Review

Point-of-care technologies for molecular diagnostics using a drop of blood

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Molecular diagnostics is crucial for prevention, identification, and treatment of disease. Traditional technologies for molecular diagnostics using blood are limited to laboratory use because they rely on sample purification and sophisticated instruments, are labor and time intensive, expensive, and require highly trained operators. This review discusses the frontiers of point-of-care (POC) diagnostic technologies using a drop of blood obtained from a finger prick. These technologies, including emerging biotechnologies, nanotechnologies, and microfluidics, hold the potential for rapid, accurate, and inexpensive disease diagnostics.

Blood as a target for molecular diagnostics

Blood is a bodily fluid that contains abundant information about the health status of the individual. The average human adult has a blood volume of ~5 l continuously circulating throughout the body to deliver necessary nutrients and transport metabolic waste [1]. Blood consists of 54.3% plasma, 45% red blood cells (RBCs), and 0.7% white blood cells (WBCs) by volume [2]. Plasma is composed of proteins, nucleic acids, and nutrients or waste products, and it maintains electrolyte balance and protects the body from infection and blood disorders [3–5]. Serum is produced by removal of blood-clotting factors from plasma [6] and is the main source of samples used in blood-based molecular diagnostics. The levels of molecular constituents in blood are directly associated with the physiological state of the body, therefore, detection of these molecules in serum is often used for prevention, identification, and treatment selection for a variety of diseases.

Traditional technologies for molecular diagnostics in blood include ELISA, PCR, and mass spectrometry (MS) [7]. However, these technologies are limited to laboratory use because they rely on sample purification and sophisticated instruments, are time and labor intensive and expensive, and require highly trained operators. In addition, the sensitivity of some of these technologies is unsatisfactory for detecting trace levels of biomarkers. Therefore, there is still a great challenge to develop simple, inexpensive, rapid, and easy-to-use technologies for POC blood molecular diagnostics. A typical POC assay is affordable, specific, sensitive, portable, rapid, and user friendly, which also makes it suitable for use in low-resource settings [8,9]. The first true POC device was the urine dipstick, which was developed in 1957 to measure urinary protein [8]. Glucose meters and lateral flow devices are currently the most widely used devices in POC blood molecular diagnostics, although they are not applicable if highly quantitative, sensitive, and high-throughput measurements are required. Emerging technologies, including biotechnologies, nanotechnologies, and microfluidics, hold the promise to improve the capabilities for future POC disease diagnostics [10–13].

In this review, we discuss recent developments in new technologies for molecular diagnostics using a drop of blood obtained from a finger prick. Technological developments for low-volume blood diagnostics may facilitate rapid, accurate, and inexpensive diagnosis of disease in the hospital clinic or self-monitoring at home. Taking blood from a finger prick is relatively painless, and it is suitable for POC and pediatric disease diagnostics because of the small samples required. Here, we provide a survey of applicable new technologies for measuring proteins, nucleic acids, and other molecules (e.g., hormones, metabolites, and drugs) as well as downstream molecular analyses based on cancer cells isolated from the blood. We discuss the advantages and disadvantages of each method (Table 1).

Detection of proteins

Proteins are well known to be required for numerous biological functions and processes, ranging from enzymatic reactions to hormone synthesis, maintenance of metabolic equilibrium, and tissue repair [14]. For clinical applications, levels of certain protein biomarkers directly reflect disease stages and have been regarded as one of the most convenient clinical sources for disease diagnosis. Blood

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Keywords: point-of-care diagnostics; molecular diagnostics; proteins; nucleic acids; biomolecules; circulating tumor cells.

