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TRENDS

Nanofiber-integrated miniaturized systems: an intelligent platform for cancer diagnosis

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Abstract
Cancer diagnostic tools enabling screening, diagnosis, and effective disease management are essential elements to increase the survival rate of diagnosed patients. Low abundance of cancer markers present in large amounts of interferences remains the major issue. Moreover, current diagnostic technologies are restricted to high-resourced settings only. Integrating nanofibers into miniaturized analytical systems holds a significant promise to address these challenges as demonstrated by recent publications. A large surface area, three-dimensional porous network, and diverse range of functional chemistries make nanofibers an excellent candidate as immobilization support and/or transduction elements, enabling high capture yield and ultrasensitive detection in miniaturized devices. Functional nanofibers have thus been used to isolate and detect various cancer-related biomarkers with a high degree of success in both on-chip and off-chip platforms. In fact, the chemical and functional adaptability of nanofibers has been exploited to address the technical challenges unique to each of the cancer markers in body fluids, where circulating tumor cells are prominently investigated among others (proteins, nucleic acids, and exosomes). So far, none of the work has exploited the nanofibers for cancer-derived exosomes, opening an avenue for further research effort. The trend and future prospects signal possibilities to strengthen the implementation of nanofiber-miniaturized system hybrid for a next generation of cancer diagnostic platforms both in clinical and point-of-care testing.

Keywords Cancer diagnosis · Nanofibers · Electrospinning · Miniaturized analytical systems · Point-of-care diagnostics · Liquid biopsy

Introduction

Cancer has emerged as the second leading cause of mortality worldwide in which the World Health Organization has estimated 9.6 million deaths in 2018, and most cases have been occurring in low- and middle-income countries [1]. Early detection and effective treatment remain the key factors for increasing survival rates of cancer-diagnosed patients. The development of cancer diagnostic tools with high accessibility, applicability outside of well-equipped clinical settings, low complexity, and high affordability will facilitate screening and early diagnosis, and will provide needed information for further cancer managements by physicians (Fig. 1). Microfluidic-based analytical systems hold a significant promise for developing such diagnostic devices [2] as multiple steps can be integrated into a compact portable single device, that is suitable for system automation. The low sample and reagent consumption lead furthermore to minimal assay costs.

Cancer markers refer to any substances that are generated by cancerous cells or other cells in response to cancer. Their abnormal levels or distinct molecular profiles of cancer markers in comparison to healthy individuals could potentially signal the existence of cancer and be useful for cancer management. In recent years, progress in identifying cancer-derived markers in body fluids has revolutionized cancer diagnosis, shifting from conventional surgical biopsy to “liquid biopsy” [3]. Sampling and analysis of cancer markers from body fluids such as blood, urine, and saliva have gained more

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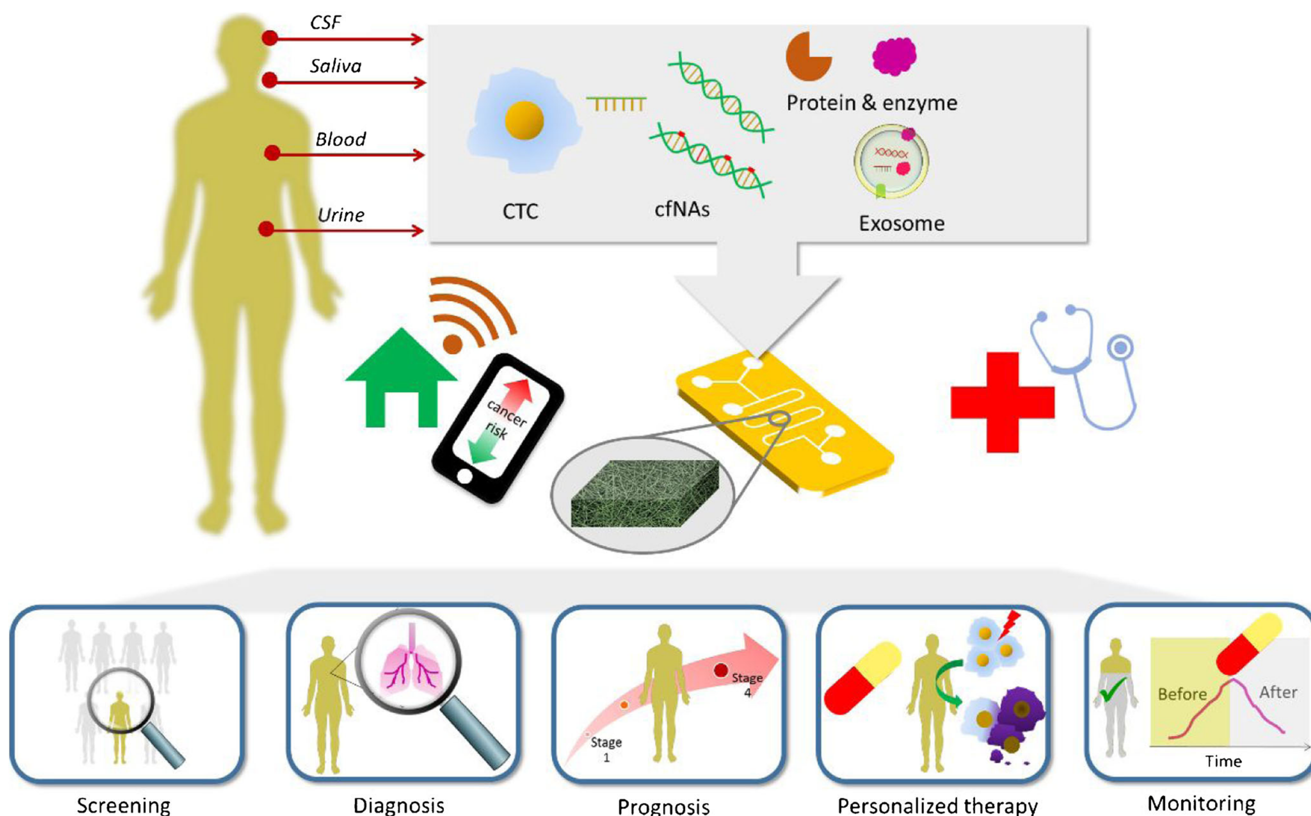


Fig. 1 Nanofibers integrated microfluidic analytical systems and their application in clinical and point-of-care cancer diagnostics based on liquid biopsy. CSF = cerebrospinal fluid

popularity due to their non- or minimal invasiveness, simplicity, and suitability for high throughput assays. Circulating tumor cells (CTCs), proteins, cancer-related enzymes, circulating-free nucleic acids (cfNAs), and exosomes released directly or indirectly from tumor cells into the circulation system are considered as potential cancer markers that provide broad clinical utility at all levels, ranging from screening to monitoring of therapeutic efficacy and recurrence (Fig. 1) [4].

