Programmable materials for efficient CTCs isolation: From micro/nanotechnology to biomimicry

Jianwen Hou | Xia Liu | Shaobing Zhou

1 Key Laboratory of Advanced Technologies of Materials, Ministry of Education, School of Materials Science and Engineering, Southwest Jiaotong University, Chengdu, China
2 School of Life Science and Engineering, Southwest Jiaotong University, Chengdu, China

Correspondence
Shaobing Zhou, Key Laboratory of Advanced Technologies of Materials, Ministry of Education, School of Materials Science and Engineering, Southwest Jiaotong University, Chengdu 610031, China. Email: shaobingzhou@swjtu.edu.cn

Jianwen Hou and Xia Liu contributed equally to this work.

Funding information
The National Natural Science Foundation of China (Nos. 51725303, 51903214, 21903065, 52033007), the Science and Technology Project of Sichuan Province (2020YFSY0017 and 2021YFH0125), Key Project of Sichuan Department of Science and Technology (No. 2020YFSY0017), and the Fundamental Research Funds for the Central Universities (Nos. 2682020CX03, 2682020CX54)

Abstract
Escaping from primary tumors and entering into blood flow, circulating tumor cells (CTCs) contain significant information for both the original tumors and metastasis mechanisms. CTCs detection has become an effective liquid biopsy of tumors and shows great promise in early cancer detection, disease monitoring, prognosis and personalized medicine. Despite the urgent need from clinics, CTCs isolation from blood is still a huge challenge due to their extreme rareness in blood. Well-defined micro/nanostructures and nature-inspired hierarchical architectures offer unique avenues to address this challenge by matching well with the special physical properties of CTCs to sort cells or forming local topographic interactions to strengthen cell adhesion, thereby improving the CTC-isolation performance. In this review, we first summarize researches on CTCs isolation with diverse micro/nanostructured substrates, which mainly include nanomaterials, microfluidics, DNA nanostructures, micromotors and in vivo detection devices. In sequence, various CTC-isolation biomimetic architectures, which are inspired by different natural creatures (e.g., viruses, cells, extracellular matrix [ECM], plants, and animals), have been highlighted. At last, remaining challenges and future perspectives in designing CTC-isolation platforms for clinical applications are also discussed.

KEYWORDS
biomimicry, CTCs, hierarchical architectures, liquid biopsy, micro/nanostructures

1 INTRODUCTION

Circulating tumor cells (CTCs), the cancer cells shedding from primary tumors and penetrating into the bloodstream, may cause new metastases which account for 90% of all cancer deaths.1–3 It has been proved that the number of CTCs can act as an important marker of tumor progression and metastasis.4 Additionally, CTCs could provide indicative valuation information for early diagnosis, therapeutic efficacy, as well as cancer prognosis.5,6 Compared with routine clinical analysis, which is typically realized by surgical removal or tumor biopsy to collect disseminated tumor cells, enumeration of CTCs from peripheral blood has emerged as a “liquid biopsy” and is readily operated in clinical application.7 Though urgently needed from clinics, the CellSearch® system is the only CTC-detection...
Various approaches have emerged for efficiently separating rare CTCs from blood. And these isolation strategies could be divided into biochemical methods and biophysical methods. Biochemical methods selectively isolate CTCs by recognizing unique biochemical markers (EpCAM, HER2, and EGFR) expressed on CTCs surface. Biophysical methods separate CTCs utilizing the physical property differences of CTCs and blood cells, such as cell deformability, size, density, and adhesiveness. Although these strategies have made some progress in separating CTCs, they still have drawbacks and limitations. Specifically, the expression level of biochemical markers on the tumor cells can be changed because of epithelial to mesenchymal transition (EMT) process and the heterogeneous nature of CTCs. Moreover, the size of some CTCs is found to be the same or even smaller than that of WBCs. To maximize the isolation efficiency, some researchers developed a variety of dual-model separation strategies by combining biochemical and biophysical methods. Zhao et al. reported a multifunctional microbead-based density gradient centrifugation method for efficient isolation of CTCs. They synthesized gelatin nanoparticle-coated silica microbeads (SiO$_2$@Gel MBs) and modified them with anti-EpCAM and anti-CD146 antibodies. Both EpCAM-positive and EpCAM-negative CTCs could be obtained using the developed optimized gradient centrifugation medium after the cells were incubated with SiO$_2$@Gel MBs, which could effectively increase the size and density of targeted cells. Jiang et al. developed a fluorescent microspheres-based separation platform by combining affinity-based microspheres attachment and size-based exclusion assay. CTCs were first size-amplified and simultaneously labeled by antibody-functionalized fluorescent polystyrene microspheres, then isolated by a pyramidal microcavity array. These combinations of biochemical and biophysical methods show superior cell isolation performances. However, it is still challenging to efficiently isolate and subsequently profile CTCs because of the extreme rarity of CTCs in the bloodstream.

Well-defined micro/nanostructures and nature-inspired hierarchical architectures may offer unique avenues to address these challenges. For biochemical method, nanostructured substrates can promote the formation of local topographic interactions between nanoscaled cell surface components (e.g., filopodia and microvilli) and the underlying nanostructure topography, which is beneficial to improve the capture efficiency. For biophysical method, the basic parameters of these structures can be precisely controlled to match well with the special physical properties of CTCs for sorting cells accurately. With the rapid advancement of micro/nanotechnology and biomimicry, various programmable materials based on well-defined micro/nanostructures and bio-inspired hierarchical architectures have been ingeniously designed for highly efficient separation of scarce CTCs from blood.

Herein, this review will focus on the development of programmable materials for CTCs isolation. First, diverse micro/nanostructured substrates, which mainly include nanomaterials, microfluidics, DNA nanostructures, micromotors and in vivo detection devices, will be highlighted. Subsequently, we will summarize recent progress in CTCs isolation achieved by various multiscale biomimetic architectures, which are inspired by different natural creatures, such as viruses, cells, ECM, plants, and animals. Lastly, remaining challenges and future perspectives in designing CTC-isolation platforms in clinical applications are also discussed.

