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DNA nanospheres with microfluidics: a promising platform for cancer diagnosis?

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> "…DNA nanospheres with microfluidics are a promising platform for circulating tumor cell isolaton and cancer diagnosis and prognosis."

The mortality rate from cancer in the USA reached more than 570,000 in 2012, or equal to more than 1500 Americans each day [1]. More than 90% of cancer deaths result from metastasis [2], hence methods for early-stage cancer detection are vital for the reduction of mortality and they will have a significant medical and social impact. However, according to the Early Detection Research Network of the National Cancer Institute, 'there are no validated molecular biomarker tests for the early detection of any cancer' [3], even though a handful of biomarkers have been approved by the US FDA.

It is known that, during metastasis, some cancer cells escape from the primary tumor through exfoliation, entering the bloodstream and becoming circulating tumor cells (CTCs) in the peripheral blood stream [2,4]. Some of these CTCs acquire the capability to colonize secondary sites, spreading tumors to distant organs [2,4]. Therefore, CTCs have the potential to serve as important biomarkers for early diagnosis of cancer metastasis [2,4]. An additional benefit is that CTC enumeration is less invasive than biopsy, while providing a quantifiable method for cancer diagnosis and prognosis.

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However, CTCs are extremely rare; the number of CTCs is in the order of one to 100 cells in 1 ml of blood [4]. For comparison, other cells in 1 ml of blood include approximately 5×10^9 erythrocytes, 7.8×10^6 leukocytes and approximately 3.5×10^8 platelets [5]. Therefore, detection of CTCs in peripheral blood is a needle-in-a-haystack challenge. Currently, CellSearch® (Veridex LLC, NJ, USA) is the only FDA-approved method, and it relies on antibody-coated magnetic particles for isolation of CTCs from blood [4]. One of its key drawbacks is low capture efficiency.

Microfluidics could play an important role in enhancing the capture efficiency of CTCs. Microfluidics is a multidisciplinary field that involves microfabrication to make devices, microflows to study fluids in the micron scale and a variety of other applications. One aim of microfluidics is to shrink an assembly of laboratory instruments onto a device the size of a computer chip, known as a 'lab-on-a-chip'. This is analogous to reducing a room-sized computer of the 1950s to a laptop today. Examples of commercially available microfluidics-enabled apparatuses include the Bioanalyzer 2100 from Agilent Technologies (CA, USA) and the BioMark™ HD System from Fluidigm Corp. (CA, USA).

The application of microfluidics to cell capture affords a number of advantages. First, cells in a sample must diffuse from the bulk of the solution to the solid surface before interacting with the immobilized antibodies, as in CellSearch. Diffusion distance is in the millimeter scale; however, the diffusion distance in microchannels is in the micrometer scale, significantly increasing the interaction opportunities between CTCs and capture agents. As a result, the capture efficiency should increase, along with decreased incubation time. Second, microchannels offer a significantly higher surface-to-volume ratio than macroscale reaction vessels. Consequently, the amount of capture agents on the surfaces could be in excess, driving their reactions with CTCs to completion. In addition, micropillars and other geometries may be created to enhance the surface areas. Third, microfluidics enables integration with other components, such as mixers and detectors. Mixing can propel CTCs to the proximity of channel surfaces, thus, enhancing capture efficiency. Detection components may allow the sorted cells to be further analyzed using on-chip biological assays, speeding up the process compared with conventional methods in which sorting is performed first, followed by analysis in another apparatus.

Applying microfluidics to CTC isolation has been recently demonstrated by a few research groups [6–12]. Using the same CTC definition as in CellSearch, Nagrath and coworkers isolated CTCs in blood samples from breast cancer patients; they showed that >50 CTCs/ml were enumerated in 80% of the cohort [6]. However, <7 CTCs/ml (or 50 CTCs/7.5 ml) were obtained in 88% of breast cancer patients when CellSearch was used [4]. These results suggest that the use of microfluidics is advantageous for CTC detection, with higher capture efficiency, as discussed above.

