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REVIEW

Implementation of Lab-on-a-Chip technologies in hematology: advances and challenges

Implementación de las tecnologías Lab-on-a-Chip en Hematología: avances y desafíos

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ABSTRACT

Advances in biomedical engineering, electronics, and bioinformatics are catalyzing the transition from conventional laboratories to Lab-on-a-Chip technologies. This technology shows potential for application in areas with a strong diagnostic component, such as hematology. This article was developed with the aim of describing the principles, advances, and challenges of implementing Lab-on-a-Chip technologies in hematology. Guided by the principles of microfluidics, these technologies enable tests ranging from complete blood counts to more complex ones such as flow cytometry. The ability to perform multiple analyses in parallel, its portability, and speed could greatly improve care in the care unit or at the patient's bedside, leading to early and timely diagnosis. However, component integration issues, manufacturing complexity, robustness, reliability, sensitivity, and lack of standardization remain real problems that hinder its development. Its development, although still slow, and integration with artificial intelligence techniques will favor diagnosis and treatment in hematological practice.

Keywords: Hematology; Clinical Chemistry Tests; Microfluidics; Equipment and Supplies; Indicators and Reagents; Biomedical Engineering; Computational Biology.

RESUMEN

Los avances en materia de ingeniería biomédica, electrónica y bioinformática catalizan la transición del laboratorio convencional a las tecnologías *Lab-on-a-Chip*. Esta tecnología muestra potencial de aplicación en áreas con un fuerte componente diagnóstico, como es el caso de la hematología. El presente artículo se desarrolló con el objetivo de describir los principios, avances y desafíos de la implementación de las tecnologías *Lab-on-a-Chip* en Hematología. Guiados por principios de la microfluídica, estas tecnologías permiten realizar pruebas que van desde un hemograma, hasta otras más complejas como una citometría de flujo. Las capacidades de realizar múltiples análisis en paralelo, su portabilidad y rapidez pudieran mejorar en gran medida la atención en la unidad de cuidados o en la cabecera del paciente, llegando al diagnóstico temprano y oportuno. Sin embargo, los problemas de integración de componentes, la complejidad de fabricación, la robustez, fiabilidad, sensibilidad y falta de estandarización continúan siendo problemas reales que frenan su desarrollo. Su desarrollo, aunque aún lento, y la integración con técnicas de inteligencia artificial favorecerá el diagnóstico y tratamiento en la práctica hematológica.

Palabras clave: Hematología; Pruebas de Química Clínica; Microfluídica; Equipos y Suministros; Indicadores y Reactivos; Ingeniería Biomédica; Biología Computacional.

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INTRODUCTION

Hematology is a branch of medicine that deals with the diagnosis, treatment, and prevention of diseases of the blood and the organs that produce it. The analysis of blood components forms the basis of hematological diagnosis, with the complete blood count and blood smear having long been established as fundamental tools.[1]

The conventional process for performing hematological tests begins with the collection of a blood sample. This sample is transferred to laboratories where it is processed in large, complex equipment that requires qualified personnel and the use of reagents, which are often expensive.[2]

Lab-on-a-Chip (LOC) technology represents one of the greatest advances in the biomedical field, integrating fluid engineering and biotechnology. These devices aim to miniaturize a complex biochemical laboratory into a small, easily portable device, facilitating patient assessment in the care unit or point of care.[3]

The basis of all LOC devices is microfluidics, the science and technology of systems that process or manipulate small volumes of fluids, on the order of microliters, using channels with dimensions ranging from tens to hundreds of micrometers.[4,5] This technology, often referred to as micro total analysis systems (μTAS), allows a complete blood count to be performed or a disease to be diagnosed using small volumes of biological samples.[6]

The transition from the conventional hematology laboratory to the LOC format involves the integration of a series of fundamental processes, which until now have been understood in a conventional way in hematological practice and diagnostics. From initial sample preparation to multiparametric cell analysis, LOC devices are redefining what is possible at the point of care.

This article aims to highlight the advances and challenges of implementing Lab-on-a-Chip technologies in hematology.