2 | WELL-DEFINED MICRO/NANOSTRUCTURES FOR CTCS ISOLATION

With the rapid development of micro/nanotechnology, various micro/nanostructured materials have been constructed and show huge advantages in CTCs isolation. On the one hand, microstructures and nanostructures could match well with the microscale morphology and nanoscale components (e.g., microvilli) of the target cells, respectively. On the other hand, their high specific surface area can effectively increase the amount of recognition ligands on the substrate and the collision chances with target cells. In this section, we make an overview of the developed micro/nanostructured substrates, which mainly includes static capture materials (nanomaterials) and dynamic capture materials (microfluidics, DNA nanostructures, micromotors and in vivo detection devices) for efficient CTCs isolation. And these representative micro/nanostructured substrates and their morphological, working mechanisms, cell-isolation performances, and advantages/limitations are listed in Table 1.

2.1 | Nanomaterials

Nanomaterials with well-defined nanoscale topography, which has wide applications in tissue engineering

\[ \text{HOU et al.} \]

WELL-DEFINED Nanomaterials

medium after the cells were incubated with SiO$_2$@Gel using the developed optimized gradient centrifugation positive and EpCAM-negative CTCs could be obtained anti-EpCAM and anti-CD146 antibodies. Both EpCAM- and 5 billion erythrocytes per mL).9 Therefore, high-performance CTC-isolation materials are highly desirable for promoting biological studies and clinical applications.
| Category       | Substrate       | Principle     | C: capture yield; R: release yield; N/A: not available. | Ref. |
|----------------|-----------------|---------------|--------------------------------------------------------|-----|
| Nanomaterials  | Nanopillars     | Biochemical   | C: 45%–65%, N/A, 84%–91%                              | 21  |
|                |                 |               | C: 40%–70%, N/A, 89%                                   |     |
|                |                 |               | C: 70%                                                 |     |
| Nanofibers     |                  | Biochemical   | N/A, N/A, 96%, Improved cell affinity                 | 22  |
| Nanowires      |                  | Biochemical   | N/A, 96%                                               | 23  |
| Dendrimers     |                  | Biochemical   | N/A, N/A, Multivalent binding with enhanced stability  | 24  |
| Microfluidics  | Microcavity      | Biophysical   | 97%, N/A, 98%, Rapid and efficient                     | 33  |
|                | Ratchets         | Biophysical   | >90%, N/A, >98%, Label-free separation                 | 34  |
|                | herringbone-chip | Biophysical   | 91.8%, N/A, 95%, High-throughput                       | 35  |
|                | Membrane filter  | Biophysical   | ~90%, N/A, N/A, Negative enrichment of CTCs            | 37  |
| DNA nanostructures | 3D DNA network | Biochemical   | 84%, 99%, 66%, Multifunctional DNA strands             | 45  |
| DNA nanostructures | Aptamer         | Biochemical   | C: 80%, 95%, 78%–83%, Effective capture and specific release | 46  |
| DNA nanostructures | Tetrahedral DNA nanostructures | Biochemical | C: 87.4%, N/A, 91%, Highly ordered upright aptamers | 49  |
| Micromotors    | Platinum         | Biochemical   | C: 80%, N/A, > 90%, Simple and self-powered           | 55  |
| In vivo devices | 3D PDMS scaffold | Biochemical   | C: 40%, N/A, N/A, Effective detection of heterogeneous CTCs | 64  |
due to their excellent ECM-mimicking property, can offer a unique route to address these challenges in CTCs isolation.\textsuperscript{18–20} Compared with the flat substrates, nanostructured substrates have the following advantages: (1) nanostructured substrates have a higher surface area to contact with CTCs; (2) more capture agents can be loaded on the nanotextured substrates; (3) local topographic interactions of the underlying substrates and nanoscaled cell surface components can be enhanced by nanostructured topography. Thus, various nanomaterials have emerged for efficiently isolating CTCs.

Silicon-nanopillar (SiNP) arrays were pioneeringly applied to rare-cell capture by Wang et al. (2009).\textsuperscript{21} Densely arrayed SiNPs (100–200 nm diameter and 1–20 μm length) were produced onto silicon wafers by a wet chemical etching method, followed by grafting biotinylated anti-EpCAM onto the substrates modified with streptavidin (Figure 2A). The results revealed that the cells capture yield on the SiNP substrates (45%–65%) was 10-fold higher than that on the flat Si substrates (4%–14%). Compared with the rounded cells with few nanoscaled cellular protrusions adhered on the flat substrate, the cells on the SiNP substrates displayed many 100–200 nm
interdigitated cellular protrusions. This validated that enhanced local topographic interactions between SiNPs and nanoscaled cell surface components played a main role in vastly increasing the capture performance of cells. Different from the above reported vertically oriented SiNPs employed for CTCs enrichment, Zhang et al. applied ultralong horizontally packed inorganic nanofibers into CTCs detection (Figure 2B).22 Densely packed calcinated TiO$_2$ nanofibers (TiNFs) with the size regime of 100–300 nm in diameter could be prepared by electrospinning the mixture solution of polyvinyl pyrrolidone (PVP)/titanium n-butoxide (TBT). Then the streptavidin-coated substrates were freshly grafted with biotinylated anti-EpCAM for testing capture performance of cancer cells. The capture efficiency of cells on TiNF substrate was more than 18 times than that of the flat Si substrate after incubating with EpCAM-positive cells (BGC823 and HCT116). And over 45% of spiked cells (i.e., HCT116) maintained viable after isolating from the artificial blood samples. It was observed that target cells adhered on the TiNFs surface exhibited completely outspread pseudopodia. Moreover, the sizes of the TiNFs and the pseudopodia matched well to achieve sufficient contact and strong adhesion force, showing that the reformatative substrate/cell affinity was caused by the enhanced local topographic interactions between the cell components and the horizontally packed TiNFs. Combining horizontal with vertical ITO nanowire branches, Wang et al. further constructed a novel three-dimensional (3D) fractal nanobiointerface for efficiently capturing cancer cells.23 Higher efficiency (89% vs. 67%) and shorter time (35 minutes vs. 45 minutes) could be achieved using this fractal nanobiointerface when compared to ITO nanowire array without branches. What is more, environment scanning electron microscopy (ESEM) images showed that the cells on the fractal nanobiointerface exhibited most filopodia (Figure 2C), which could be attributed to the combined action of the vertical and horizontal nanowire branches. Nanoscale poly(amidoamine) (PAMAM) dendrimer, which is well known for its easy deformability and
the capability to preorganize/orient ligands, is thought to be an ideal mediator for forming multivalent binding effect, which was important to significantly improve the performance of CTCs isolation from peripheral blood. As shown in Figure 2D, the capture yield of cancer cells on the dendrimer-immobilized surfaces (70%) was greatly enhanced than that on the PEGylated surfaces (20%) for the simulated clinical samples, indicating that the multivalent binding effect of dendrimer played a key role in enhancing the cell detection sensitivity. This was mainly attributed to that the 3D structure of the dendrimer could effectively reduce the deformation energy (entropy) of dendrimer ligands to recognize their receptors, facilitating the formation of local multivalent binding. Moreover, capture efficiency could be increased by seven times by the addition of E-selectin, indicating that E-selectin-mediated cell rolling could synergistically cooperate with multivalent binding for substantially enhancing the tumor cell detection. Besides, many other types of nanomaterials, such as nanodots, nanosheets, nanofilms, and nanoparticles, have also been introduced to enhance capture efficiency.

