Charney and C. M. Mueller, *J. Am. Diet. Assoc.*, 2004, **104**, 1258.
63 H. Honda, A. R. Qureshi, O. Heimbürger, P. Barany, K. Wang, R. Pecoits-Filho, P. Stenvinkel and B. Lindholm, *Am. J. Kidney Dis.*, 2006, **47**, 139.
64 A. Shenkin, *Clin. Chem.*, 2006, **52**, 2177.
65 W. Laiwattanapaisal, T. Songjaroen, T. Maturos, T. Lomas, A. Sappat and A. Tuantranont, *Sensors*, 2009, **9**, 10066.
66 C.-J. Huang, C.-C. Lu, T.-Y. Lin, T.-C. Chou and G.-B. Lee, *J. Micromech. Microeng.*, 2007, **17**, 835.
67 C.-C. Lin, C.-C. Tseng, C.-J. Huang, J.-H. Wang and G.-B. Lee, *Biomed. Microdevices*, 2010, **12**, 887.
68 W.-B. Lee, Y.-H. Chen, H.-I. Lin, S.-C. Shiesh and G.-B. Lee, *Sens. Actuators, B*, 2011, **157**, 710.
69 Y.-N. Yang, H.-I. Lin, J.-H. Wang, S.-C. Shiesh and G.-B. Lee, *Biosens. Bioelectron.*, 2009, **24**, 3091.
70 M. Wolf, D. Juncker, B. Michel, P. Hunziker and E. Delamarche, *Biosens. Bioelectron.*, 2004, **19**, 1193.
71 M. C. Peoples and H. T. Karnes, *Anal. Chem.*, 2008, **80**, 3853.
72 S. Choi, A. Lajevardi-khosh and J. Chae, *2011 16th International Solid-State Sensors, Actuators and Microsystems Conference*, 2011, 2223.
73 G. Lee, I. Park, K. Kwon, T. Kwon, J. Seo, W.-J. Chang, H. Nam, G. S. Cha, M. H. Choi, D. S. Yoon and S. W. Lee, *Biomed. Microdevices*, 2012, **14**, 375.
74 L. Gervais and E. Delamarche, *Lab Chip*, 2009, **9**, 3330.
75 K. Sun, N. Ramgir and S. Bhansali, *Sens. Actuators, B*, 2008, **133**, 533.
76 A. Kumar, S. Aravamudhan, M. Gordic, S. Bhansali and S. S. Mohapatra, *Biosens. Bioelectron.*, 2007, **22**, 2138.
77 J. S. Mitchell, T. E. Lowe and J. R. Ingram, *Analyst*, 2009, **134**, 380.
78 K. S. Jaya Rao, S. G. Srikantia and C. Gopalan, *Arch. Dis. Child.*, 1968, **43**, 365.
79 M. Azahar Ali, S. Srivastava, P. R. Solanki, V. Varun Agrawal, R. John and B. D. Malhotra, *Appl. Phys. Lett.*, 2012, **101**, 084105.

80 S. Aravamudhan, A. Kumar, S. Mohapatra and S. Bhansali, *Biosens. Bioelectron.*, 2007, **22**, 2289.
81 A. Wisitsoraat, P. Sritongkham, C. Karuwan, D. Phokharatkul, T. Maturos and A. Tuantranont, *Biosens. Bioelectron.*, 2010, **26**, 1514.
82 N. Ruecha, W. Siangproh and O. Chailapakul, *Journal of Electronic Science and Technology*, 2010, **8**, 82.
83 S. Aravamudhan, N. S. Ramgir and S. Bhansali, *Sens. Actuators, B*, 2007, **127**, 29.
84 M. A. Ali, P. R. Solanki, M. K. Patel, H. Dhayani, V. V. Agrawal, R. John and B. D. Malhotra, *Nanoscale*, 2013, **5**, 2883.
85 J. Steigert, M. Grumann, M. Dube, W. Streule, L. Riegger, T. Brenner, P. Koltay, K. Mittmann, R. Zengerle and J. Ducrée, *Sens. Actuators, A*, 2006, **130–131**, 228.
86 W. Schrott, M. Nebyla, L. Meisterová and M. Přibyl, *Chem. Pap.*, 2010, **65**, 246.
87 E. P. Kartalov, D. H. Lin, D. T. Lee, W. F. Anderson, C. R. Taylor and A. Scherer, *Electrophoresis*, 2008, **29**, 5010.
88 E. Kartalov, J. Zhong, A. Scherer, S. Quake, C. Taylor and W. French Anderson, *BioTechniques*, 2006, **40**, 85.
89 P. Jothimuthu, R. A. Wilson, J. Herren, X. Pei, W. Kang, R. Daniels, H. Wong, F. Beyette, W. R. Heineman and I. Papautsky, *Electroanalysis*, 2013, **25**, 401.
90 J. Hix, C. Martinez, I. Buchanan, J. Morgan, M. Tam and A. Shankar, *The American Journal of Clinical Nutrition*, 2004, **79**, 93.
91 A. Kaufmann, *Anal. Bioanal. Chem.*, 2012, **403**, 1233.
92 S. J. Lehotay and J. Hajšlová, *TrAC, Trends Anal. Chem.*, 2002, **21**, 686.
93 L. M. L. Nollet, F. Toldrá, *Food analysis by HPLC*, CRC Press, 2012.
94 R. A. Frazier and A. Papadopoulou, *Electrophoresis*, 2003, **24**, 4095.
95 L. Asensio, I. González, T. García and R. Martín, *Food Control*, 2008, **19**, 1.
96 Y. T. Atalay, S. Vermeir, D. Witters, N. Vergauwe, B. Verbruggen, P. Verboven, B. M. Nicolaï and J. Lammertyn, *Trends Food Sci. Technol.*, 2011, **22**, 386.
