Cancer Cells

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Self-Propelled Microrockets to Capture and Isolate Circulating Tumor Cells

*Weiwei Gao and Omid C. Farokhzad**

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The first report of circulating tumor cells (CTCs) can be traced back to 1869 when Thomas Ashworth, an Australian physician, observed tumor cells in the blood of a patient who succumbed to advanced metastatic cancer.[1] Since then cancer research has proved the critical roles played by CTC in the metastatic spread of carcinomas. In addition, CTCs contain key information of how tumor genotypes evolve during the cancer progression. Therefore, technologies that can yield purer CTC populations from blood samples are powerful tools to provide early and noninvasive detection of cancer, along with the prediction of treatment responses and tumor progression. Despite these significances, in reality, CTCs are extremely rare. A few CTCs shed from metastatic tumors mingle with the approximately 10 million leukocytes and 5 billion erythrocytes in 1 mL of blood, making their detection and isolation a formidable technological challenge.[2]

Given the relative paucity of CTCs in circulation, the existing technologies for their detection are based on two distinct steps: the enrichment of the CTCs from blood followed by the confirmation of CTCs in the purified sample. Approaches to enhance the enrichment or confirmation of CTC are an extremely promising area of investigation. CellSearch is the only FDA (U.S. Food and Drug Administration)-approved assay up to date and it is available for detection of CTCs from the blood of patients with breast, prostate, and colon cancers. The assay is based on immunomagnetic separation of epithelial cell adhesion molecule (EpCAM) positive cells from whole blood followed by analysis of immunostained candidate CTCs. AdnaTest which is still under development is based on immunomagnetic separation of CTCs followed by multiplex real-time polymerase chain reaction (RT-PCR) for quantification of tumor-associated RNA transcripts. The latter approach while theoretically more sensitive has the limitation of lacking quantitative or morphological information of CTCs; however, it may serve a complementary role in CTC detections.

Nanotechnology has enabled a variety of increasingly sensitive and reproducible techniques to detect human CTCs from blood samples.[3] For example, a number of strategies have been developed to isolate CTCs based on their distinguishable physical properties from circulating erythrocytes and leukocytes including size, density, charge, migratory properties, and specific cell-type-related characteristics such as melanocytic granules in melanoma cells. Meanwhile, tumor-associated antigens and immunoseparation methods by flow cytometery or immunomagnetic techniques remain the more definitive tool to discriminate CTCs from other cells in circulation. More recently, EpCAM-functionalized microposts within microfluidic channels have been developed to capture CTC under precisely controlled laminar flow conditions to potentially decrease the number of CTC loss and false negative results.[4]

In a recent issue, a team of researchers led by Liangfang Zhang and Joseph Wang at the University of California, San Diego (UCSD) reported a novel approach to capture CTCs.[5] In their work, a self-propelled "microrocket" was developed to selectively pick up CTCs as it navigated through a cell mixture and subsequently transported the captured cells to desired locations. Recent advances in nanotechnology have witnessed a number of self-propelled cargo transport platforms.[6] As illustrated by the microrocket developed by the UCSD team, they are likely to open many opportunities for simple, fast, and effective capture and isolation of biological targets from complex medium.

Markedly, the microrocket developed by the UCSD team offers a new example of how nanotechnology enables the assembly of multiple functionalities into nano- or microscale devices, which can be subsequently applied to overcome biomedical challenges. In this case, it is the clever and intricate assembly of energy harness, power generation, motion control, and biological functionalization that eventually leads to the use of microrockets to isolate CTCs.

The microrocket developed by Wang, Zhang, and coworkers consists of a rolled-up metal sheet with platinum, iron, and gold from the inside out. The inner platinum layer converts peroxide to oxygen and water. As the hollow center of the microrocket is tapered, the oxygen bubbles vent only through one opening and thus produce a unidirectional propelling force. The mid iron layer allows researchers to steer the microrocket by using an external magnetic field. The outer gold layer can be decorated with antibody molecules that target carcinoembryonic antigen (CEA) over-expressed in colorectal, gastric, and pancreatic cancers. The specificity of

[*] W. Gao, Prof. Dr. O. C. Farokhzad
Laboratory of Nanomedicine and Biomaterials
Department of Anesthesiology, Brigham and Women's Hospital
Harvard Medical School, 75 Francis Street, Boston, MA 02115 (USA)
E-mail: ofarokhzad@zeus.bwh.harvard.edu

the antibody allows microrockets to detect and capture the cells of interest and bypass the non-targeted cells. Assisted by an external magnetic field, microrockets can then tow the captured cells to a predetermined destination.

To achieve successful CTC isolations by using the microrocket, Wang, Zhang, and co-workers have overcome multiple obstacles. For example, a sufficient power supply for self-propelled microscopic machines in general is a challenging task, as fluids appear extremely viscous in microscopic scale. The challenge becomes formidable in this work since the microrocket needs to work in biological fluids and load CTCs of considerable sizes. To overcome this challenge, the team adopted a previously developed catalytic microtubular "jet engine".[7] The hollow shape of the engine minimizes the microrocket weight and a rolled-up design further stretches the surface-to-volume ratio of the engine. As a result, the microrocket acquires adequate power that allows it to travel at a relatively high speed (ca. 85 $\mu m\,s^{-1}$ in a diluted serum medium). Remarkably, this speed only dropped negligibly from 85 to 80 $\mu m\,s^{-1}$ in the same medium after loading a CTC. Wang, Zhang, and co-workers also faced another challenge to integrate bioactive antibodies without interfering with the "power system" of the microrocket. The team again took the advantage of the microtubular jet engine, which confined the catalytic sites solely to its inner surface. As a result, the outer surface was spared for chemical modifications, allowing the team to fabricate a layer of gold and link anti-CEA antibody by using common conjugation reactions. An additional challenge the team encountered is that the microrocket needs to be easily maneuvered, especially when it transports the captured CTCs. To better steer the microrocket in complex medium, the team increased the thickness of the iron layer to gain a larger magnetic force. Collectively, the microrocket developed by Wang, Zhang and coworkers has proved that microscopic machines powered by miniature motors can be biologically functionalized and transport large cellular cargos in biological fluids despite the high ionic strength and viscosity.

Cargo-bearing microscopic machines, as illustrated by the microrocket developed at UCSD, can find a great number of biomedical applications in vitro including dynamic material assembly, guided cargo transport, and motion-based molecular sensing. However, tremendous challenges exist prior to applying these micro- and nanoscale machines for in vivo applications, which may include more effective targeted drug delivery, stem cell recruit, and in situ tissue repair. Among these challenges, efficient and biocompatible power generations require particular attention. The microrocket developed by the UCSD team harnesses the energy from the H_2O_2 added to the medium. Clearly, for future in vivo applications, alternative energy sources have to be exploited. One approach is to engineer fuel-free nanomotors by generating propulsion through external electromagnetic fields. As one example, the same team at UCSD recently reported a "fuel-free" magnetically driven metal nanowires and achieved precise and tunable forward ("pushing") and backward ("pulling") locomotion.[8] Meanwhile, inspired by complex mechanical tasks performed by motor proteins in living life, scientists have used protein building blocks to assemble biological motors for powering and manipulating nanoscale components.[9] These developments provide many opportunities for future in vivo applications that call for exploration. We envision that with sustained developments, self-powered microscopic machines may facilitate a wide range of biomedical applications in advanced cargo delivery and tissue engineering.[10,11]

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