The presence of cancer markers in relatively low number among high abundance of interferences from the sample matrix, especially in peripheral blood, remains a major challenge that calls for efficient isolation of biomarkers and highly sensitive detection platforms [2]. Nanomaterials have been widely implemented in microfluidic analytical systems, with the goal to boost capture efficiency and detection sensitivity by exploiting their high surface area-to-volume ratio characteristic [5, 6]. As such, especially nanofibers prepared by electrospinning (Fig. 2a) have emerged as a promising nano/micro interface for microfluidic-based analytical systems for the next-generation cancer diagnostics [7–9]. Here, the nanofibers can serve as various functional elements within the microfluidic cancer diagnostic devices, which include isolation, release, enrichment, and detection. Special and unique features of electrospun nanofibers such as the immense specific surface area and the generated 3D porous structures with high interconnectivity are highly beneficial in microfluidic cancer diagnostic devices. In particular, they facilitate high collision rates between the functional interface and cancer markers, making capture and detection more efficient than with non-3D porous nanomaterials. Thus, nanofiber microfluidic analytical hybrid systems could be envisioned as a powerful and robust combination to cope with the current challenges.

The study of nanofibers for cancer diagnosis in microfluidic devices is nascent, but rapidly growing, demonstrating their superior functionalities and performances in comparison to other materials. Over the past few years, nanofibers have been continually being investigated both on-chip and off-chip as an efficient concentrator and a sensitive transducer. This article highlights the state-of-the-art with respect to the various cancer markers, as each exhibits specific challenges and requirements that directly affect design and function of the nanofibers and the microfluidic systems. While exosomes are not yet isolated and detected with the help of nanofibers, current (nano)materials are being explored to serve similar purposes. Improvements afforded through future nanofiber strategies are hence proposed. The article concludes with an outlook of the current trend as it relates to progress made in biosensor research, diagnostics, and cancer research, paving the way to advance the field.

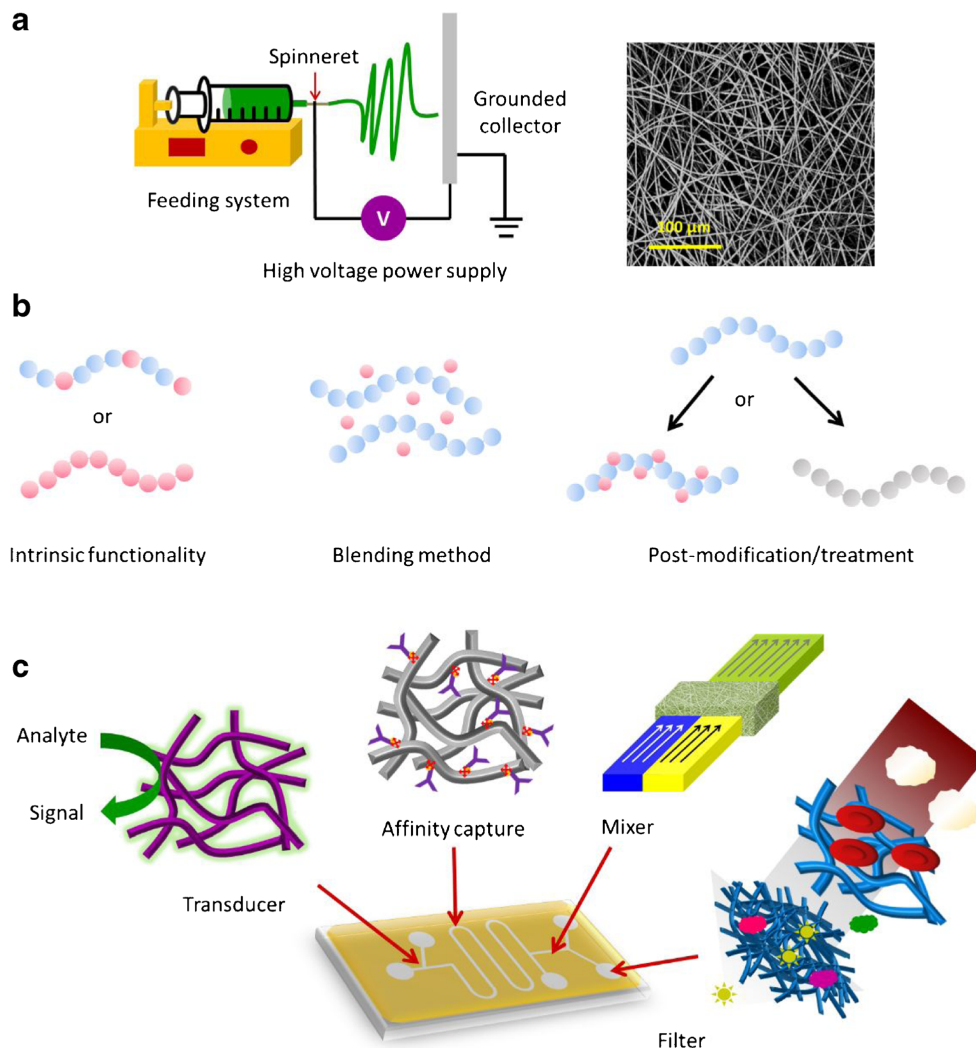


Fig. 2 (**a**) Electrospinning setup (left) and non-woven nanofiber membrane (right). (**b**) Various modification strategies of nanofibers. (**c**) Electrospun nanofibers as versatile components in microfluidic analytical systems

Why electrospun nanofibers?

Electrospinning requires a simple and inexpensive instrumental setup making it eligible to many limited-resource settings. The setup consists of a high-voltage power supply, a syringe pump, a spinneret, and a collector (Fig. 2a). In general, a spinning solution is pushed through a spinneret with a constant flow rate. Application of high voltage between the spinneret and a grounded collector plate results in repulsive forces at the hanging droplet. Once the force overcomes the surface tension, a polymer jet is ejected towards the collector, forming nanofibers with diameters ranging from tens to hundreds of nanometers (depending on solution properties and process conditions). The setup is not only suited for mass production but also highly compatible to miniaturized system integration, which enable the fabrication of low-cost devices.

Nanofibers provide an inherently large surface area. Furthermore, they can easily be modified with biorecognition molecules or functional nanomaterials. These functional chemistries can be realized by various approaches, e.g., through intrinsic properties of the native polymer, through blending additives to the polymer solution, and by post-modification/treatment of as-spun nanofibers (Fig. 2b). Even multifunctionalities within one nanofiber are easily achievable in this manner.

The electrospinning process creates 3D porous nanofiber mats. Therefore, nanofibers are easier to handle than other 1D nanomaterials, e.g., nanowires or nanotubes, but retain the benefits of 1D characteristics. When integrated into microchannels, these 3D mats can span throughout the entire channel volume providing a great number of binding and/or reaction sites in a confined space of the overall miniaturized systems. Furthermore, unlike 0D and 2D nanomaterials, e.g., magnetic nanoparticles and graphene, these nanofiber mats hold their functional entities in place and uniformly distributed with no tendency of agglomeration under varying assay conditions. The high interconnectivity of the 3D fibrous structure can serve as a mixing unit in microfluidic systems [10, 11]. Consequently, this strategy enables efficient capturing and sensitive detection along with a wide dynamic range for

on-chip applications in cancer diagnosis and can even lead to much fast-response times [11].