2.2 Microfluidics

The microfluidic technique, which could precisely handle fluids in the microscale with high precision and sensitivity, has emerged as a promising tool for in vitro CTCs capture and isolation. Compared with conventional methods, microfluidic technique shows many advantages such as satisfactory sensitivity, automatic operation, and multiplexing capabilities. First of all, the microfluidic technique with large surface-area-to-volume ratios could trap rare CTCs using a very small sample volume in a highly sensitive way. Moreover, it could simultaneously achieve sample collection, isolation, and analysis using a one-step process, significantly reducing their processing time and improving chances of capturing viable CTCs. Last but not the least, many advanced nanotechnologies and different functional units can be facilely integrated into microfluidic platforms for multiple analysis of CTCs. Recently, various CTC-isolation microfluidic devices have been developed.

Microfluidic techniques mainly include “label-free strategies” and “affinity-based strategies.” The label-free strategies achieve CTCs separation utilizing the physical property differences between normal blood cells and CTCs. Size and deformability, which are considered as two commonly separation criteria, are usually employed in separating cells. Compared with the size of normal blood cells (2–20 μm), most epithelial-derived CTCs showed much larger cell size (14–26 μm). Based on the size difference, Hosokawa et al. constructed a microfluidic device to achieve size-based separation of CTCs. A nickel microfilter made up of 100 × 100 holes with 8–11 μm diameters was coupled to the microfluidic device (Figure 3A). The capture yield of CTCs was approximately 97% when the microfluidic device was applied to an artificial sample (10–100 lung carcinoma NCI-H358 cells per mL blood). Additionally, the viable cells account for approximately 98% after recovery, showing this platform could separate CTCs in a friendly way. Cell deformability is another approach to improve the selectivity of distinguishing CTCs from hematological cells. Due to having an enlarged nucleus, the nucleus-to-cytoplasm ratio and deformability of tumor cells are greater and less than that of leukocytes. Inspired by this, Park et al. designed a deformability-based microfluidic device composed of a matrix of tapered constrictions to separate CTCs (Figure 3B). The tapered constriction matrix was composed of 2048 columns and 32 rows. The pore size of the constrictions remained unchanged along each row and increased (from 2 to 18 μm) from the top to the bottom of the matrix. The infused cells diagonally proceeded over the funnel matrix. RBCs could only pass through the funnel top due to their high deformability. While CTCs and white blood cells (WBCs) proceeded diagonally before reaching an obstructive funnel row, then they proceeded horizontally to separate outlets. The capture yield of CTCs on this microfluidic device could reach up to >90% for artificial samples, which were prepared by mixing cancer cells into unprocessed normal blood samples. And the capture yield of CTCs from clinical samples with this chip could be 25 times greater than that with the conventional CellSearch system.

