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Review

Droplet microfluidics for single-molecule and single-cell analysis in cancer research, diagnosis and therapy

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ABSTRACT

Genetic and functional heterogeneity of tumor cells represents major obstacles to cancer research, detection and effective treatment. In order to develop new therapeutic approaches in an era of personalized medicine, it is important to understand the functional characteristics of DNA, RNA, and proteins at the single-molecule level in individual cancer cells. Droplet microfluidics has emerged as a new tool that offers advantages in analyzing single molecules and single cells for high-throughput analysis with exceptional sensitivity. In this review, we highlight some recent reports that employed droplet microfluidics for cancer research, diagnostics, and therapeutics, and offer a view on future applications.

Contents

1. Introduction

Molecular and cellular heterogeneity in malignant tumors represents major hurdles to developing effective cancer therapy [1], so accurate analysis of individual biomolecules, such as DNA, RNA, and proteins, in cancer can help to improve understanding of the development, the progression and the classification of tumor types at the cellular and genetic levels, and that may further aid the development of new diagnostic and therapeutic approaches [2]. Currently, much of our knowledge of cancer biology is based on information obtained from bulk experiments using traditional population-averaged approaches, such as Western blotting, proliferation assays, DNA sequencing or cytotoxicity assays [3]. While these approaches have been useful for understanding features of cancer, key information, such as molecular distributions, functional variations, and drug-target interactions, may be hidden at the levels of the single molecule and the single cell. Also, isolation and analysis of single molecules and single cells in a high-throughput manner with large sample volumes are essentially impossible to implement using the traditional methods. There is also a pressing need for new diagnostics that can detect rare cancer

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biomarkers including circulating tumor cells (CTCs) that exist at low concentrations in a complex mixture of biological samples. In the past few decades, great emphasis has therefore been placed on developing powerful technologies that can dissect complex biological processes of cancer to the single-molecule or single-cell levels, including flow cytometry, atomic force microscopy, optical and magnetic tweezers, micro-needle manipulation, and single-molecule fluorescence spectroscopy [4].

In particular, droplet microfluidic devices recently emerged as powerful tools that allow precise encapsulation of single molecules or single cells within monodisperse microdroplets for high-throughput analysis with exceptional sensitivity. These devices utilize two immiscible fluids in microfluidic channels to create rapidly monodisperse water-in-oil (w/o) microspheres or nanospheres, called droplets [5], which serve as individual reaction vessels with volumes ranging from a few femtoliters (fL) to nanoliters (nL) (Fig. 1). Importantly, the droplets generated can be further manipulated for mixing [6], merging [7], diluting [8], splitting [9], sorting [10] and thermocycling [11] required for particular assay protocols. It has also been shown that these droplets are stable at ambient conditions for months, and cells can be cultured within the droplets for up to a week. Furthermore, various analytical approaches can be used for precise, high-throughput droplet analysis, including fluorescence detection (using photomultiplier tubes or avalanche photodiodes), mass spectrometry, electrochemical detection, and surface-enhanced Raman scattering [12]. These features of droplet microfluidic systems not only permit high-throughput analysis using minimal amounts of reagents but also reduce solute-surface interactions and cross contamination of reagents. Importantly, the droplet systems can achieve high detection sensitivity by concentrating the targets in small confined compartments [13]. These properties make possible the characterization and the isolation of individual cells for further analysis of genetic materials and proteins. Specifically, various assays, including gene and protein expression, enzyme kinetics, cell proliferation, differentiation and signaling, can be achieved using droplet microfluidics.

Several recent excellent reviews discussed aspects of droplet microfluidics, including device fabrications and manipulation, single-cell encapsulation and analysis, and pathogen detection [12,14–21]. In this review, we highlight the most recent advances in the use of droplet microfluidic technologies for single-biomolecule and single-cell analysis in cancer research, diagnostics and therapeutics. We also discuss advantages and potential challenges of droplet microfluidic systems, and finally future trends in the hope that we may stimulate new directions in applications in elucidating complex, heterogeneous biological processes, and developing new diagnostics and therapeutics.