Lastly, the morphology of electrospun fiber and mat can be rationally tuned by manipulating processing conditions and material properties. For example, thinner fibers can be obtained from lower polymer concentration, higher conductivity, and higher voltage. Also, micron-sized fiber mats tend to have larger pore sizes than nano-sized fiber mats [12]. This allows the flexibility to assign electrospun nanofibers as functional units in microfluidic chip for cancer diagnostic systems. For instance, when employing nanofiber mat in affinity-based capturing, fiber mat with pore diameter larger than cancer and blood cells may be suitable while smaller pore size would fit better for smaller cancer markers, e.g., proteins, nucleic acids, and exosomes. Moreover, various pore sizes of fiber mat would be suitable to serve as a filtering unit based on the size-exclusion principle. Envisioned capabilities of nanofibers in microfluidic analytical systems are illustrated in Fig. 2c.

Functional nanofibers for cancer diagnosis

Circulating tumor cells

CTCs (10–20 μm) are shed from primary tumors or secondary metastatic sites and enter the blood circulation system. The detection of CTCs in whole blood greatly benefits cancer diagnosis at early and metastatic stages, as well as the monitoring of cancer recurrence. In addition, molecular analysis of CTCs is highly valuable for personalized cancer therapy as they carry information of the original tumor cells. The major challenge of CTC isolation is their rarity (1–10 CTCs/mL blood) and their existence in peripheral blood that contains a high abundance of red (RBC) and white (WBC) blood cells (10^6–10^9 cells/mL). Nanofibers have been extensively exploited for (a) CTC capturing with high recovery yield [13], (b) releasing captured CTCs with high purity and viability, and (c) facilitating further downstream processes including cell enrichment and analysis. Nanofibers for CTCs, in general, should be less prone to non-specific binding of RBCs and WBCs, and biocompatible to enhance specific capture and cell viability, respectively.

(a) CTCs express recognition sites that can be targeted by affinity-based capturing. The anti-epithelial cell adhesion molecule (anti-EpCAM), anti-CD146, folic acid (FA), hyaluronic acid (HA), and DNA aptamers have been conjugated to nanofibers for capturing CTCs [13]. Zhang et al. first employed nanofibers for static CTC capturing [14]. Here, TiO_2 nanofibers were deposited onto a flat silicon substrate and later activated to enable a covalent link with streptavidin prior to conjugation to biotinylated anti-EpCAM. The capture experiments proved that the nanotopography of nanofibers strengthened CTC-substrate affinity, which was stronger than that for the flat silicon surface. CTC capture experiments were carried out in a static mode and required a 1-h incubation period for a maximum capture yield of ~ 80%. When CTCs were spiked into blood samples, the capture efficiency was unfortunately reduced to almost 50% in comparison to experiments using culture medium. Hou et al. fabricated poly (lactic-co-glycolic acid) (PLGA) nanofibers modified with anti-EpCAM, deposited on a glass slide and integrated into a microfluidic system, known as PN-nanovelcro chip (Fig. 3a (i and ii)) [15]. Here, comparable capture efficiencies from the spiked samples in a culture medium and whole blood were obtained (Fig. 3 (iii)). This is likely due to the merit of cell capturing in dynamic systems. However, it should be noted that the capture yield of ~ 90% required the integration of a chaotic mixer at the top part of the microfluidic chip to increase cell-substrate contact frequency (Fig. 3a (iv)). After successful capture, the chip can be disassembled. Its transparency, unlike when using silicon nanopillar substrate [16], allows for laser microdissection technology to selectively cut the CTC-nanofiber region for subsequent molecular analysis with high purity.

(b) Nanofibers have been modified with other functional moieties enabling a programmable release of the CTCs with desirable purity and viability (Fig. 3b). Release can be achieved through different ways depending on physical and chemical properties of the modified nanofibers. For example, nanofibers coated with the thermo-responsive groups such as poly(*N*-isopropylacrylamide) (PNIPAAm) display hydrophobic properties at 37 °C, encouraging efficient CTC capture by aptamer and non-specific binding of blood cells via hydrophobic interaction [17]. Reducing the temperature to 20 °C makes the PNIPAAm to elongate and to subsequently become hydrophilic, resulting in the detachment of all blood cells not captured by strong affinity agents. Furthermore, the elongated state of PNIPAAm enables the accessibility of complementary sequences to hybridize with the aptamers, leading to subsequent release of intact CTCs from the interface without contamination with foreign agents. Alternatively, chemically cleavable sites, e.g., disulfide bonds, have been used as an intermediate linker between the nanofibers and biorecognition molecules. Upon introduction of cleaving agents, e.g., glutathione (GSH), CTCs can be released. Here, the concentrations of the releasing agents have to be carefully studied to ensure the CTCs' viability [18, 19]. In a quite different approach, conducting polymer nanostructures can facilitate an electrical stimulation which triggers detachment

of CTCs [20, 21]. It has been shown that the applied voltage of − 0.8 V for 15 s does not significantly reduce the viability of released MCF7 cells [21]. Furthermore, when + 1.0 V is applied for 5 s right after the releasing voltage, the membrane integrity of WBCs breaks down, resulting in an agglomeration of WBCs that remained trapped in the channel. Thus, WBC contamination was reduced from released CTC suspension in this strategy.

Recently, Xiao et al. have shown a significant difference in the purity of the released CTCs when using randomly oriented and aligned nanofiber mats [19]. The aligned nanofiber mats had a significantly lower tendency of blood cell attachment in comparison to their random counterpart, under both dynamic and static conditions. It is assumed that WBCs get increasingly trapped in the random nanofiber mat which could not be detached easily during washing steps. This study also demonstrated that dynamic capturing in microfluidic systems enhanced the purity of CTCs captured by both aligned and randomly oriented nanofiber mats (~ 2-folds higher in comparison to static modes).

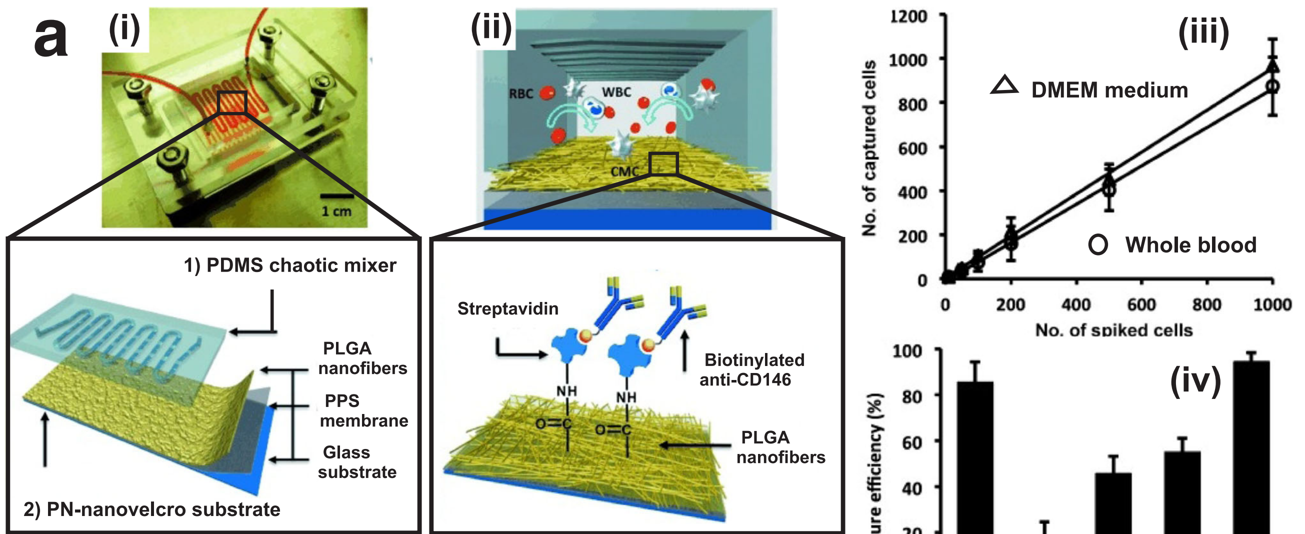
(c) Highly biocompatible nanofibers such as PLGA enable cancer cell cultivation after capture as proposed by Xu et al. [22]. Here, HA-functionalized PLGA nanofiber membranes were integrated into a microfluidic device to selectively capture HeLa cancer cells via their CD44 receptors at a very low cell concentration (20 cells/mL serum-free culture medium). Continuous media perfusion maintained the viability of the captured cells for several days. This work could be highly beneficial for personalized drug testing [23] with real-time monitoring capability, and molecular interrogation for patients suffering from cancer metastasis.