The affinity-based strategies mainly utilize the recognition between expressed specific biomarkers on the cancer cell surface (e.g., CD45 and EpCAM) and their corresponding aptamers or antibodies coated on the surface of microfluidic platform. And these strategies could be divided into positive selection which isolates CTCs by targeting biomarkers expressed on cancer cells (EpCAM, HER2, and EGFR) and negative selection, which removes hematopoietic cells by targeting antigens expressed on blood cells (CD45). One representative example of positive selection was the so-called CTC-chip, which was developed by Toner’s group. The CTC-chip contained 78,000 microposts within 19 mm × 51 mm active capture area. The dimensions of microposts and their gap were 100 μm × 100 μm and 50 μm, respectively. And every three rows of the equilateral triangular arrays were vertically shifted by 50 μm throughout the chip, maximizing the chip–cells interactions. And the CTC-chip could successfully recognize CTCs from the blood of all the patients (115/116) with various metastatic cancers (breast,
pancreatic, prostate, lung, and colon cancer). However, the interaction between CTC-chip substrate and target cells were limited by laminar flow. To overcome this problem and disrupt streamlines, they developed a high-throughput microfluidic by integrating herringbones or surface ridges into the device wall, which could maximize collisions between the antibody-modified microfluidic walls and target cells. The herringbone-chip consisted of a 1” × 3” glass slide fixed to a polydimethylsiloxane (PDMS) structure, which contained eight microchannels with patterned chevrons or herringbones on their upper surface. The height of the channels and grooves were 50 μm and 40 μm, respectively. The angle between the axis of the channel and the herringbones was 45°, and the principal wave vector was $2\pi/100 \text{ μm}$. And the herringbone-chip could identity CTCs from the blood of patients (14/15) with metastatic prostate cancer. This was mainly due to that the herringbone-induced microvortices could cause “shift” of cells travel by disrupting the laminar flow streamlines, when compared with, thus increasing the collision frequency between cells and chips than that of a traditional flat-walled microfluidic device (Figure 3C). While the positive selection is highly effective, it usually suffers from a biased population due to tumor heterogeneity and varying cell expression induced by epithelial to mesenchymal transition. In order to solve this problem, the negative selection method to isolate CTCs is proposed. It could selectively deplete WBCs by directly targeting their well-known overexpressed membrane biomarkers (e.g., CD45), thus achieving enrichment of CTCs from blood. Chu et al. developed a monolithic device using 3D printing technology by integrating a commercial membrane filter into immunoaffinity-based cell-capture microfluidic and for negative directly enriching CTCs from whole blood (Figure 3D). The cell-capture section consisted of 4–32 stacked microfluidic layers with 475-μm pitch and 175-μm height. Inside the microfluidic layers, 200-μm microposts were separated by 200 μm from each other and shifted by 10 μm in every row to enable sufficient collision with WBCs, which may follow different flowlines in laminar flow. Followingly, a membrane filter with 3 μm-pore size was used to filter the leukodepleted blood for eliminating anucleated blood cells. This device could eliminate the need of RBCs-lysing sample preparation step. And enriched tumor cells showed ~90% recovery rate after isolation from artificial samples, which were mixed with breast, prostate, or ovarian cancer cells. Moreover, prostate cancer CTCs could also be directly isolated from a 10 mL clinical whole blood sample by this device.
As a naturally occurring biomacromolecule, DNA has attracted considerable attention in CTCs detection due to some of their unique features, such as good affinity, high controllability, ease of synthesis, and inherent biocompatibility. Additionally, exquisite DNA nanostructures with various sizes, dimensions, and shapes can be formed due to the precisely programmable nucleotide base pairing of DNA. When used for CTCs isolation, DNA molecules (e.g., aptamer) have many advantages over antibodies: (1) aptamers are more stable under various harsh conditions and can be easily modified with various chemical groups; (2) aptamers are easier to recognize cell membrane markers due to their small size, which could enhance the recognition performance in identifying distinct subpopulations; and (3) cell release can be achieved under mild conditions (e.g., nuclease or the complementary strand of an aptamer), which is beneficial to maintain cell vitality.

As shown in Figure 4A, a 3D DNA network was prepared by rolling circle amplification (RCA) to capture and isolate CTCs. The diameter of DNA network ranged from <500 nm to 5 μm, which consisted of repeating adhesive aptamer domains, could extend as long as 20 μm into the solution and “wriggled” in 3D form. Significantly greater capture yield and higher cell purity were shown on the multivalent DNA networks than that on antibodies and monovalent aptamers. Additionally, cell release could be obtained after cleaving the DNA networks using restriction enzymes, which guaranteed the cell vitality for molecular analysis. Shen et al. designed a novel NanoVelcro Chip by grafting two DNA-aptamers onto silicon nanowires (SiNWs) in a stationary device setting.
It is noteworthy that invertible capture and release of CTCs could be realized just by toehold-mediated strand displacement triggered by DNA. And this paves a new way of achieving dynamic capture and release of cells in a noninvasive manner.

2.4 Micromotors

Micromotors, which could move autonomously by effectively transforming various energy sources into driving powers, have paved a new way for various biomedical applications, such as precision surgery, targeted delivery, and motion-based biosensing disease marker. For biosensing application, self-propelled micromotors could be used for capturing and transporting target substances by providing efficient fluid mixing. Inspired by this feature, Balasubramanian et al. pioneeredly applied micromotor to CTCs isolation. The micromotor was a reeling metal sheet composed of platinum, iron, and gold from the inside out (Figure 5A). The inner platinum layer could convert peroxide into water and oxygen, providing the driving force for the micromotor motion. The sandwiched iron layer, which was sensitive to an external magnetic field, could steer the motion direction of the micromotor. The outer gold layer could be readily functionalized with anti-carcinoembryonic antigen (anti-CEA) that specifically targets CEA, which were overexpressed in about 95% of pancreatic, gastric, and colorectal cancers. As a result, target cancer cells in both phosphate-buffered saline (PBS) and serum could be selectively captured and effectively transported by this micromotor. The micromotor moved at the rate of 85–80 μm/s before and after capturing cancer cells in serum environment, indicating the micromotor had a high towing force. The specific recognition of the CEA+ cancer cells to micromotors modified with anti-CEA mAb was proved by control experiments. Compared with the control groups (group 1: anti-CEA mAb-modified micromotors and the pancreatic cancer cells without antigen [CEA−]; group 2: SAM-modified micromotors without the mAb and the CEA+ pancreatic cancer cells), only the micromotors modified with anti-CEA mAb could trap the target CEA+ cancer cells. Furthermore, the micromotor could specifically identity target cancer cells from cell mixtures, offering a novel approach based on immuno-micromachine for in vitro CTCs detection without sample preprocessing. Carbon nanotube (CNT), which has a larger surface area and rich carboxyl groups due to its special hollow tube structures, is thought to be an ideal substrate for developing ultrafast supersensitive and biosensing systems. Inspired by this, Banerjee et al. developed a chemically powered CNT-based micromotor for specific CTCs isolation. The CNT-based micromotor...
system was constructed by loading Fe₃O₄ nanoparticles and transferrin (Tf) onto the inner and outer surface. And the CNT-based micromotor could efficiently propel itself by the thrust of O₂ bubbles formed by the decomposition of H₂O₂ catalyzed by Fe₃O₄ nanoparticle. Cell capture studies showed that Tf-CNT-Fe₃O₄ particles could efficiently trap TF⁺ cancer cells (~85%) from an artificial CTC-like suspension in just 5 minutes. And such self-powered micromotor provides a new approach for efficiently extracting CTCs from biological fluids.

