Prostate cancer and microfluids – Current status and Future applications

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Abstract

Introduction

Microfluidic systems aim to detect sample matter quickly with high sensitivity and resolution, on a small scale. With its increased use in medicine, the field is showing significant promise in prostate cancer diagnosis and management due, in part, to its ability to offer point-of-care testing. This review highlights some of the research that has been undertaken in respect of prostate cancer and microfluidics.

Methods

Firstly, this review considers the diagnosis of prostate cancer through use of microfluidic systems and analyses the detection of prostate specific antigen, proteins, and circulating tumour cells to highlight the scope of current advancements.

Secondly, this review analyses progressions in the understanding of prostate cancer physiology and considers techniques used to aid treatment of prostate cancer, such as the creation of a micro-environment.

Finally, this review highlights potential future roles of microfluidics in assisting prostate cancer, such as in exosomal analysis.

Conclusion

In conclusion, this review shows the vast scope and application of microfluidic systems and how these systems will ensure advancements to future prostate cancer management.

Introduction

Microfluidics is the study of systems that can perform analysis on microscopic quantities of fluid using equipment on a micrometre scale. Due to adapted fabrication techniques for silicon processing from the electronics industry, the microfluidic system is also commonly referred to as a microfluidic chip. A microfluidic chip contains features which have at least one linear dimension in the submillimetre diapason. In terms of volume such miniaturisation translates into microlitre, nanolitre or picolitre volumes of fluid processed on-chip at a time. One immediate consequence of using small volumes is the reduction of sample and reagent consumption per assay, thus making the analysis more affordable and environmentally friendly than the conventional detection methods. Miniaturisation in microfluidics also leads to process intensification due to surge in surface-to-volume ratio and decrease of diffusion times, which essentially facilitates faster analysis. Typically, five core components are required in a fully integrated microfluidic system to allow for autonomous analysis: sample introduction, movement, purification, detection and analysis (1). Microfluidic systems aim to detect sample matter quickly with high sensitivity and resolution, while significantly reducing the invasiveness of sample collection and volume of the analysis. This, coupled with the small scale of equipment used, the high level of automation and its relatively cheap production, is leading to an increase in its use in medicine (2, 3).

In addition, the field of microfluidics is showing significant promise in cancer diagnosis and management (4, 5). Analysis of urine, blood, and other tissue fluids on a microfluidic scale has yielded detection of circulating tumour cells and cancer specific biomarkers, as well as permitting growth and study of tissue on a single cell level.

Prostate cancer is the third most common cancer in the United Kingdom and second most common cause of cancer death in men (6, 7). The current gold standard for prostate cancer diagnosis is the invasive method of transrectal ultrasound guided biopsy (8). There is a significant amount of debate currently taking place internationally regarding a screening process for prostate cancer, as it is known that early prostate cancer diagnosis is pivotal in ensuring increased survival rates through simple and effective treatment (9). However, biomarkers for prostate cancer can be non-specific. Sensitive microfluidic detection systems, with their ability to detect clinically significant levels of certain markers with just small quantities of fluid, together with a small size permitting point of care testing, may be able to offer instantaneous cancer diagnosis and as such, revolutionise cancer screening. Microfluidic systems may prevent a patient from the morbidity associated with prostate biopsies and allow for appropriate diagnosis, as well as to encourage further study to assist with cancer management (10). Furthermore, in noting recent developments to cope with the evolving coronavirus pandemic, such as the recommendations for remote consultations and only performing high priority or emergency surgery, a system that avoids the need for biopsy and minimal patient contact may prove helpful (11).

This review seeks to summarise past and ongoing microfluidic prostate cancer research to identify key advancements in detection, therapeutics, and physiological understanding.

For this review, PubMed was used to search for citations as electronic resources. The database was searched with no restrictions on language, date published or country of origin. We used database specific combinations for the terms ‘prostate’ and ‘microfluid’ to appear in

the paper title. Further papers were subsequently chosen to help elaborate on these papers, as well as to show potential future directions of research.

Prostate Specific Antigen

Prostate specific antigen (PSA), is a protein biomarker that has been found to be the single most significant and clinically used predictive factor for identifying men at increased risk of prostate cancer (12, 13). Evidence suggests that a PSA level of 4ng/mL or higher is clinically significant in cancer biopsy and a level less than 0.4ng/mL, or even ≥ 0.2ng/L checked with a second confirmatory level, is significant in monitoring for cancer recurrence after radical prostatectomy (14-17). Some studies even suggest that the exact figure is not important, but a rising PSA of two or more subsequent occasions is a better determinant (18). However, PSA levels are also increased in a variety of other, non-malignant prostate conditions, including prostatitis and benign prostatic hyperplasia (19). Therefore, much research has occurred into the microfluidic detection of PSA to both increase the ability to detect PSA from small fluid quantities and to enhance diagnostic ability, through differentiation of PSA more indicative of cancer.