97 S. Neethirajan, I. Kobayashi, M. Nakajima, D. Wu, S. Nandagopal and F. Lin, *Lab Chip*, 2011, **11**, 1574.
98 Y.-F. Chen, L. Jiang, M. Mancuso, A. Jain, V. Oncescu and D. Erickson, *Nanoscale*, 2012, **4**, 4839.
99 A. Manz, N. Graber and H. M. Widmer, *Sens. Actuators, B*, 1990, **1**, 244.
100 V. Dolník, S. Liu and S. Jovanovich, *Electrophoresis*, 2000, **21**, 41.
101 A. Escarpa, M. C. González, A. G. Crevillén and A. J. Blasco, *Electrophoresis*, 2007, **28**, 1002.
102 A. Escarpa, M. C. González, M. A. López Gil, A. G. Crevillén, M. Hervás and M. García, *Electrophoresis*, 2008, **29**, 4852.
103 G. Monti, L. De Napoli, P. Mainolfi, R. Barone, M. Guida, G. Marino and A. Amoresano, *Anal. Chem.*, 2005, **77**, 2587.
104 H. Ueno, J. Wang, N. Kaji, M. Tokeshi and Y. Baba, *Journal of Separation Science*, 2008, **31**, 898.
105 A. G. Crevillén, A. J. Blasco, M. C. González and A. Escarpa, *Electrophoresis*, 2006, **27**, 5110.
106 V. Blazek and R. A. Caldwell, *Int. J. Food Sci. Technol.*, 2009, **44**, 2127.
107 V. K. S. Hsiao, J. R. Waldeisen, Y. Zheng, P. F. Lloyd, T. J. Bunning and T. J. Huang, *J. Mater. Chem.*, 2007, **17**, 4896.
108 S. Yang, F. Guo, B. Kiraly, X. Mao, M. Lu, K. W. Leong and T. J. Huang, *Lab Chip*, 2012, **12**, 2097.
109 S. Yang, M. I. Lapsley, B. Cao, C. Zhao, Y. Zhao, Q. Hao, B. Kiraly, J. Scott, W. Li, L. Wang, Y. Lei and T. J. Huang, *Adv. Funct. Mater.*, 2013, **23**, 720.
110 B. Pérez-López and A. Merkoçi, *Trends Food Sci. Technol.*, 2011, **22**, 625.
111 A. G. Crevillén, M. Avila, M. Pumera, M. C. González and A. Escarpa, *Anal. Chem.*, 2007, **79**, 7408.
112 F. Fernández, K. Hegnerová, M. Piliarik, F. Sanchez-Baeza, J. Homola and M.-P. Marco, *Biosens. Bioelectron.*, 2010, **26**, 1231.
113 Y. Gao, F. Guo, S. Gokavi, A. Chow, Q. Sheng and M. Guo, *Food Chem.*, 2008, **110**, 769.
114 Y. B. Zheng, T. J. Huang, A. Y. Desai, S. J. Wang, L. K. Tan, H. Gao and A. C. H. Huan, *Appl. Phys. Lett.*, 2007, **90**, 183117.
115 Y. B. Zheng, L. Jensen, W. Yan, T. R. Walker, B. K. Juluri, L. Jensen and T. J. Huang, *J. Phys. Chem. C*, 2009, **113**, 7019.
116 Y. B. Zheng, B. Kiraly, P. S. Weiss and T. J. Huang, *Nanomedicine*, 2012, **7**, 751.
117 Y. J. Liu, Q. Hao, J. S. T. Smalley, J. Liou, I. C. Khoo and T. J. Huang, *Appl. Phys. Lett.*, 2010, **97**, 091101.
118 B. K. Juluri, Y. B. Zheng, D. Ahmed, L. Jensen and T. J. Huang, *J. Phys. Chem. C*, 2008, **112**, 7309.
119 C. Situ, M. H. Mooney, C. T. Elliott and J. Buijs, *TrAC, Trends Anal. Chem.*, 2010, **29**, 1305.
120 S. Vyawahare, A. D. Griffiths and C. A. Merten, *Chem. Biol.*, 2010, **17**, 1052.
121 W. Laiwattanapaisal, J. Yakovleva, M. Bengtsson, T. Laurell, S. Wiyakrutta, V. Meevootisom, O. Chailapakul and J. Emnéus, *Biomicrofluidics*, 2009, **3**, 14104.
122 M. Kamruzzaman, A.-M. Alam, S. H. Lee and T. D. Dang, *Sens. Actuators, B*, 2013, **185**, 301.
123 K. S. Lok, S. Z. binte Abdul Muttalib, P. P. F. Lee, Y. C. Kwok and N.-T. Nguyen, *Lab Chip*, 2012, **12**, 2353.
124 M. Kamruzzaman, A.-M. Alam, K. M. Kim, S. H. Lee, Y. H. Kim, A. N. M. H. Kabir, G.-M. Kim and T. D. Dang, *Biomed. Microdevices*, 2013, **15**, 195.
125 D. Hoegger, P. Morier, C. Vollet, D. Heini, F. Reymond and J. S. Rossier, *Anal. Bioanal. Chem.*, 2007, **387**, 267.
126 L. Cai, Y. Wu, C. Xu and Z. Chen, *J. Chem. Educ.*, 2013, **90**, 232.
127 J. L. Garcia-Cordero, L. M. Barrett, R. O'Kennedy and A. J. Ricco, *Biomed. Microdevices*, 2010, **12**, 1051.
128 H. Hwang, Y. Kim, J. Cho, J. Lee, M.-S. Choi and Y.-K. Cho, *Anal. Chem.*, 2013, **85**, 2954.