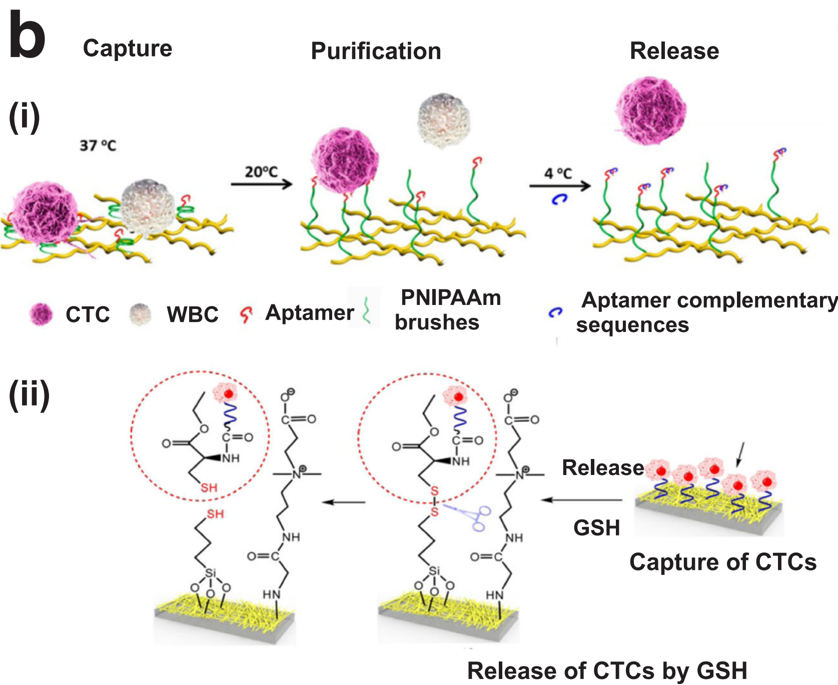
As CTCs are the precursors in metastatic cancers, their accurate quantification in the blood stream provides valuable clinical information for diagnosis and treatment. Various techniques have been proposed to address this task. For example, small fractions of inorganic heterogeneous nanofibers such as CuO-NiO/carbon nanofibers and CuO/Co_3O_4, decorated with FA, have been used as an artificial enzyme that catalyzes hydrolysis of ammonia borane (AB) (Fig. 3d). The production of H_2 from AB can be detected via a portable pressure meter in a closed reaction vessel and correlates to the quantity of CTCs [24, 25]. This strategy enables a convenient way for quantifying specific cancer cells with limits of detection (LODs) as low as 50 and 100 cells/mL in PBS and whole blood, respectively. This could be a useful platform for early point-of-care cancer diagnosis. The as-mentioned examples well-illustrated high versatility of electrospinning to generate functional composited nanofibers that are not limited only to organic materials in which their utility can be extended beyond capturing CTCs.

In conclusion, nanofibers provide a broad range of functionalities for cancer diagnosis based on CTCs in both off-chip and on-chip systems, including capturing, releasing, culturing, and quantifying. However, their full potential has not been harnessed yet, especially regarding 3D porous structure-induced mixing feature. It should be noticed that using nanofibers for CTC capturing in the proposed microfluidic systems still required the integration of additional chaotic mixing elements to induce maximum capture efficiency. As evident in some studies, capture yields of using nanofiber strategies without the mixer only provided results similar to those with polymer films and not different from silicon nanowires or nanopillars [15, 26]. I assume that an important impediment was the clogging of the nanofiber mats. To address this issue, the average pore size of fiber membrane should be enlarged and entering blood samples could be diluted. In fact, various techniques can be used to enlarge the pore sizes [12]. For example, Pham et al. reported pore sizes of 20 to 45 μm when generating fibers with thickness between 4 and 10 μm [27]. As Nellore et al. could successfully filter CTCs from whole blood by aptamer-modified 3D graphene oxide foams (pore size of 20–40 μm) [28], such a strategy should also be feasible for nanofiber mats. Mixing occurring within the 3D porous structures and an optimization of the collision rate are also worthwhile studying [10] as these will lead to higher CTC capturing yields. Further obvious improvements for CTC analysis based on nanofiber microfluidic hybrid systems include the use of receptors other than the EpCAM as tumor heterogeneity and the loss of EpCAM expression during epithelial-mesenchymal transition require multiple recognition strategies [28]. Furthermore, the integration of multifunctional modules enabling capture, release, lysis, and analysis of CTCs within one device would be valuable for clinical diagnosis [29].

Cancer marker proteins

Cancer-derived marker proteins are produced either by cancer cells themselves or by other cells in response to the cancer and are primarily found in blood serum and, sometimes, in urine. Epidermal growth factor receptor 2 (EGFR2 or ErbB2), alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA), and prostate-specific antigen (PSA) are classical examples of standard protein markers presence in plasma or serum used for diagnosis of various cancers nowadays [31]. Current challenges associated with cancer diagnosis based on protein detection are their low abundance with large numbers of protein interfering, no existing replication technique, and instability under ambient environment that necessitate highly sensitive detection techniques. Although ELISA is the current standard method for routine clinical diagnostics with satisfying LODs


a
(i)
1 cm
(ii)
RBC
WBC
CMC
(iii)
DMEM medium
Whole blood
No. of captured cells
No. of spiked cells
1) PDMS chaotic mixer
PLGA nanofibers
PPS membrane
Glass substrate
2) PN-nanovelcro substrate
Streptavidin
Biotinylated anti-CD146
PLGA nanofibers