Microfluidic platforms for prostate specific antigen diagnostics

Detection of PSA using microfluidics began in 2007, and the intention was to improve upon the commonplace use of the Enzyme Linked Immunosorbent Assay (ELISA). Initial studies used an immobilised anti-PSA antibody attached to a horseradish peroxidase enzyme. When activated by PSA, in the presence of hydrogen peroxide, this enzyme catalyses the oxidation of 4-tert-butylcatechol, which can then be quantified. This method of PSA detection showed a detectable response equivalent to the concentration of total PSA in the serum. As compared to ELISA, this microfluidic method revealed significant enhancement in sensitivity. Additionally, the overall assay time of this cost-effective method was 70 minutes shorter than the 100 minutes required for ELISA test kits, and resulted in no reduction in sensitivity (20).

Three years later, Triroj et al. created a different design to a microfluidic chip to rival the earlier research (9). They used a system that generated a current proportional to the number of immune complexes created between PSA and a PSA antibody. Their system worked on the basis that PSA, from spiked buffer solution, would compete with a PSA-enzyme conjugate, on the microfluidic chip, for the binding site on an anti-PSA antibody. The release of the PSA enzyme-conjugate, after competitive replacement, caused a proportional reduction in electrical current on the microfluidic chip. This method was able to detect PSA in just 7 seconds and highlighted a promising approach in electrical PSA detection (9).

Electric based detection methods were employed once again in 2019, when a portable, robust, low cost microfluidic device to sense PSA was generated. This device used a screen-printed electrode coated with a glass solution using sol-gel based approach. It was able to detect PSA in human serum with concentrations as low as 1pg/L through chronoamperometry and square wave voltammetry. This microfluidic chip employed an anti-PSA antibody fixed onto the surface of a gold electrode, so that chronoamperometry could be used to detect the electrochemical signals subsequently produced on PSA binding. Currents proportional to PSA concentrations were created with sensitivity and reproducibility (21). In contrast to the earlier method employed by Triroj et al., this technique used an

increase in current as opposed to a decrease, providing capacity for a wider range of PSA concentrations.

As a result of the opening of new avenues for microfluidic PSA detection, some teams have employed the use of aptamers, which are artificial capture probes of specifically designed sequences of nucleic acids, to detect specific proteins. The first team to apply aptamers in PSA detection used a novel, love-wave biosensing device on a polydimethylsiloxane microfluidic template, in an attempt to allow for highly sensitive and specific PSA detection (22). However, this study, which relied on aptamer detection of PSA, only obtained a detection limit of 10ng/mL, which was not low enough for real sample detection. Thus, more work was required to permit point-of-care use as well as real time monitoring.

Therefore, two years later, the concept of aptamers was used once again to create a magnetic controlled system that exhibited photocurrent response from PSA concentrations as low as 0.001ng/mL. This new technique released trigger DNA when PSA successfully bound an aptamer, and this subsequently caused the formation of a large amount of glucose oxidase. This glucose oxidase then reacted with a graphene oxide - bismuth ferrite combination to produce a quantifiable photocurrent in the microfluid system. This system was effectively employed to analyse PSA in human serum, displaying good accuracy with a low detection limit, highlighting a clear improvement to earlier aptamer trials (23).

Outside of antigen-based detection mechanisms, research has taken place into the use of ultrasonic waves to separate PSA from whole blood samples. This use of these ultrasonic waves, coupled with a microfluidic channel was seen to appropriately assist in separation of plasma from whole blood, which could then be further analysed for PSA. Though this method was able to detect PSA at clinically relevant levels, it was limited by the multi-step nature of the method which would not allow for point-of-care testing without further development (24).

Microfluidic point-of-care testing

The research methods described above were successful in a laboratory environment. However, to make microfluidic detection of PSA more accessible to patients and healthcare professionals, research was conducted by Barbosa et. al to enhance microfluidic PSA testing of whole blood using a smartphone device. Their research showed that an iPhone camera and fluorescence detection could be used to detect PSA in a clinically significant range from 0.9 to 60ng/mL (25). However, the team noted that improvements were required in optimising the enzymatic reaction with the PSA, and that a more robust UV light source was required as opposed to an iPhone. Therefore, later in 2017, the same team did further work to enable point-of-care testing through use of a flat-bed scanner. The team engineered a microfluidic method to successfully highlight PSA at concentrations between 10-100ng/L, by using gold nanoparticles with silver enhancement conjugated to an anti-PSA antibody. Although this data set did show significant variability at higher PSA concentrations, direct optical detection was not possible without the use of enzymatic amplification or fluorophoresis. However, as this study utilised a flat-bed scanner, the team successfully showed continued activism in producing low cost point-of-care testing, although the team did also note that vast improvements were necessary to improve one step particle detection (26).