### 2.5 In vivo devices

In vivo CTC detection technology, which can monitor the dynamic change of CTC number, may offer new tools for more in-depth cancer diagnosis and metastasis research. Nowadays there are mainly two types of in vivo CTC detection technologies: GILUPI CellCollector and in vivo flow cytometry (IVFC). GILUPI CellCollector mainly consists of a bioclean stainless steel medical wire, which is modified with antibody and placed in the vein for 30 minutes to enrich CTCs in vivo. And the detection rates of CTCs are 70% and 72% for early and late cancer stages, respectively. IVFC technology could monitor circulating cells within living animals in real-time based on various contrast principles, such as fluorescence excitation and emission, photothermal effect, photoacoustic effect. While these two technologies have made some progress in CTCs detection, their further applications are limited by that they are unable to capture typical CTCs from blood. Vermesh et al. developed a new technology named MagWIRE for intravascularly retrieving and enriching CTCs. MagWIRE, a flexible self-contained magnetic wire, could be encapsulated in a standard intravenous catheter and readily inserted into a superficial blood vessel due to its small diameter, flexibility, and biocompatible plastic sheath. First the CTCs in the blood were labeled by injected magnetic particles coated with antibody. And the magnetic fiber could capture the labeled cells when the entire blood volume flowed past. Then the bound targets could be released into the buffer for downstream analysis when displacing the magnets from the MagWIRE sheath. And viable model CTCs could be captured by the in vivo labeling and pass within 10 seconds. Moreover, the capture efficiency was improved by 10–80 times and 500–5000 times than a 5 mL blood draw and the commercially available Gilupi CellCollector, respectively. Cheng et al. developed a flexible 3D CTC-Net probe for in vivo intravascularly capturing CTCs. The CTC-Net, which consisted of a 3D elastic poly(dimethylsiloxane) (PDMS) scaffold composed of connected macro pores with 40–250 μm size, could accommodate a large number of immobilized antibodies and improve cell-substrate collision frequency. The CTC-Net was first functionalized with anti-EpCAM antibody and then stucked into a blood vessel using an indwelling needle after compression. Finally, a 3D “fishing-net” structure for capturing CTCs could be formed in the vessel
after the compressed CTC-Net fully unfolded (Figure 5B). Extremely low concentration of rare CTCs (less than 1 cell/mL) could be collected by the CTC-Net, permitting diagnosis of early-stage tumors. Moreover, captured CTCs could also be downstream analyzed due to the recompression and regain of the CTC-Net. The CTC-Net could successfully isolate dozens of CTCs from tumor-bearing rats before cancer metastasis, showing great potential in CTC-based early diagnosis, metastasis research, and targeted therapy of cancer.

3 NATURE-INSPIRED HIERARCHICAL ARCHITECTURES FOR CTCs ISOLATION

Natural selection has resulted in the evolution of numerous materials and structures, which have been optimized for a broad variety of functions. And this also provides a vast database of optimized solutions to technical problems with the survival of biological organisms, such as virus, cells, plants, and animals. In this section, various CTC-isolation biomimetic architectures inspired by nature creatures (nanoscale viruses, microscale cells and ECM, macroscale plants and animals) are systematically summarized. And these different morphological and unique features, working mechanisms, cell isolation performances, and advantages/limitations of representative biomimetic architectures were listed in Table 2.

3.1 Cells

As the basic structural element and functional unit, cells play key roles in various fundamental biological processes, such as the immune invading capability of red blood cells (RBCs), adhesive property of platelets to surgical sites and CTCs, and virus phagocytosis by host cells. Inspired by these, a variety of biomimetic platforms mimicking cell morphologies or utilizing the functions of cell membranes have been exploited for CTCs isolation.

Inspired by leukocytes morphologies, Meng et al. developed a unique micro/nano hierarchical biointerface by combining chemical vapor deposition (CVD) and thermal oxidation. As shown in Figure 6A, the hierarchical biointerface, which was assembled by leukocyte-inspired...
**TABLE 2** Summary of nature-inspired hierarchical architectures for CTCs isolation

| Category          | Object                      | Principle   | Yield | Purity | Viability | Advantages/Limitations                                         | Ref. |
|-------------------|-----------------------------|-------------|-------|--------|-----------|------------------------------------------------------------------|------|
| Cells             | Leukocyte                   | Biochemical | C: 62.3% | N/A    | N/A       | Micro/nano hierarchical biointerfaces                             | 82   |
|                   |                             | C: 90.3%    |       |        |           |                                                                  |      |
|                   |                             | R: 99%      |       |        |           |                                                                  |      |
|                   |                             | C: 91.77%   |       |        |           |                                                                  |      |
|                   |                             | C: 91.2%    |       |        |           |                                                                  |      |
| Cancer cell       |                             | Biophysical | N/A   | 99%    |           | Cell Imprinting without antibody                                 | 83   |
| Platelet-leukocyte hybrid membrane | Biochemical | 96.98% | N/A    | 97.6%   | Fluidity-enhanced multivalent binding                            | 88   |
| Leukocyte membrane nanovesicles | Biochemical | N/A | 97.6% | N/A      | Rigid-flexible DNA nanoclaws                                    | 89   |
| Viruses           | Virus infection             | Biochemical | >90% | 85%    | N/A       | Rigid-flexible DNA nanoclaws                                    | 93   |
| M13 bacteriophage | Biochemical                | >45%        |       | >84%   |           | Nanotentacle-based structure                                    | 95   |
| ECM               | Magnetic dynamic microinterface | Biochemical | C: 85% | 84.5% | N/A       | Natural biofeedback mechanism                                   | 104  |
| Plants            | Rose petals                 | N/A         | 85%   | 98%    |           | High cell purity and viability                                  | 105  |
| Straws            | Biochemical                 | C: 84%      | N/A   | 99%    |           | In situ manipulation of CTCs                                    | 106  |
| Animals           | NanoOctopus                 | C: ~95%     | 96.7% | 94%    |           | Ultrahigh sensitivity and specificity                            | 107  |
| Octopus-chip      | Biochemical                 | C: 89.4%    | N/A   | 96%    |           | High capture efficiency and viability                           | 108  |