b
Capture
Purification
Release
(i)
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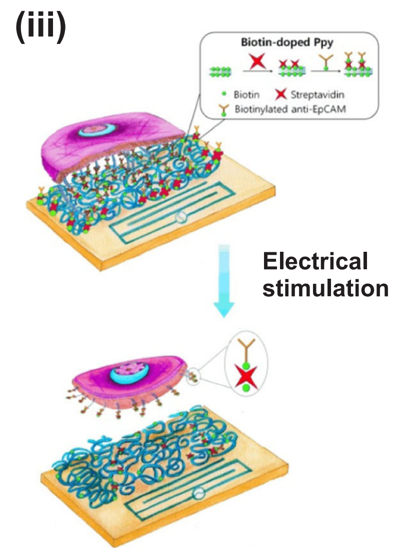
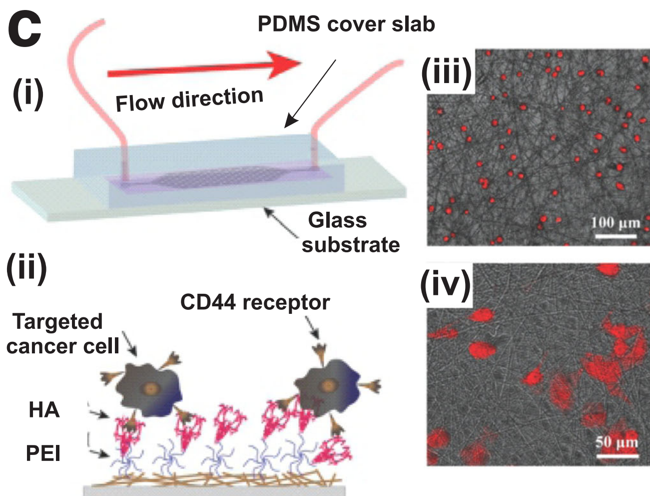

(iv)
Capture efficiency (%)
PN-nanovelcro chips
PN-nanovelcro chips (no anti-CD146)
PN-nanovelcro chips (no herringbone)
anti-CD146-coated PLGA film
anti-CD146-coated SiNW substrate
(iii)
Biotin-doped Ppy
Biotin
Streptavidin
Biotinylated anti-EpCAM
Electrical stimulation


c
(i)
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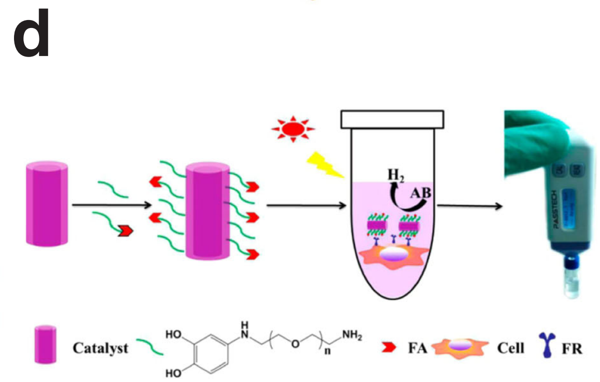
d
Catalyst
FA
Cell
FR

Fig. 3 Applications of nanofibers for CTCs. (**a**) Capturing cells. (i) The PN-nanovelcro chip for capture circulating melanoma cells (CMCs). (ii) Capture mechanism of the PN-nanovelcro CMC chip. (iii) Comparison of capture efficiency when spiked CMCs to culture medium and whole blood. (iv) Cell capture efficiency with various chip configurations. Reprinted with permission from Ref [15] (Copyright 2013 John Wiley and Sons). (**b**) Various controlled release strategies. (i) Thermo-responsive polymer induced property change. Reprinted with permission from Ref [17] (Copyright 2017 American Chemical Society). (ii) Chemical cleavage. Reprinted with permission from Ref [18] (Copyright 2018 American Chemical Society). (iii) Electrical triggered detachment. Reprinted with permission from [30] (Copyright 2014 John Wiley and Sons). (**c**) Cultivation of CTCs. (i) Microfluidic chip configuration. (ii) CTC capturing platform. (iii) Captured HeLa cells. (iv) Growth of HeLa cells after 3 days. Reprinted with permission from Ref [22] (Copyright 2017 Royal Society of Chemistry). (**d**) Quantification of cancer cells by a portable pressure meter. (FR, folate receptor). Reprinted with permission from Ref [24] (Copyright 2017 American Chemical Society)

[32], large sample volume and reagent consumption, multiple processes involved, and long incubation periods (several hours) make them unsuitable for early cancer diagnosis and point-of-care-based testing. Various recent examples show that nanofiber strategies can provide highly sensitive diagnostic tests for these protein markers. In all cases, specific characteristic features of the biomarkers are mirrored by specific functionalities on the nanofibers.

Nanofibers have proven themselves as an excellent matrix for anchoring biorecognition molecules (e.g., antibodies and aptamers) against cancer marker proteins [33–35]. Metal oxide nanofibers are widely employed as they contain plenty of –OH groups which facilitate further treatment and conjugation to bioreceptors of interest. In addition, they have been employed as signal transduction materials when incorporating functional entities, e.g., nanoparticles, into the nanofibers [36, 37].

Zinc oxide (ZnO) nanofiber (50–150-nm diameter)-modified anti-ErbB2 were successfully employed for label-free immunoassay based on impedance spectroscopy with excellent LODs and a remarkable dynamic range for breast cancer detection [33]. Here, the LOD of 1 fM (4.34×10^{-5} ng/mL) and dynamic range of 1.0 fM–0.5 μM could be realized due to a large number of antibody binding sites available for the capture of ErbB2 protein. It should be noted that this outstanding analytical performance is normally hard to achieve from label-free-based detection or even label-based detection schemes [38]. This example highlights the benefit gained from large surface area of electrospun nanofibers in boosting detection sensitivity as well as extending dynamic range. In another study by the same group, TiO_2 nanofibers were physically adsorbed to graphene foam (pore size of 580 μm) and further conjugated to anti-ErbB2 via covalent bonding [35]. The graphene-TiO_2 nanofibers/anti-ErbB2 were manually inserted into a PDMS microfluidic channel (Fig. 4a (i)), to serve as a working electrode for label-free detection again based on impedance and also on differential pulse voltammetry. LOD and linear range of ErbB2 detection were comparable for both applied detection techniques and similar to their previous study [33], implying that the miniaturized system in this study only provides for a convenient immunoassay process regarding binding and washing but does not improve analytical sensitivity. It could be assumed that relatively large pore size of the graphene scaffold does not assist in mixing or improvement of collision rates as discussed above, which in turn can be addressed by conducting nanofiber mats, especially carbon nanofibers (CNFs), with average pore diameter of a few micrometers. Nanofiber mats would also assist in overcoming the here-described impractical chip fabrication. Lastly, even though selectivity towards some foreign proteins commonly present in serum samples [33] or closely related proteins (ErbB3 and ErbB4) [35] was evaluated, applicability in actual plasma or serum is needed.

Transduction nanomaterials, e.g., nanoparticles and carbon nanotubes, can be anchored onto nanofiber surfaces with high loading capacity. For example, quantum dots (QDs) have been decorated onto nanofibers and further modified with DNA aptamer specific to PSA [37]. Here, the absence of PSA allows the hybridization between complementary DNA-attached gold nanoparticles (AuNPs) probes and the QD-anchored aptamers, resulting in signal quenching of the immobilized QDs (Fig. 4b (ii)). On the contrary, when PSA bound to the aptamers, higher fluorescent intensity from QD can be generated due to less quenching capacity from DNA-AuNP-hybridized probes. With the porous network of nanofiber mats, a low LOD down to 0.46 pg/mL and linear range of 0.001–100 ng/mL could be obtained, which was 3 orders of magnitude greater than that of using a planar membrane. Besides that the immense surface area available in the nanofiber membrane enabled a larger linear range due to high loading capacity of DNA aptamers, the excellent performance could also be attributed to the greater accessibility of AuNP probes and PSA inside the 3D porous network. Detection of PSA in real serum samples from prostate cancer patients was also demonstrated. The results were comparable to those obtained from standard technique-based chemiluminescence analysis carried out by the hospital. Furthermore, the proposed platform is highly promising for the detection of multiple cancer marker proteins when various types of QDs are used [39].