Microfluidic detection of cancer specific prostate specific antigen

Although many of the above mentioned methods show significant promise in PSA detection, they are of limited value, as the PSA detected is not specific for prostate cancer (19). However, there does exist research that is looking specifically at microfluidic PSA detection to identify prostate cancer. Gao et al, based in China, have shown significant promise since 2016 in their use of microfluidics for PSA detection. This team utilised a Surface-Enhanced Raman Scattering (SERS) technique with microfluidics to successfully isolate and quantify PSA (27), and two years later were able to highlight the significance of their preliminary research.

With the knowledge that a low level of free PSA, but a high total PSA highlights a person more at risk of prostate cancer, the team in China attempted to isolate these two forms of PSA in a dual microfluidic platform (28, 29). The technique of microfluidic detection of free and total PSA had previously been employed by Chiraco et al in 2013, who designed a microfluidic electrochemical impedance spectrography system that permitted detection of free and total PSA through use of two separate antibodies (30). However, Gao et al, in contrast, used their earlier employed SERS technique to detect PSA in human serum through the creation of quantifiable magnetic immune complexes, to enable the clinically significant detection of both free and total PSA in samples in only 5 minutes (29). On the other hand, this study was on a small scale using only five samples from people with known prostate cancer and had a significant drawback in the use of an external pump which reduced the capacity for point-of-care testing.

Consequently, further research was conducted by Gao et al to increase the capacity for point-of-care testing. They designed a capillary pump on their microfluidic chip design to help reduce sensor size, improve reliability and to allow for sensitive and rapid detection of PSA. Though a new chip had to be loaded for each sample analysis, the PSA concentration was detected in a linear range, with ability to analyse below the clinically significant level of 4ng/mL, in only 5 minutes, without an external pump. Thus the standard of their previous research to detect PSA levels with high sensitivity at concentrations as low as 0.01ng/L was maintained and a method more akin to point-of-care testing was created (31).

Multiple detection targets

To boost the accuracy of prostate cancer diagnostic tests, molecular targets such as miRNA as well as the proteins they code for, can be tested for at the same time as serum PSA (32, 33). As opposed to their simple detection alone, testing for these molecular targets, in addition to PSA, is thought to improve upon the limitations of a simple PSA assay. Though multiple detection targets have yet to be employed with PSA in microfluidic prostate cancer analysis, it is a route that could see much success moving forward.

In 1999, Bussemakers et al. found the highly prostate cancer specific DD3 (differential display code 3) protein. This protein was not found in any other cell of the body aside from prostate cells and was found to exhibit an almost 100-fold overexpression in malignant areas compared to benign areas within the same prostate tissue. In contrast, PSA alone was not able to identify significant differences between benign and malignant tissue within the same prostate. The protein levels of DD3 were also found to directly correlate with cancer severity. Further work highlighted that the aetiology of this protein was a non-coding microRNA, found

in prostate cells. Thus the presence of this protein and microRNA was highlighted to be a promising marker for both diagnosis and potential treatment of prostate cancer (34).

Therefore in 2002, the same research team developed a method for accurate quantification of the miRNA responsible for DD3 protein creation using the reverse transcription polymerase chain reaction. As well as corroborating earlier results, the team showed that even in tissue with less than 10% tumour cells, DD3 miRNA expression was significantly increased. The expression of this miRNA was found to be much higher than some other upregulated proteins in cancer cells, such as those involved in telomerase activity. With such high levels of miRNA in tumour cells, the team predicted the presence of the miRNA would be advantageous in fluid detection even on a small scale; thus, showing possible applicability to microfluidics. Interestingly, the team were not able to find the functionality of the DD3 protein and also found that there was no correlational activity of tumour stage with protein levels (35).

Later research in California looked further into analysis and quantification of the gene encoding the DD3 prostate specific miRNA, which came to be known as Prostate Cancer Gene 3 (PCA3). In this study, the PCA3 miRNAs and PSA mRNAs were collected and quantified in men undergoing prostate cancer biopsy and correlated with their prostate biopsy results. The two collected mRNAs were then put together to calculate a patient specific ratio. The specificity of this PCA3:PSA mRNA ratio, to detect prostate cancer, was found to be 79% as compared to 28% specificity for use of serum PSA levels alone. Therefore, this study showed that analysis of multiple detection targets enhances prostate cancer diagnosis, allowing for earlier treatment. The study also showed 89% specificity in detecting normal patients, reducing the need for over diagnosis, as currently seen with PSA. The study also showed applicability of cancer recurrence screening (36).