C: capture yield; R: release yield; N/A: not available.
Viruses

WBC hybrid membrane (HM-IMBs) and modified them (ure6B). The cell-imprinted structures were fabricated by combining boronate affinity and cell imprinting (Figure 6D).83 The cell-imprinted structures were fabricated by combining boronate affinity and cell imprinting (Figure 6D). Then the natural leukocyte membrane used for preparing biomimetic nanointerface had various biological functions. First of all, recognition ligands can be laterally rearranged for high-affinity binding due to the membrane’s fluidic nature. And recruitment and accumulation of other targeted biomarkers and recognition ligands will occur after a binding event takes place, achieving synergistic multivalent target binding. Second, nonspecific adhesion of blood cells can be greatly reduced because of the cell-resistant property of the leukocyte membrane.80 Third, cell damage caused by interfacial collisions can be greatly reduced by the soft yet flexible nanovesicle layer, which serves as a cushion between the capture substrate and target cell. In addition, a capture platform was prepared by interrogating a DLD-patterned microarray onto the microfluidic chip (DLD-Chip) with size-dictated interaction. This microdevice functionalized with capture ligands could isolate highly pure target CTCs with high capture efficiency through increasing substrate–CTCs collision and decreasing substrate–blood cells interaction. Compared with a chip functionalized with monovalent aptamer, this fluidic biomimetic nanointerface showed significant affinity increase by four orders of magnitude and sevenfold higher capture yield in blood. Moreover, low nonspecific adhesion of blood cells and high CTCs viability (97.6%) could be achieved by this soft nanointerface. And this fluidity-reinforced multivalent binding strategy showed high potential in clinical applications as the chip could successfully detect CTCs in all cancer patient samples (17/17).

3.2 | Viruses

As a natural phenomenon in biological systems, viral infection of mammalian cells is a complex process driven by the synergistic effects of multiple biomolecules for efficient recognition.91,92 Inspired by the identifying mechanism of virus infection, Wang et al. developed virus-mimetic magnetic DNA nanoclaw (MDNCs) for specifically targeting and efficiently isolating CTCs (Figure 7A).93 The MDNCs were prepared by RCA, which was followingly hybridized with a specific part of the tandem DNA strand. Combining the flexibility of single-strand DNA (ssDNA) with the rigidity of double-strand DNA (dsDNA), MDNCs showed a high capture efficiency (82.3% ± 7.1%) to MDA-MB-231 cells,
which was attributed to the loaded cell-targeting multiple antibodies (Abs). In addition, high capture efficiency and specificity from clinical samples could also be achieved by the rigid-flexible MDNCs. M13 bacteriophage is a well-known virus for its large surface area (18,700 nm²), which has a width of 6 nm and length of ∼900 nm. And the virion is mainly composed of the 2700 copies of the pVIII coat protein, which account for 87% of virus mass and 99% of virus surface. Inspired by this, M13 bacteriophage-based “nanotentacle”-structured magnetic particles were prepared for isolating CTCs from whole blood. As shown in Figure 7B, the M13-bacteriophage immobilized to magnetic particles was modified with PEG and further conjugated with tethered monoclonal antibodies specific for the epidermal receptor 2 (HER2). The nanotentacle-structured magnetic particles showed outstanding sensitivity to target
3.3 ECM

ECM is composed of dynamic meshwork of cross-linked proteins and plays an important role in various cellular processes such as adhesion, migration, survival, and differentiation. As the composition and organization of the ECM change with time which are caused by various external or internal biological stimuli, the dynamically changeable cell-ECM interactions can provide various architectural, mechanical, and biochemical signals for controlling relevant cell behaviors. As mimics of the natural ECMs, dynamic biointerfaces are an emerging frontier in the field of biomaterial science and has been widely applied in cell-based fundamental studies and tissue engineering. Recently it is found to be an ideal tool for the capture and release of CTCs. Pan et al. developed a novel dynamic biointerface by combining mussel-inspired peptide mimics and invertible catecholboronate chemistry. Biomimetic peptides containing a catechol-containing sequence and a cell-binding sequence at each end was designed. The dynamic biointerface was obtained by binding mussel-inspired peptides to a substrate grafted with polymers containing phenylboronic acid (PBA) via catechol–boronate interactions. The resultant biointerface could capture MCF-7 cells with high selectivity (98.1% ± 0.7%) from the mixture (1:1) of MCF-7 cells and HL60 cells. Moreover, 99% of the MCF-7 cells could be released in a sugar-responsive manner and the dynamic biointerface exhibited excellent reusability for capture and release of tumor cells. Subsequently, they constructed a magnetic dynamic microbiointerface by grafting the biomimetic peptide onto magnetic microbead functionalized with PBA-containing polymer brushes. As shown in Figure 7C, magnetic microbeads were first modified with 3-methacryloyloxypropyltrimethoxysilane (MPTS), which could initiate the polymerization of hydrophilic monomer 2-hydroxyethyl acrylamide (HEAA) and saccharide-sensitive monomer acrylamidophenylboronic acid (AAPBA). The hydrophobic HEAA polymer brushes could guarantee the high purity of targeted cells as it had good resistance to nonspecific cell adhesion. The dynamic magnetic platform not only showed selective cancer cell capture (~85%) and sugar-responsive release of them (> 93%) in cell culture medium, but also could isolate a decent number of target cells (~23) from artificial CTC blood samples (1 mL spiked with 100 cancer cells). In view of the high capture efficiency and remarkable selectivity, the ECM-inspired dynamic biointerfaces with reversible property and programmable features showed high potential for developing rare-cell detection platforms.