In a similar anchoring strategy, the activity of cancer-related enzymes, e.g., protease and telomerase, can be quantified [40, 41]. Nanofibers have shown their capability to anchor specific peptide labeled with fluorophores for specific cleavage by the target protease (metalloproteinases-9, MMPs) (Fig. 4a (iii)) [42]. The detection of released fluorophore was detected downstream at the detection zone of the microfluidic device. The combination of nanofibers and microfluidic system resulted in efficient mixing that provides the enhanced mass transfer and reduced diffusion distance between anchored peptide substrate and MMPs, thus enabling a faster response time (30 min) and LOD down to 10 pM, which is much faster than that of MMP-9 assay kit that requires 4 h. Also, multiplex sensing systems are highly

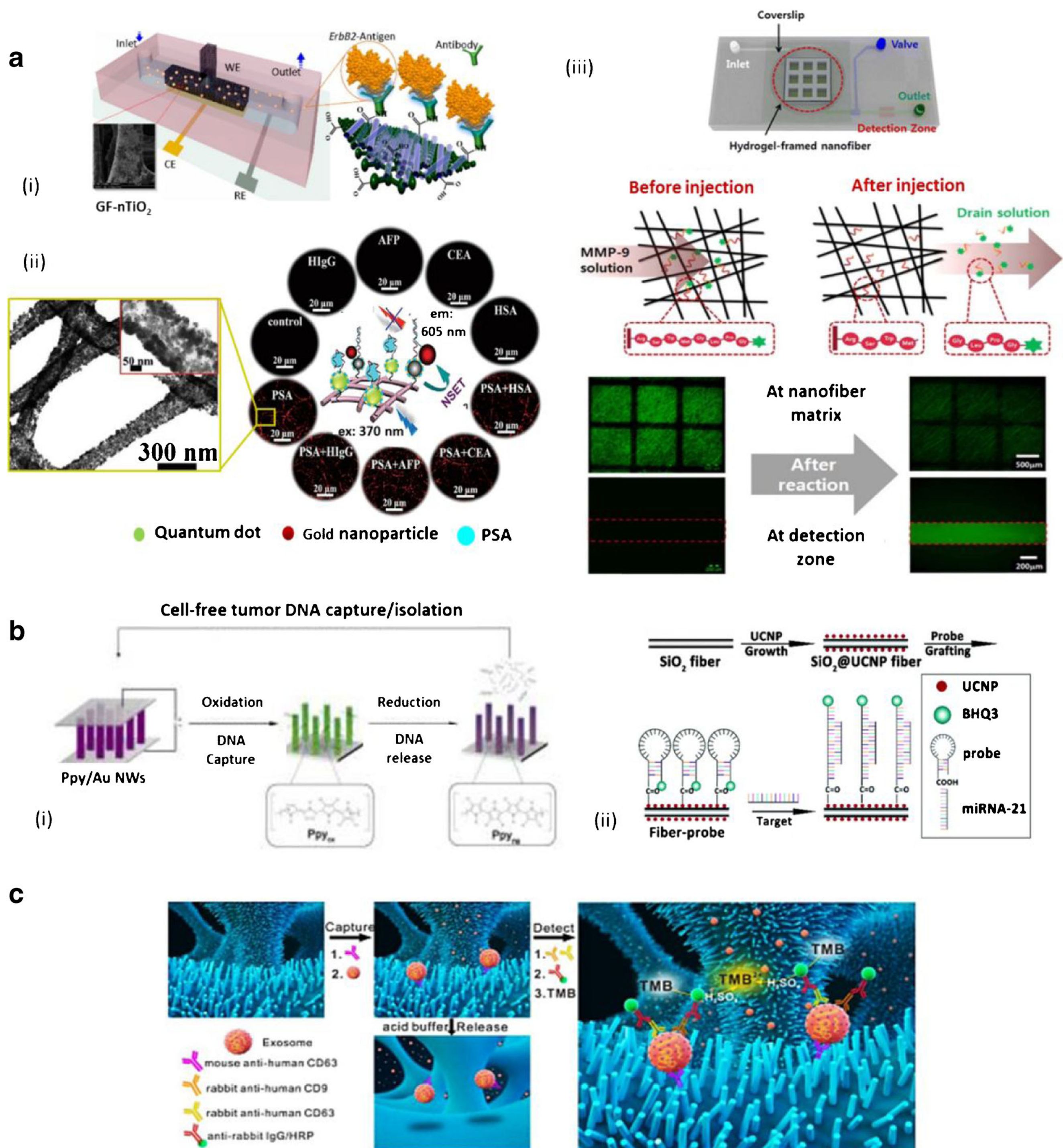

Fig. 4 Applications of nanofibers for small cancer markers. (**a**) Capturing protein markers and detection by electrochemical- (i) and optical-based techniques (ii), and protease enzyme assay (iii). Reproduced with permissions from Ref [35] (Copyright 2016 American Chemical Society), Ref [37] (Copyright 2017 Royal Society of Chemistry), and Ref [42] (Copyright 2016 American Chemical Society), respectively. (**b**) Capture and release cfNAs by tuning charges of conducting polymers (i) [48] (This is an open-access article distributed under the terms of the Creative Commons Attribution (CC BY-NC) license (https://creativecommons.org/licenses/by-nc/4.0/)), and detection of miRNA by UCNPs (ii). Reproduced with permissions from Ref [51]. (**c**) The capture, detection, and release of exosomes by ZnO-chip device. Reproduced with permissions from Ref [61] (Copyright 2018 Elsevier)

feasible with this platform especially when immobilized multiple peptide substrates onto the nanofiber matrix in separate microfluidic channels. Alternatively, various labels could be used for a specific peptide substrate. In addition to fluorescent label, electrochemically active molecule such as ferrocene was tagged with a peptide substrate and electrochemically detected by CNF

supports [43], which also possibly permits multiplex sensing systems similar to the aforementioned scheme.

Circulating-free nucleic acids

cfNAs derived from cancer cells are considered as a potential marker for cancer screening, prognosis, and monitoring the efficacy of anticancer therapies [44]. cfNAs including DNA, mRNA, and miRNA are thought to be released from cancer cells into the blood stream via apoptosis or necrosis similar to normal cells. In general, increased levels of cfNAs in blood plasma in comparison to healthy subjects could reflect the pathological process of cancer development. In addition, the alterations of their sequences and specific methylations can be assessed and exploited as a tool for wide applications in clinical diagnostics [45] that is more informative, specific, and accurate than protein biomarkers. In comparison to the cancer markers mentioned earlier, the extraction of cfNAs is considerably easier and harmless to its physical native condition.