2. Nucleic-acid analysis

Detection and analysis of nucleic acids (NAs: DNA or RNA) are fundamentally crucial for cancer research, diagnosis and therapy. Analysis of NAs at the single-molecule level is particularly important because it allows us to understand the genotypic characteristics of each molecule, so helping to identify abnormal genes. In this section, we summarize recent reports on the use of droplet microfluidics for detection, quantification and sequence analysis of NAs at the single-molecule level in cancer research.

Polymerase chain reaction (PCR), one of the most robust NA-amplification tools, has been coupled to droplet microfluidics to become what is commonly known as droplet digital PCR (ddPCR) [11,22–24]. In ddPCR, the sample is diluted and partitioned into millions of separated droplets. Each droplet contains, on average, one or no target sequence of interest [25]. After PCR amplification, the numbers of target-DNA molecules in the sample can be determined by counting the number of 'positive' *versus* 'negative' droplets. Detection of somatic mutations in tumor-associated genes can provide highly useful information for cancer diagnosis, treatment and prognosis. However, detecting a few copies (~1%) among many wild types is a challenge with conventional qPCR because of its limited sensitivity and high signal/noise ratio. ddPCR allows discrete counting and quantification of mutants to overcome such limitations. For example, using ddPCR, Pekin et al.

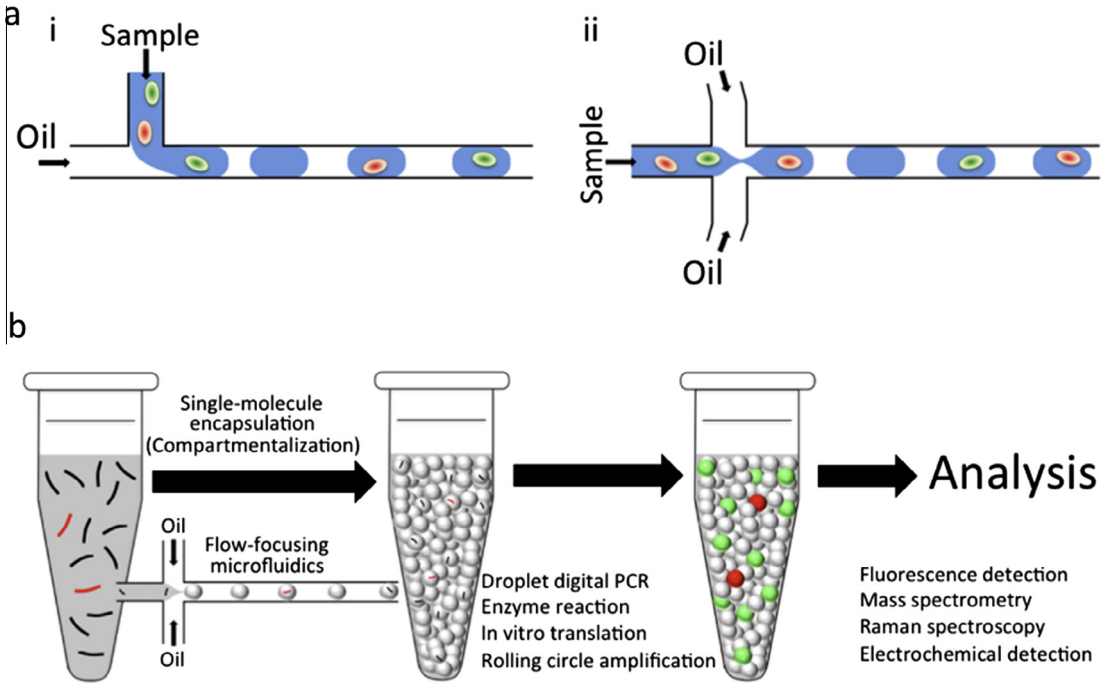

Fig. 1. Single-cell (a) and single-molecule (b) encapsulation and analysis using droplet microfluidics. (a) The two most commonly used approaches for droplet generation: (i) T-junction (ii) flow focusing. (b) Rare target molecules in complex biological sample are compartmentalized, amplified and analyzed using droplet microfluidics at the single-molecule level.