3.4 Plants

As mentioned above, nanostructures have been proved to play an important role in improving CTCs capture performance. In view of the fact that the CTCs sizes are on the micrometer length scale, microstructures with nanotextures are thought to have the potential for further enhancing cell capture. On the one hand, more antibodies can be immobilized on the microstructured topographies with a larger surface area, thereby improving the capture performance of CTCs by increasing the collision chances between antibodies and membrane receptors. On the other hand, microscale topographic structures could offer better size match and physical contact with the targeted cells. Therefore, intricately hierarchical structures with both microstructures accommodating cell size and nanostructures matching the cellular pseudopods have great potential in further enhancing CTCs capture.

Various plants in nature (such as straws, cactaceae, and rose petals, etc.) have hierarchical structures with multiple levels. Inspired by nature, Dou et al. fabricated a novel 3D hierarchically structured surface by mimicking the micro- and nanostructures of natural rose petals. As shown in Figure 8A, an imprint pattern-transfer technique was used to construct the rose petal-mimicking hierarchical surface, which was composed of microconcave/convex structures with 20–30 μm depths/heights and nanofolds with 500–600 nm widths. Then the hierarchical micro/nanostructures were grafted with anti-EpCAM by a disulfide bond for capturing CTCs and releasing cells in a stimulus-responsive manner. The cell capture ability of these hierarchical substrates was six times higher than that of polydimethylsiloxane (PDMS) surfaces functionalized with anti-EpCAM under static conditions at the concentration of 100 cells/mL. Compared with flat polydimethylsiloxane (PDMS) surfaces functionalized with anti-EpCAM. Moreover, high release efficiency (85%) and ideal cell viability (98%) could be obtained after the substrates were treated with glutathione (GSH). Inspired by the structure of straws, Yang et al. constructed a microfluidic device integrated with hierarchical microtubes for simultaneously capturing and chemical manipulating cancer cells in situ (Figure 8B). The hierarchical spiky microstraw arrays (HS-MSA) were prepared by repeated steps of material depositions and etching, followed by nanospikes growth under hydrothermal conditions. And high capture efficiency (~84%) and strong specificity could be obtained using the anti-EpCAM-functionalized 3D
FIGURE 8 Various plants/animals-inspired CTC-isolation platforms. (A) Bioinspired hierarchically structured surfaces mimicking the nano- and microstructures of natural rose petals for efficient CTCs isolation. Reproduced with permission. Copyright 2017, American Chemical Society. (B) Microfluidic device integrated with hierarchical spiky microstraws for efficiently capturing and in situ manipulating cancer cells. Reproduced with permission. Copyright 2019, Wiley-VCH. (C) Magnetic microparticles functionalized with multivalent aptamers acting as “Regenerative NanoOctopus” for efficiently capturing target cells from whole blood. Reproduced with permission. Copyright 2019, American Chemical Society. (D) Octopus-bioinspired multivalent aptamer-functionalized DLD-patterned microfluidic chip (AP-Octopus-Chip) for improving CTCs isolation. Reproduced with permission. Copyright 2019, Wiley-VCH

HS-MSA micro/nanostructure for capturing cancer cells. After the HS-MSA was integrated into a microfluidic device, extracellular drug could be delivered to the captured cells in situ with ideal dose, spatial, and temporal controls through the inner fluidic conduits of hollow microstraws, achieving precise chemical regulation of the extracellular microenvironment. The microfluidic device could simultaneously achieve both CTCs detection and cell manipulation in situ, offering a new tool for high-throughput screening drugs in personalized therapy.

3.5 Animals

Many animals in nature have evolved special structures for preying. For example, multivalent trailing tentacles have been evolved by the octopus for hunting. These long tentacles containing repeated adhesive units (e.g., mucus) could extend into the flow and maximized the touch with flowing targets, thereby enhancing the capture efficiency. Inspired by this, Chen et al. developed a so-called “NanoOctopus” device for capturing cells from blood (Figure 8C). The NanoOctopuses consisted of a magnetic microparticle (MP) acting as octopus head and grafted long single-stranded DNA sequences mimicking tentacles. And more than 500 repeating “suckers” of DNA aptamer sequences contained in each DNA sequence could specifically recognize target biomarker proteins on cell membranes. On the one hand, the binding affinity could be effectively improved by multivalent binding between the target cells and the multimeric aptamer. On the other hand, the aptamer accessibility to cell receptors and steric hindrance from the particle surfaces could be respectively increased and decreased by the long tentacle DNA strands (∼2.7 μm). The capture yield and purity of target cells in whole blood were 88% ± 6% and 96.7% under the condition that the concentration of WBCs was 5000 times higher than that of CCRF-CEM cell. The results showed that the NanoOctopuses could be successfully applied to clinical samples, which was verified by that the NanoOctopuses could isolate PTK7-expressing cancer cells (100% detection) from 33 AML patients.
Song et al. developed an aptamer-tailed octopus chip (AP-Octopus-Chip) by combining octopus-mimicking nanostructures with a size-dictated immunocapture chip for improving capture efficiency. They first synthesized the AuNPs and used the freeze-thaw method to immobilize thiolated aptamer on them. On the one hand, local topographic interactions and the aptamer stability could be improved by AuNP surface modified with the aptamer, which offered a rough interface and protected DNA probes from nuclease degradation. On the other hand, excess thiol molecules with good biocompatibility could disrupt the Au–S bonds readily, achieving the effective release of target cells with high cell viability for further analysis. Then a DLD-patterned microfluidic chip was integrated with the multivalent aptamer-functionalized AuNPs. As shown in Figure 8D, CTCs had full access to AuNP-SYL3C modified-micropillar as they could cross streamlines based on the DLD principle. While blood cells had low contact chances as they stayed within the initial flow streamline due to their smaller size than CTCs. The results showed that the multivalent aptamer–antigen binding affinity and the capture yield were improved 100-fold and more than 300% than that of a chip modified with monovalent aptamer. Moreover, up to 80% release efficiency and 96% cell viability could be obtained using a thiol exchange reaction, showing this method was fully compatible with downstream analysis.