Circulating tumor DNAs (ctDNAs) are a fraction of cfDNAs in which their length are longer (> 200 bp vs. 70–200 bp) and the concentration is highly variable depending on the stage and type of cancer. Unfortunately, they are present in relatively low amounts (ng/mL level, < 0.1–10% of the total cfDNA detectable in human blood [46]) and are prone to generate false negative signals due to their degradation by nucleases, in particular when applied directly to real samples. Additionally, capturing of short fragment cfDNA is difficult to achieve by current isolation and purification methods. Consequently, cancer diagnosis based on ctDNA requires highly efficient capturing and very sensitive detection methods, similar to CTC and protein markers. Positively charged nanofiber membranes with small pore size could rationally trap negatively charged ctDNA and further induce releasing through changing the surface charge of nanofibers.

Strategies addressing these challenges employ conducting micro- or nanostructure into microdevices to efficiently capture and release cfDNA by taking advantages of reversible changes of redox states [47, 48]. For example, the charge of polypyrrole (Ppy) can be modulated by the applied electric fields where oxidation and reduction potentials induce positive and negative charges, respectively. This facilitates electrostatic interaction or repulsion of cfDNA in capture and release mechanisms, respectively. Lee et al. have proven that the DNA recovery yielded from electropolymerized Ppy nanostructures outperformed the other extraction techniques, i.e., using THP protocol, magnetic nanoparticles, and Qiagen kit for low (10–100 bp)- and middle (100–2 k bp)-length DNAs [47]. Later, the same group coated vertically arranged gold nanowires with Ppy (Ppy/Au NWs) to increase the active surface area (Fig. 4b (i)) [48]. With this strategy, they have demonstrated that the capture and release efficiency of Ppy/Au NWs were 4 times greater than those using the flat surface. Interestingly, the maximum capturing efficiency was independent of nanowire diameters but releasing efficiency required optimum dimension, i.e., 1-μm NWs reached almost 100% whereas 500-nm NWs and 10-μm NWS exhibited 80%. To test capture and releasing efficiency in real sample, DNAs with 100-bp length were spiked into blood plasma with concentration ranging from 0.001 to 500 ng/mL. Without further plasma processing, over 90% efficiency of DNA capture and release was obtained for DNA concentrations higher than 1 ng/mL. Using nanofibers instead of nanowires will likely improve the capture and release efficiency at low cfDNA concentrations.

Circulating miRNAs, single-stranded non-coding small RNA molecules, are identified as a potential cancer biomarker for several types of cancer [49]. They can be found in various samples such as blood, serum, saliva, and urine. Their low abundance and extreme short length (typically 17–25 nucleotides) introduce immense difficulties in capturing either by complementary sequences or adsorption, and in their detection. Thus, development of nanofibers for circulating miRNA should point towards the promotion of fast analysis protocol, specific and strong capturing of miRNAs, and high-sensitive detection. Recently, upconverting nanoparticle (UCNP)-incorporated nanofibers have been employed for sensitive detection of miRNA-related cancers based on fluorescence resonance energy transfer (FRET) [50, 51]. As an example, Wang et al. grew UCNPs (30–50 nm) on SiO_2 nanofiber support with highly uniform distribution along the nanofibers (Fig. 4b (ii)). A molecular beacon (MB)-linked quencher was later grafted onto UCNP-decorated nanofibers, enabling close proximity between the quencher and the UCNP and thus reducing red-light emission at 660 nm. The hybridization of target miRNA and MB probes therefore resulted in an opened hairpin structure, enabling the emission of photoluminescent signals. This detection scheme leads to an LOD down to 2 nM for miRNA-21 (a miRNA sequence associated with lung cancer) and was able to discriminate one-base and three-base mismatched miRNA-21 and miRNA-195, demonstrating its ability to investigate DNA mutations that are vital for cancer detection.

To improve hybridization and hence increase capture efficiency of miRNAs, peptide nucleic acids (PNAs) are becoming a potential candidate to functionalize nanofibers [52]. In addition, cationic conducting polymer combined with PNAs is of interest to enhance binding stability of miRNA [53]. Here, polythiophene (PT) could promote strong capturing of all miRNAs in the sample matrix via electrostatic interactions. The subsequent addition of biotinylated PNA (b-PNA) sequences enabled the selective miRNA assay through monitoring the PT–miRNA–b-PNA triplex formation. Further introduction of avidin-coated AuNPs specifically bound to b-PNA leads to an LOD of 400 pM of miRNA-21, quantified with a quartz crystal microbalance. However, even though the proposed technique showed the detection of miRNA-21 in the plasma sample, they still needed commercially available extraction kits for miRNA isolation to minimize the potential

interference associated with physisorption of plasma components.

Circulating tumor exosomes

Exosomes are vesicles released from most cell types including cancer cells. Cancer-derived exosomes have caught significant attention as a potential marker for cancer detection and monitoring because they carry informative biomolecular contents, e.g., proteins, nucleic acids, and enzymes that uniquely reflect the original cells from which they were secreted [54, 55]. The size of cancer-derived exosomes is typically in the range of 30–100 nm, similar to exosomes secreted from normal cells. They can be found in various body fluids, e.g., blood, urine, saliva, and breast milk, in a significant large quantity (10^9–10^{12} exosomes/mL) and comparable to the amount of normal exosomes (10^{11} exosomes/mL) [56]. The role of exosomal proteins and nucleic acids as diagnostic biomarkers has been extensively studied in recent years as shown in previous review articles [54, 55, 57]. For example, exosomal proteins presented on the surface such as CD24, EpCAM, and CA-125 are related to ovarian cancer, and miRNA-encapsulated exosomes such as miR-101, miR-372, and miR-373 are biomarkers for breast cancer [55]. Detection of exosomal nucleic acids is considered more attractive than cfNAs as the nucleic acids are protected from degradation by nucleases during circulation. A major problem of using cancer-derived exosomes in clinical diagnosis is the difficulty in isolation and detection among the other normal exosomes or extracellular vesicles. Thus, research efforts have attempted to develop efficient and reliable techniques for addressing the issue [57]. It is envisioned that either positively charged nanofibers or recognition molecule-anchored nanofibers could serve as a potential candidate for isolating exosomes with high efficiency.

While nanofiber-integrated microfluidic devices have not yet been realized for this challenge, their excellent performance as described for CTCs is an indicator that this will be studied and addressed in the near future. The ideas of using other nanomaterials to enhance capture efficiency of cancer-derived exosomes in microfluidic devices have been reviewed [58]. Yasui et al. recently demonstrated the ability to capture them from urine samples by using nanowire-integrated PDMS microfluidic system [59]. The nanowires with dimension of ca. 100 nm wide and 2000 nm long are embedded into a PDMS substrate. The positively charged nanowires allow electrostatic attraction to the negative surface of exosomes. After exosome lysis and analysis of miRNA content off-chip, the system showed superior benefits over conventional ultracentrifugation such as greater collection efficiency (894 miRNA/mL urine vs. 13 miRNA/mL urine) and required shorter times for isolation (40 min vs. 300 min). Positively charged nanofiber-integrated microfluidic device can well serve this task [60].

