COMMENTARY

Pushing the boundary of cancer diagnostics through microfluidic technologies

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Precision cancer therapy involves the clinical diagnosis of the molecular characteristics of cancer and thereby designing appropriate therapeutic strategy to treat the cancer. In the past decades, the discovery of cancer-specific biomarkers and the advancement of DNA technology has greatly aided the molecular characterization of cancers. Improvements in bioimaging techniques and artificial intelligence (AI)-assisted image analysis further improved the precision of cancer diagnosis. Nevertheless, clinical diagnostic technologies used to screen for effective treatment options lagged behind. The hurdles not only lay in the limitation of real-life sample supply for method development, but also in the lack of appropriate models to recapitulate the dynamic *in vivo* scenario. Furthermore, the lengthy and expensive trials to fulfil the legal requirements of clinical diagnostic tools deters most translational efforts of primitive discoveries.

THE WINDING ROAD

The earliest efforts to study cancer involves the random induction of tumor growth by exposing mice to tar.[1] Subsequently, culturing of cancer cells became feasible in both two-dimensional and three-dimensional settings. Technically, adherent monocultures were proven to be more robust in recapitulating the prominent cancer hallmark of cell proliferation. Conceptually, all cancers were thought as a single type of disease and thus one-fits-all synthetic drugs were robustly screened and applied. Because of the limitation of technical and conceptual advances, predicting, preventing nor combating cancer has neither been satisfactory in the last century.

A subsequent breakthrough in precision cancer therapy was the discovery of molecular biomarkers and the development of therapeutic drugs targeting these biomarkers. The first targeted therapy is tamoxifen, which binds the estrogen receptor in breast cancer and was approved by the FDA in 1977. However, the approved application was based on the tissue origin and phase of the cancer type, rather than the molecular target. As more and more molecular biomarkers were discovered and characterized, the conceptualization of distinguishing cancers based on their molecular subtypes instead of tissue origin gradually came into light. Subsequently, application of the PAM50 panel for molecular subtyping of breast cancer marked a new era for the diagnosis and prognosis of cancer.[2] Nevertheless, early diagnostic tests are restricted to either DNA-based PCR methods or tissue-based immunohistochemical methods. The wind turned around when the Human Genome Project brought down the costs of next generation sequencing (NGS) technologies, from which companion diagnostic companies emerged. NGS-based clinical diagnosis provides invaluable evidence for the administration of targeted therapy, but remains less useful for immunotherapy. To date, the decision to perform immunotherapy relies on tissue biomarkers such as tumor mutation burden (TMB) and PD-1/PD-L1, or less often, predictive biomarkers from liquid biopsy. The difficulty of applying liquid biopsy to cancer diagnosis mainly lies in the reliability and reproducibility of these tests to discriminate cancerous materials from normal materials and to obtain unambiguous readout signals despite the dynamic changes of the body fluids.

LIGHT AT THE END OF THE TUNNE

Microfluidic technologies, also commonly known as "lab-on-chip", are built on miniature devices in which micro-, even nano-, volumes are applied.