4 CONCLUSIONS AND PERSPECTIVES

In summary, we have reviewed various advanced material interfaces based on well-defined micro/nanostructures and nature-inspired hierarchical architectures for CTCs isolation. Despite the promising results achieved by these interfaces, most of them still stayed in the laboratory. And there are many challenges to be addressed before these systems can be applied clinically.

First, the heterogeneity among CTCs can not be ignored as most systems capture CTCs based on the affinity strategies. The expressed biomarkers on the CTCs membrane vary between different cancers and patients. Currently, EpCAM is a widely used targeted receptor while it is not expressed in some malignant cells, leading to false negatives or positives. Some researchers present the cocktail of the multiplex antibodies method to overcome this obstacle, while their clinical application is severely limited by the expensiveness of antibodies and complexity of surface modification. Future work may be focused on the screening of new affinity reagents, which are stable, cheap and can recognize a broad spectrum of CTC phenotypes.

Additionally, the label-free methods seem to have greater potential for clinical application since they do not need any affinity reagents. The purity is the main concern of these methods as the physical properties of some blood cells are similar to that of CTCs. Fox example, Marinucci et al. reported that CTCs had the same or smaller size than leukocytes. This will also inevitably introduce false positives results. Further efforts need to be devoted to searching for the unique properties of CTCs, which are completely different from that of other cells.

Finally, nature-inspired multiscale structures, which can achieve structural and functional integrity, may open new avenues to address the challenges of CTCs isolation. Although promising results and some progress have been achieved by these nature-inspired hierarchical materials in the light of detection sensitivity and capture efficiency, there is still a long way to go before the biomimetic technology comes into practical application. Current researches are mainly concentrated in improving the capture efficiency by just simulating the morphologies of nature creatures, while how to guarantee the purity and viability of cells is often ignored. Thus, future endeavors should focus on the design of integrated multifunctional intelligent materials for CTCs isolation.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

The authors acknowledge the financial support of the National Natural Science Foundation of China (Nos. 51725303, 51903214, 21903065, 52033007), the Science and Technology Project of Sichuan Province (2020YFSY0017 and 2021YFH0125), Key Project of Sichuan Department of Science and Technology (No. 2020YFSY0017), and the Fundamental Research Funds for the Central Universities (Nos. 2682020CX03, 2682020CX54).

ORCID

Shaobing Zhou https://orcid.org/0000-0002-6155-4010

REFERENCES

1. J. P. Thiery, H. Acloque, R. Y. Huang, M. A. Nieto, Cell 2009, 139, 871.
2. C. L. Chaffer, R. A. Weinberg, Science 2011, 331, 1559.
3. V. Plaks, C. D. Koopman, Z. Werb, Science 2013, 341, 1186.
4. J. S. De Bono, H. I. Scher, R. B. Montgomery, C. Parker, M. C. Miller, H. Tissing, G. V. Doyle, L. W. Terstappen, K. J. Pienta, D. Raghavan, Clin. Cancer Res. 2008, 14, 6302.
5. M. Yu, A. Bardia, N. Aceto, F. Bersani, M. W. Madden, M. C. Donaldson, R. Desai, H. Zhu, V. Cumaillas, Z. Zheng, Science 2014, 345, 216.
6. M. Poudineh, E. H. Sargent, K. Pantel, S. O. Kelley, Nat. Biomed. Eng. 2018, 2, 72.
7. Z. Shen, A. Wu, X. Chen, Chem. Soc. Rev. 2017, 46, 2038.
8. K. C. Andree, G. van Dalum, L. W. Terstappen, Mol. Oncol. 2016, 10, 395.
**AUTHOR BIOGRAPHIES**

**Jianwen Hou** received his BS degree from Northwest University in 2010 and obtained his PhD degree under the supervision of Prof. Jinghua Yin from Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, in 2016. From 2017 to 2019, he carried out postdoctoral research with Prof. Itamar Willner at The Hebrew University of Jerusalem. Subsequently, he joined Southwest Jiaotong University as an associate professor. His current research is mainly focused on the development of polymer-based platforms for disease theranostics and tissue regeneration.

**Xia Liu** received her BS degree from Sichuan Normal University in 2010 and PhD degree from Changchun Institute of Applied Chemistry, Chinese Academy of Sciences in 2016 under the supervision of Prof. Zhenxin Wang. During 2014–2015, she studied in The University of Liverpool as a joint PhD student under the supervision of Dr. Haifei Zhang. During 2017–2019, she worked with Prof. Itamar Willner at The Hebrew University of Jerusalem as a Postdoctoral Research Fellow. After that, she joined Southwest Jiaotong University as an Associate Professor. Her research focuses on surface modification, DNA-based smart materials and single-molecule mechanics.

**Shaobing Zhou** received his BS degree in polymer chemistry from Sichuan University (1996) and his PhD in organic chemistry from Chengdu Institute of Organic Chemistry, Chinese Academy of Sciences (2003). He was promoted in 2005 to full professor in the School of Materials Science and Engineering, Southwest Jiaotong University. His research interests include the synthesis and characterization of biodegradable polymers, drug-delivery systems, shape-memory polymer composites, and electrospun biodegradable polymer fibers as a tissue engineering scaffold. He is the author or coauthor of more than 150 refereed articles and 15 Chinese patents/patent applications. He has been cited over 6000 times and has an h-index of 50.

**How to cite this article:** Jianwen Hou, Xia Liu, Shaobing Zhou. *VIEW*. 2021;20200023. 
https://doi.org/10.1002/VIW.20200023