0167-7799/$ – see front matter
 http://dx.doi.org/10.1016/j.tibtech.2014.01.003

Table 1. List of POC platforms for molecular diagnostics

Platform	Affordable	Specific	Sensitive	Portable	Rapid	Multiplex	Quantitative	User-friendly	Refs
Proteins									
ELISA-based methods									
LFA	+++	+++		+++	+++			+++	[18]
Integrative mChip	++	+++	+	++	+++		+	+++	[19]
Digital ELISA		++	+++				++		[20]
Plasmonic ELISA	++	++	+++		+		++		[21]
Silicon nanoribbon chip	+	+++	+++		+	++	++	+	[22]
GMR sensor		+++	+++		++	+++	++		[23]
IBBC		+++	++			+++	++		[24,25]
P-ELISA	+++	+++	+	+++	+	+++	++	++	[26–28]
V-Chip	+++	+++	++	+++		+++	+++	++	[29]
Non-ELISA-based methods									
MPS		++	+++			++	+		[32]
GFP–AuNPs	++				+++	++			[31]
AuNPs (colorimetric)	+++	++	+	++	+++	+	++	+++	[33]
AuNPs (DLS)	++	++	+++		+++		++	++	[34,35]
Nucleic acids									
DNA									
μCICS		++	+++		++		++	+	[44]
TAm-Seq		++	++			++	+		[45]
CCP	+	++	+++		++		++	++	[46]
RNA									
HPD-SENS	+	+++	++		+	++	++		[48]
EMRS	+	++	+++		+	+	++		[50]
Nanopore sensor		++	+++		++		++		[51]
Other types of biomolecules									
Graphene glucose sensor	+++	++	++		++		++	++	[52]
PGM–aptamer sensor	++	+	++	+++	+		+++	+	[56]
DMF		+	+++				+		[57]
Amperometric sensor	+	++	++	+	+		++	++	[60]
CTCs									
Immunomagnetic assay	++	++	+	+	++		+	++	[66]
Immunoassay chip	+	++	++		+		+	+	[67–70]
Size-based microchip	+	+						+	[71]
Dielectric separation	+	+			+			+	[73]

+++, high; ++, intermediate; +, low. Data for blank cells are not traceable through literature search.

contains >20 000 different proteins, with concentrations ranging from <1 ng/l (troponin) [15] to 50 g/l (serum albumin) [16,17]. Thus, there are abundant blood proteins available as candidate biomarkers for disease detection.

ELISA-based methods

Currently, most methods for blood protein analysis are based on ELISA, which serves as the clinical gold standard. In traditional ELISA methods, colorimetric or fluorescent readout signals are used to visualize the binding of a protein to a specific recognition molecule [12]. Despite the development of numerous new ELISA-based technologies for protein detection, many challenges remain to their application in POC diagnostics. Among these are improvements to increase sensitivity, multiplicity, quantification, portability, speed of operation, and clarity of readout, and reduce cost.

The traditional ELISA requires repeated washing steps, which makes the method time consuming and cumbersome. The lateral flow assay (LFA) or immunochromatographic assay, originally introduced in 1987, is considered the most successful commercial technology that overcomes these limitations [18]. This technology combines the principles of thin-layer paper chromatography and ELISA, allowing rapid separation of plasma components in a drop of blood in a few minutes. Recently, a new technology was developed that integrates fluid handling and silver reduction in a microfluidic chip (mChip) and can simplify ELISA. Diagnosis of HIV based on this device requires minimal equipment, analysis can be completed within 20 min, and it requires as little as 1 μl of blood [19]. Although these devices are simple to use, these technologies still exhibit many limitations, such as low sensitivity, results that are only semiquantitative, and low throughput.

The ability to measure low concentrations of disease biomarkers can improve the standard of care in resource-limited areas. Sensitivity can be improved by reducing the ELISA reaction volume to ensure a high concentration of fluorescent substrate [20]. By confining the fluorophore-generation reaction to 50 fl, a digital ELISA method was developed to detect proteins in serum at subfemtomolar concentrations. An alternative method to improve sensitivity is to introduce new signal amplification approaches into ELISA. Controlling the growth of gold nanoparticles (AuNPs) by catalase results in a color change, and its incorporation into an ELISA method (plasmonic ELISA)