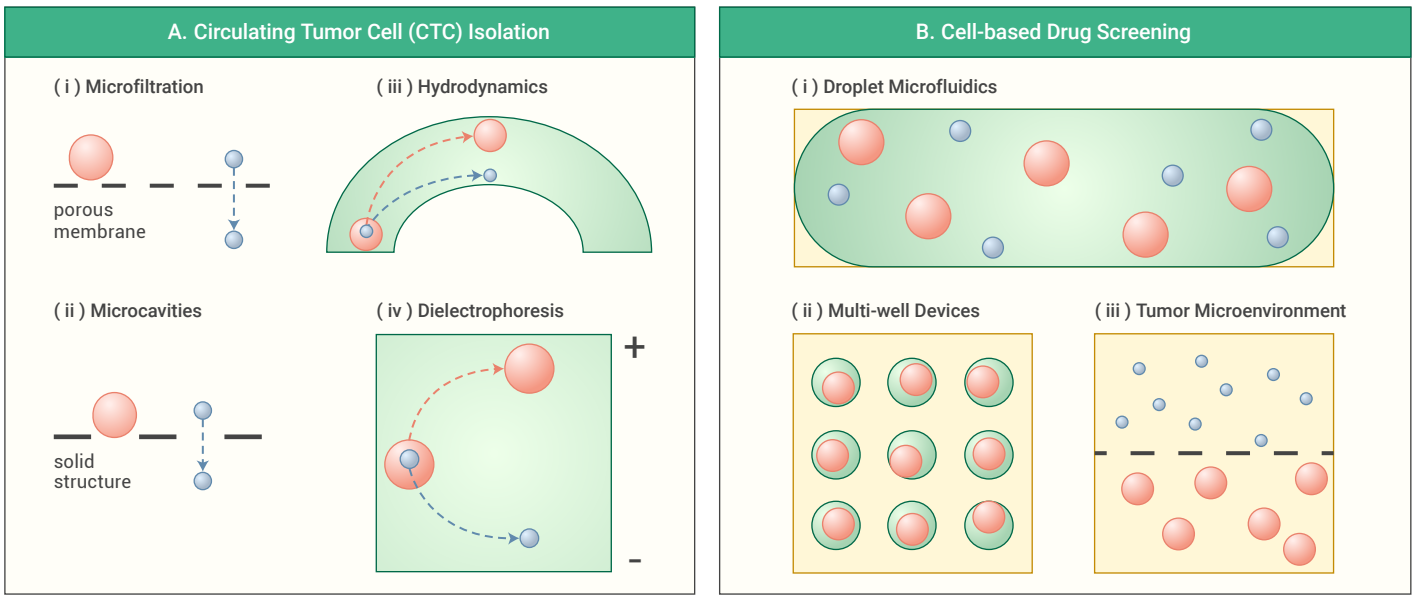

Figure 1. Examples of microfluidic technologies applied to (A) circulating tumor cell (CTC) isolation and (B) cell-based drug screening (A) Aside from antibody-based methods, reports of physical separation of CTCs include (i) microfiltration, which separates cells by a porous membrane that allows cells below certain size or with substantial deformability to pass through; (ii) microcavities, which exploits solid microstructures to separate cells; (iii) hydrodynamics, which separates cells by their flow dynamics in solution; and (iv) dielectrophoresis, which makes use of the electric charge of cells to separate CTCs. (B) Cell-based drug screening can be done using (i) droplet microfluidics, in which cells are suspended in water-in-oil droplets; (ii) multi-well devices, which contain microfabricated wells similar to a miniaturized multi-well plate; and (iii) microfabricated devices that allow the co-culture of various cells types in the tumor microenvironment in confined compartments to analyze the intercellular interactions.

Traditional microfluidic technologies include channel microfluidics and digital microfluidics, while paper microfluidics is also gaining steam in recent years. Channel microfluidics makes use of pressure pumps to generate the principal driving force, while the channel structures control fluid dynamics. Digital microfluidics mainly drives fluid movement by electrowetting on dielectric (EWOD) while dielectrophoresis contributes to solid-liquid processes. Alternatively, wetting is a major driving force in paper microfluidics, which diverts the flow by surface modification. While paper microfluidics mostly work on monophasic systems, channel and digital microfluidics work for both monophasic and biphasic systems composing of aqueous and oil phases. Because each system has their pros and cons, the choice is often determined by application.

According to the United States Food and Drug Administration (FDA), medical device submissions that use microfluidic technology has increased by more than 400% from 2013 to 2018, among which include devices for early cancer detection, but the present regulatory science gaps pose challenges for rational assessment and clinical approval.[3] The major bottlenecks of current technologies include effective translation from proof-of-principle experiments, scale-up device production, as well as the diversity of scientific principles applied to signal readout and/or data interpretation. Nonetheless, the FDA has recognized these challenges and is developing programs to overcome them. Hence, future consideration of microfluidics-based cancer diagnostic tests for clinical use is warranted.