The capture agents most commonly used in CTC research are antibodies. An alternative to antibodies is aptamers. Aptamers are oligonucleic acid sequences, such as short DNA or RNA, with a high binding affinity to a target of interest. Aptamers possess binding capability comparable with that of monoclonal antibodies, and they have been shown to bind to a variety of targets, including small molecules, proteins, nucleic acids and cells [13,14]. In

contrast to antibodies, aptamers have the following advantages: complete engineering in test tubes rather than in animals; straightforward production by chemical synthesis through DNA synthesizers; and long-term stability during storage [13,14].

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Aptamers are selected through an approach called systematic evolution of ligands by exponential enrichment [15]. In their pioneering work, Tuerk and Gold found two RNA sequences with a dissociation constant of 5×10^{-9} M with T4 DNA polymerase [15]. Cell-based systematic evolution of ligands by exponential enrichment is a newer hybrid process developed to select aptamers for target cells [16]. A wide range of DNA aptamers have been selected and shown to have strong binding affinity with a variety of cells, including acute lymphoblastic leukemia, lymphoma and hepatocellular carcinoma cells, as well as bacterial pathogens [14,16].

Aptamers have now been incorporated with microfluidic devices for tumor cell isolation from unprocessed whole blood [12]. It was found that a compromise must be made between capture efficiency and cell purity by optimizing key parameters such as device geometry and flow rate. Capture efficiency is defined as the ratio of the number of target cells captured to the number of target cells introduced. Cell purity is defined as the ratio of the number of target cells captured to the number of total cells captured. For instance, capture efficiency decreases with increasing flow rate as a result of larger shear force at a higher flow rate, and reduced interaction time between cells and binding agents on channel surfaces. However, cell purity increases with increasing flow rate owing to the fact that cells bound nonspecifically are washed away with a stronger shear force at a higher flow rate. Therefore, an optimal flow rate exists that results in satisfactory capture efficiency with decent cell purity.

To further enhance capture efficiency, we recently reported the development of a platform combining DNA nanospheres with microfluidics for CTC isolation [17]. Each nanosphere consisted of a gold nanoparticle (AuNP) that was conjugated with a number of DNA aptamers. Conjugation between gold and thio-ended DNA has been well established, and attachment of multiple DNA oligonucleotides to a AuNP has been demonstrated for various applications [18,19]. Up to 95 aptamers were attached to each AuNP, forming a DNA nanosphere (AuNP-aptamers). Multiple aptamers on each AuNP were able to interact simultaneously with several receptors on a tumor cell, resulting in multivalent binding between AuNP-aptamers and the tumor cell. It was found that the binding affinity of AuNP-aptamers was 39-times higher than aptamers themselves alone. The capture efficiency of human acute leukemia cells increased from 49% using aptamers alone to 92% using AuNP-aptamers. Results show that combining DNA nanospheres with microfluidics are a promising platform for CTC isolation and cancer diagnosis and prognosis.

Future studies will include applying this platform to the isolation of CTCs from peripheral blood samples of patients with metastatic cancer. For example, CTCs have been enumerated for a study investigating the efficacy of a chemotherapy drug combination for pancreatic cancer. Preliminary results of this double-blind study indicate that our CTC counts correlate

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very well with the clinical data. We currently use the same CTC definition as in the FDA-approved CellSearch: nucleated cells lacking CD45 and expressing EpCAM and cytokeratin (CK) [4]. However, it is very likely that this definition of CTCs, that is *EpCAM*-positive/*CK*-positive/DAPI-positive/CD45-negative cells, is too narrow because those tumor cells with little or no *EpCAM* expression are overlooked. During metastasis, tumor cells undergo epithelial-to-mesenchymal transition, in which tumor cells change from epithelial to mesenchymal morphology to acquire increased migratory and invasive capabilities [2,20]. Importantly, during epithelial-to-mesenchymal transition, some cells lose biomarkers associated with epithelial origin, such as EpCAM [2,20]. As a result, affinity ligands are needed to isolate both *EpCAM*-positive and *EpCAM*-negative cells. From this perspective, aptamers are advantageous since a panel of aptamers can be selected for both types of CTCs.

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Biographies

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