More recently, Chen et al. [61] constructed a microfluidic device that contains the interconnected 3D scaffold (a few hundred of nanometers in diameter) decorated ZnO nanowires for efficient capturing of exosomes based immunoaffinity (Fig. 4c). A large number of immobilized antibody on the ZnO nanowires and enhanced chaotic mixing due to the 3D scaffold provided an LOD of 2.2×10^7 exosomes/mL. It is conceivable that the application of nanofiber mats with smaller pore diameter will significantly lower the LOD. In addition, integrating high-detection sensitivity techniques to evaluate the number of cancer-related exosomes or analysis of molecular contents will be of interest to future studies [62–64]. Furthermore, nanofibers modified with lipid probes or aptamer sequences specific for exosomal proteins could be considered as alternative options for exosome isolation as they are less expensive, more stable, and easier to produce in comparison to antibodies.

In summary, cancer markers are typically present in a relatively low amount among a high abundance of interferences that require efficient isolation strategy in miniaturized devices which further facilitate specific enrichment and sensitive detection. Electrospun nanofiber membranes can offer various functionalities that are promising for those tasks as summarized in Fig. 5. The nanofiber membrane with pore sizes proper for each cancer marker will leverage the maximum capabilities offered by the 3D porous structure, enabling enhanced capture yield, high-detection sensitivity, and short analysis time. Nanofiber mats with pore sizes less than a few micrometers are well-suited for capturing and detecting all cancer markers except for CTCs that require larger pores in the range of a few tens of micrometers. Nanofiber-anchored specific biorecognition molecules can serve as both a capturing unit and a transducer which make them suitable for cancer diagnosis based on the detection of proteins, enzymes, and miRNA. Miniaturized device-integrated nanofibers with components

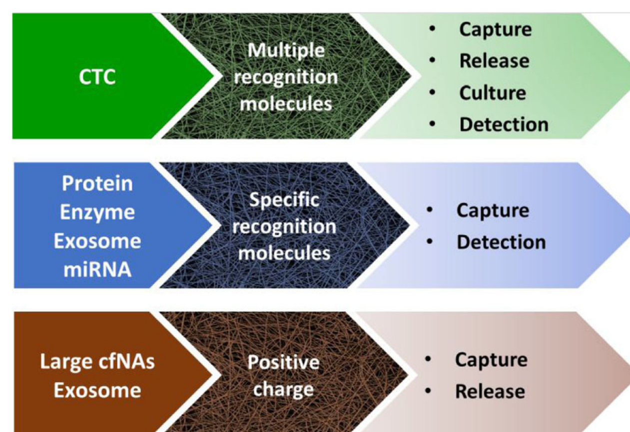

Fig. 5 Summary of ability offered by functional nanofibers for cancer diagnosis in miniaturized systems

served for downstream analysis such as releasing and detecting units will facilitate all-in-one diagnosis of CTCs, ctDNA, and exosomes. Here, non-specific capture via electrostatic interaction is reasonable for ctDNA and exosomes while specific capture via multiple recognition molecules is favorable for CTCs and exosomes.

Outlook

Platforms integrating nanofibers as functional elements into miniaturized isolation and detection platforms for cancer markers have demonstrated to be highly effective and able to address challenges and needs associated with this complex task. Features including sensing elements, controlled release units, and scaffolds for cell cultivation have already been successfully realized especially for CTCs. Future trends of this nascent field are clear and point towards increasing capabilities for the detection of all relevant biomarker classes, as well as developing platforms addressing the challenges of "real-world" point-of-care- and clinical-based testing devices for cancer diagnosis.

Foreseeable near-future research foci will hence include studies on the modification processes used to gain nanofibers with desired (bio)chemical functionalities. Currently, such modifications require tedious processes (several hours) that can even introduce adverse effects such as swelling-induced deformation of 3D porous structure and leaching of functional entities. In particular, immersion of hydrophilic nanofiber mats in modifying reagents for a long period of time could affect porosity, available surface area, and useful functionalities, ultimately resulting in the deterioration of desired performance. Possible solutions are the development of one-step electrospinning strategies that incorporate bioreceptors or signal transduction molecules directly with the polymer or during the spinning process while keeping their intrinsic functionalities. Click-chemistry enabling polymers, core-shell electrospinning, or phase-separation strategy could be an avenue to address this issue. The first strategy potentially reduces steps involved in conjugation of biorecognition molecules. The latter techniques allow for actual one-step electrospinning that enable functional entities to be presented on the nanofiber surfaces. Also, molecular imprinting technology could be adapted to nanofibers for the creation of recognition sites as it convinces with its provided durability, chemical stability, and low production cost [65]. Furthermore, high surface area and high collision rates not only increase favorable interaction between cancer markers and nanofibers but also promote undesirable surface fouling. Therefore, dual functionalities of the nanofiber polymer should be developed to provide ultra-low fouling properties in addition to (bio)recognition to avoid the many matrix-related interferences observed with the complex bodily fluids [66]. Block copolymers containing a hydrophobic core and zwitterionic polymer branches or other hydrophilic residues could be an efficient strategy to fabricate highly stable nanofibers with anti-fouling properties.

Transduction and assay strategies that will be most convincing in the future will rely on label-free and multi-analyte capabilities to lower assay complexity and increase assay throughput. Both of these can be accomplished using optical and electrochemical strategies widely used in biosensors already and demonstrated in nanofibers recently [67, 68]. Also here, the already-demonstrated intrinsic multifunctionality of nanofibers, the chemical diversity of their polymers, and doping strategies are excellent indicators for successful future research efforts.

A major future effort lies in the actual integration and miniaturization of nanofiber-based chip systems as the majority of current processes are cumbersome, complex, and at best useful for lab-based production scale. Strategies have to be found for the device assembly that are amenable to mass production while preserving the delicate 3D porous architectures, which is of special concern with the more brittle metal oxide and carbon-based nanofibers. In situ-induced generation of desired properties of nanofiber fiber mats has a great potential to alleviate many of the issues. In fact, miniaturized systems can then also include formats suitable as point-of-care or even wearable devices.

As a growing number of cancer-related markers that are found in other sources than blood, e.g., saliva, urine, and even exhaled breath, wearable devices are indeed a solution for effective and simple cancer screening and monitoring. Also, for lab-based miniaturized systems, focusing on other cancer-associated markers will be of especial interest in the near future. Exosomes in fact show superior characteristics over CTCs, including higher abundance, being rich in molecular information, and providing an inherent ability for early detection. Albeit the entire process including exosome isolation, molecular content extraction, and detection with sensitive enough performance bears a plethora of challenges, a nanofiber–microchip hybrid system undoubtedly can provide the needed capabilities.

Even though nanofiber-integrated miniaturized systems have promised the next generation of cancer diagnosis as seen from many proof-of-concept studies, their translation into clinical applications still needs to compete with the well-established technologies in central laboratories especially in terms of overall performance and cost. More importantly, high reliability of the developed devices must be met to prevent mis-diagnosis, psychological stress, and unnecessary treatments. Strong cooperation between clinicians and technology developers is needed to facilitate their applicability in the sophisticated context of cancer diagnosis.

Overall, while being a nascent research area in the field of cancer diagnosis, miniaturized analytical systems with integrated multifunctional nanofibers are worth the research effort

as they can provide solutions to the many challenges faced. Intensive pre-clinical validations by practitioners can then follow the same pathways other miniaturized analytical devices have already as these are necessary to translate the technology to real-world applications, ranging from screening to personalized therapy, with high accessibility in all-resourced settings.

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Compliance with ethical standards

Conflict of interest The author declares that she has no conflict of interest.

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