DEVELOPMENT

Principles of Microfluidics: Fluid Behavior and Microscale Phenomena

The behavior of fluids in LOC devices is fundamentally different from that in the macroscopic world. The flow is mostly laminar, meaning that fluids move in parallel layers without the turbulent mixing that characterizes larger-scale flows.[5,6,7] In this state, surface forces, such as surface tension and capillarity, together with viscous forces, dominate over inertial forces.[3] This physical status has a significant impact on device design. For example, mixing two fluids cannot be achieved by agitation; instead, it must rely on molecular diffusion across the interface between the fluid layers. To facilitate this, chip designers often create long, winding channels (serpentines) that increase the interfacial contact area and residence time, allowing diffusion.[8]

Furthermore, when analyzing blood, specific microfluidic phenomena come into play. The Fåhræus-Lindqvist effect describes the decrease in the apparent viscosity of blood as the channel diameter decreases below 300 μm. This is because red blood cells (RBCs) migrate toward the center of the channel, where the flow velocity is higher. As a result, a cell-free layer forms, a region near the channel walls that is rich in plasma and has lower viscosity. These two effects are not only crucial for understanding blood flow dynamics in microchannels, but are also actively exploited to design passive plasma separation systems.[9]

This physical paradigm shift is one of the reasons why LOC design is a highly specialized field. It requires a counterintuitive engineering approach and represents a conceptual barrier that contributes to the gap between laboratory prototypes and robust commercial products.[10]

Architecture of an LOC System

An LOC device is an integrated system that emulates a sequence of laboratory operations.6 Its architecture consists of several key elements, including microchannels, pumps and valves, and integrated sensors. Microchannels are conduits with precise, predefined characteristics through which samples and reagents flow. The pumps and valves are the structures responsible for precisely controlling the movement of fluids within the chip.[11,12] The integrated sensors are responsible for detection, the final step in the analysis. LOCs integrate a variety of microsensors capable of translating the biochemical interactions of the fluid into useful quantifiable signals. To achieve high-quality and accurate results, a variety of microsensors have been developed, which can be:

- Optical: these record changes in fluorescence, absorbance, and chemiluminescence. They tend to be useful in immunoassays and nucleic acid detection.[13,14]
- Electrochemical: detect changes in amperometry and potentiometry generated by chemical reactions. These microsensors form the basis for measuring glucose and other metabolites.[15]
- Plasmonic: these are sensors that use metallic nanostructures (usually gold) to detect molecular bonds on their surface through surface plasmon resonance. This allows the detection of interactions, chemical and structural changes.[16]
- Photoacoustic: this is a hybrid technique that combines optical excitation of the sample using laser pulses and detection of the resulting sound waves. The acoustic waves vary depending on the content of the sample.[17]

Sample preparation

Sample preparation is one of the most complex and error-prone steps in hematological analysis. Integrating this step directly into the chip is a requirement for achieving a *sample-to-answer* system that minimizes user/operator interaction and maximizes reliability.

Plasma separation

Many bioassays, mainly immunoassays and clinical chemistry tests, require blood plasma free of blood cells. This is usually achieved by centrifuging blood samples, a complex process to miniaturize. However, LOC systems are able to overcome this barrier

by applying passive principles of microfluidics. By circulating blood through microchannels, the Fåhræus-Lindqvist effect causes formed elements of the blood to migrate toward the central axis of the channel, creating a flow of plasma near the channel walls. By creating a bifurcation in the channel, it is possible to selectively divert (skim) this layer of plasma into a second channel, resulting in effective separation without moving parts or external forces.[9]

Cell Isolation

In many hematological tests, it is necessary to isolate certain cell lines. LOC devices are capable of doing this using different techniques. Size filtration strategies are useful in these cases, as they incorporate membrane systems with defined pore sizes that separate cells based on their size and deformability.[18,19] Another useful technique is affinity separation, where the inner surfaces of the microchannel are functionalized with capture molecules, simulating antigen/antibody interactions. When blood samples circulate inside the chip, cells expressing the corresponding antigen on their surface bind to the antibody and become immobilized, while the rest of the cells continue to flow.[15] Similarly, active techniques that use external force fields for cell manipulation are employed. Techniques such as magnetophoresis[8], electrophoresis[20,21] and acoustophoresis are useful for this purpose.

Sample analysis

Chip Flow Cytometry

Flow cytometry is a mainstay of cell analysis in hematology and related fields such as immunology, genetics, rheumatology, and cell biology; however, the instruments required to perform it are large, complex, and expensive.[22] µ-cytometry or flow cytometry on a chip seeks to replicate this powerful capability in a portable and affordable format.[23] In these systems, a cell suspension is introduced and a hydrodynamic approach (sheath flow) is used to align the cells in a single row, passing one by one through the analysis point. At this point, one or more light beams are collimated, where detectors simultaneously measure optical signals (forward light scattering, side light scattering, fluorescence) useful for hematological analysis.[24]

Its applications are wide-ranging;[25] includes differential leukocyte counting, identification of lymphocyte subpopulations (T, B, NK), and diagnosis and classification of leukemias and lymphomas through the detection of abnormal phenotypes.