Circulating tumor cell (CTC) isolation is one area of intense interest for microfluidics-based cancer diagnostic test. Since CTCs were isolated and characterized in 1958,[4] cancer prognosis based on CTC counts in peripheral blood have been extensively studied. The presence of CTCs often suggests poor prognosis and potential metastasis. Nevertheless, the miniscule number of CTCs in blood and the lack of selection biomarkers posed huge challenges. Effective diagnostic test development merely occurred after surface markers became available, which led to the development of antibody-based microfluidic devices to isolate CTCs. Because the antibodies are directly conjugated to the devices, these devices must be kept under the stringent preservation conditions of the antibodies, thus increasing the cost of shipping and storage. Moreover, the sample loading rate must be lowered to satisfy the antibody-binding kinetics, and hence, the robustness of these devices is compromised. Alternatively, pursuits to harness the physical separation of CTCs from blood include microfilters, microcavities, hydrodynamics, and dielectrophoresis (Figure 1A). However, the accuracy of these methods to specifically separate CTCs from numerous cell types in the blood are subject to skepticism. Therefore, the physical separation methods are considered to be more suitable for extraction of CTCs from blood, which are then confirmed by specific biomarkers. Until now, none of the published microfluidic approaches succeeded in obtaining FDA approval. For example, the CTC-iChip was once considered a promising technique, but soon lost momentum because of technical difficulties in biomarker selection as well as conceptual complications of cancer heterogeneity and cellular subtyping. Consequently, the first and only FDA-approved CTC detection device is CellSearch®, which uses antibody-conjugated magnetic beads to isolate CTCs. The failure of all development efforts in microfluidic technologies to isolate CTCs highlights the lack of technical advantage of microfluidic technologies in cell separation without equipment improvement to enable automatic and user-friendly interfaces that biologists and clinical scientists recognize. Furthermore, no technology can overcome the insufficiency of our biological understanding, although new technology aids in exploring novel biological phenomena, which in turn, feeds our biological understanding. For instance, the role of extracellular vesicles (EVs) in cancer has evolved from a mere metastasis mediator to key intercellular signaling messengers among various players in the tumor microenvironment and beyond.[5] However, the act of discriminating between EVs secreted by cancerous and healthy cells can be challenging itself, let alone the application of EV signals as predictive biomarkers. In this regard, microfluidic technologies offer the tools to isolate and analyze EVs using body fluids such as blood, saliva and urine in a minimally invasive manner.

Aside from CTC isolation, cell-based drug screening is another field of intense interest for cancer diagnostic development in the microfluidics field. Screening methods based on droplet microfluidics, multi-well devices and tumor microenvironment reconstruction have all been tried (Figure 1B). Moreover, preliminary data showed that screening of therapeutic cells is also feasible. However, these devices merely capture the cytotoxic events but provide no clue to the biological mechanism – for example, what is the genotype of the drug-resistant cells? Moreover, given that tumors are often dissociated into single cells to be applied, even the proportion of cancerous cells cannot be estimated in these devices without additional characterization steps. Hence, addressing the issues of tumor heterogeneity and phenotyping the resistant cells will be the next move. In this regard, the modular nature of microfluidic devices may provide the solution to performing single-cell NGS or single-cell mass spectrometry. However, the compatibility issue of the screening and characterization modules can be unsurmountable. Anticipated hurdles include solvent compatibility, reagent exchange, noise reduction, *etc*, and depending on the platform, additional sample cleanup steps might be essential – and any additional steps incur sample loss. Therefore, developing novel solutions to connect different modules is essential for this application.

In summary, past achievements of microfluidic devices to address cancer diagnostic issues has laid the foundation of basic working mechanisms and established some early prototypes. Future development efforts will focus on technical improvements, such as the miniaturization of instrumentation to achieve lab-on-chip, and workflow streamlining. Chip design standardization and rigorous personnel training will pave the road to more rigorous testing of devices and impact successful commercialization in later development stages. In terms of application, liquid biopsy analysis is predicted to predominate future development efforts, which may extend to longitudinal disease monitoring. Hence, we could foresee the emergence of microfluidic technologies in clinical cancer diagnostics.

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DECLARATION OF INTERESTS

The author declare no competing interests.