Complete blood count on a chip

The complete blood count has historically been the cornerstone of blood analysis, which is why being able to perform a complete blood count on a chip (CBC) could be considered the "holy grail" of point-of-care hematology. Achieving this would have a major impact on primary care, medical care in disaster situations, low-resource settings, and emergency medicine. Although it is still an unachieved goal, several technologies are pointing toward its achievement:

- Electrical impedance counting on a chip: this technique seeks to miniaturize the Coulter principle, which is the standard for laboratory analyzers. The chip contains a microopening with electrodes on either side; as blood cells suspended in a conductive diluent pass through the opening, they interrupt the electric field and generate an impedance pulse, where the amplitude of the pulse is proportional to the volume of the cell. By setting the size threshold, the device is able to discern and quantify different cell populations.[26,27,28]
- Smartphone-based imaging cytometry: this technology harnesses the computing power of mobile phones by coupling them with compact, inexpensive optical accessories that turn them into powerful microscopes. The sample is loaded onto a disposable chip, which is monitored by the smartphone through images or videos of the cells as they flow or settle on the chip. A mobile app then uses image analysis algorithms to identify and quantify erythrocytes and leukocytes, as well as measure hemoglobin concentration through a colorimetric study of the lysed sample.[29,30] These systems have shown favorable results comparable to conventional analyzers, with the added advantage of transmitting results to a server for remote diagnosis.[31]
- Leukocyte differential: systems have been developed to perform HCC.[7,32,33] These vary in complexity, with the simplest being able to perform a three-part differential (lymphocytes, monocytes, and granulocytes) based mainly on size. More complex systems are capable, through complex algorithms and selective use of reagents, of further differentiating granulocytes into neutrophils, eosinophils, and basophils.

The potential of these technologies lies not only in the miniaturization of components, but also in the ability to integrate the entire analysis chain into a single, autonomous, and robust system. However, difficulties in achieving this integration mean that many promising LOC devices remain dependent on external devices, failing to achieve their objective.[30]

Similarly, these platforms have the potential to integrate quantitative cell count analysis with morphological studies. While conventional analyzers and blood smears are separate processes, in theory, LOCs can count cells while capturing high-resolution images of them.[34] By integrating these with artificial intelligence algorithms,[35,36] a LOC device would be able to perform both analyses simultaneously, reducing time and the need for samples.[37]

Challenges for Clinical Implementation

Despite the transformative potential of this technology, the transition from research laboratories to the patient's bedside is slow due to technical, regulatory, economic, and standardization challenges. The complexity of manufacturing, although mitigated by 3D printing techniques, has prevented high-quality, low-cost mass production. Microfabrication requires strict quality control and, therefore, expensive equipment.[3,10]

Robustness and reliability are key issues in the development of diagnostic tools. When moving from the macro to the micro

domain, these systems are prone to problems that do not affect conventional equipment, such as microchannel blockages by particles, cell aggregates, or air bubble formation.[38] Similarly, sensitivity can be affected, as decreasing the scale also decreases the number of molecules to be analyzed, which can affect the signal-to-noise ratio, requiring steps to amplify signals for the detection of low-concentration analytes.[3]

Multiplexing (the ability to perform multiple analyses in parallel) is a challenge for LOCs, as integrating several different assays with their respective reagents on a chip without cross-interference requires a more complex device, making development and validation difficult.[39]

The lack of standardization is another limitation in the adoption of these systems. There are no agreed formats, sizes, or types of fluidic, electrical, or optical connections, which means that each company develops its own patented platform, limiting interoperability and objective comparison of inter-device performance and preventing the emergence of component ecosystems.

CONCLUSIONS

Lab-on-a-Chip technology represents an advance in biomedical science with potential applications in hematology, enabling tests ranging from less complex ones such as a complete blood count to more complex ones such as flow cytometry. The ability to perform multiple analyses in parallel, its portability, and speed could greatly improve care in the care unit or at the patient's bedside, leading to early and timely diagnosis. However, component integration issues, manufacturing complexity, robustness, reliability, sensitivity, and lack of standardization remain real problems that are slowing down its development. Its development, although still slow, and integration with artificial intelligence techniques will improve diagnosis and treatment in hematological practice.

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CONFLICT OF INTEREST

None.

AUTHOR CONTRIBUTION

Conceptualization: Mayra Oliva Santos, Michael Ndlovu, Dinora Márquez Delgado.
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