Continuing with multiple detection targets, a study in India looked into the ratio of serum PSA as compared to the quantity of serum prostate secretory protein of 94 amino acids (sPSP94). Though the protein is not prostate specific, serum concentrations of sPSP94 are reflective of prostate secreted levels, with the study finding that concentrations were significantly lower in cancer patients as compared to those with benign conditions. Using ELISA, the team highlighted that the ratio of these two serum proteins (PSA:sPSP94) enhanced the specificity of prostate cancer patient identification in a patient study by 70.45%. The ratio test using both proteins together was even more sensitive than either protein alone (37). Though this was an ELISA test, it is possible that a microfluidic chip may be able to perform similar functions.

Given the success of these two studies, perhaps it is through the detection of multiple protein targets that microfluidics may be able to progress in its assistance with prostate cancer diagnosis and screening.

As we can see, though significant research has been employed to detect PSA using microfluidics, studies looking at prostate cancer specific detection is lacking, aside from the most recent studies, and those that have begun to employ multiple detection targets that do not currently use microfluidics. Therefore, perhaps the detection of an alternative prostate cancer biomarker may yield more significant promise in prostate cancer developments.

Circulating Tumour Cells

Circulating Tumour Cells (CTCs), after being both actively and passively secreted from tumours, enter blood vessels and can cause further metastases (38). The detection of these CTCs in patients allows for monitoring of cancer patients, as well as holding value in informing patients of prognosis and tailored treatment options (39). CTCs can be cancer specific, and the ability to detect prostate cancer specific proteins on CTCs has shown significant benefit above non-specific PSA detection in identifying patients suffering from prostate cancer. Microfluidics is a method that has thus been employed to both detect and analyse CTCs specific to prostate cancer.

Microfluidic detection of circulating tumour cells using antigens

Prostate Specific Membrane Antigen (PSMA), also known as folate hydrolase, is a glycoprotein expressed at increased quantities by prostate cancer cells, including CTCs, and its levels have even been noted to increase with both androgen independence and cancer aggressiveness (40, 41). In as early as 2009, a team in Louisiana attempted to use microfluidics to detect PSMA on CTCs using an immobilised anti-PSMA aptamer on a microfluidic chip. The aptamer was chosen because of its enhanced stability at room temperature as compared to antibodies. In this study, the prostate specific CTCs, after flowing through the microfluidic chip at optimal velocity, were counted by a conductivity sensor after release from the aptamer. Using this method, concentrations as small as 10 cells/mL were detected. However, a comparatively large volume of 1mL of whole blood was required in this study, which is not quite in keeping with the true purpose of microfluidics (42).

Building upon this method of PSMA detection, Esmael et al. designed an integrated permalloy-based microfluidic chip for the isolation of prostate cancer CTCs using an anti-PSMA antibody. Using this chip, the team were able to detect prostate cancer cells with high sensitivity, using a magnetic separation method which had increased sensitivity at a micro-scale once immune complexes had been formed. Though not a patient study, the team were able to detect clinically relevant concentrations of 20 cells/mL, at an acceptable throughput at 100 microlitres/minute, with almost 100% capture efficiency (43). This same team continued their work in 2019 with the same permalloy-based microfluidic chip with the addition of a multi-orifice flow fractionation filter (MOFF). This addition helped to improve upon their previous attempt, as the MOFF acted to remove free magnetic beads from whole blood samples before entering the microfluid chip. Prostate CTCs at concentrations as low as 5 cells/mL were captured in this study with up to 75% efficiency. The 100% magnetic capture ability was maintained in this second test, but this was also not a patient study (10).

As opposed to magnetic capture, other microfluidic technology has been fabricated for the trapping of CTCs, such as a label free electrochemical immunosensor for PSMA detection. This technique, employed in 2017 using gold nanoparticles functionalised with anti-PSMA antibody, showed significant ability to detect only PSMA positive cells as compared to cells from normal prostate tissue, creating a sensitive and specific ability to detect PSMA positive cells akin to CTCs (44). These studies thus far, clearly show the relative ease by which microfluidics can be employed to capture PSMA positive CTCs.

Moving forward from simple CTC detection, a team in New York after using microfluidics to detect CTCs, began to look at how this method could be used to aid therapeutics. Kirby et al utilised anti-PSMA antibodies on a geometrically enhanced immunocapture device to capture CTCs with high specificity towards PSMA+ cells. These CTCs were then treated with chemotherapeutic taxane agents such as docetaxel to monitor drug target engagement. The effects of docetaxel, in its actions to induce bundle formation through stabilising microtubules, could be readily detected by immunofluorescence staining. This experiment showed different responses of CTCs in prostate cancer patients who were docetaxel resistant and differential responses for a different taxane class – paclitaxel. This exciting research paves the way for monitoring drug target response and resistance through analysis of microfluid separated CTCs to optimise cancer treatment (45).

Aside from PSMA, researchers have attempted to detect and analyse CTCs with other antigens. In 2015, Khamenehfar et al. used a microfluidic chip to detect CTCs based on their unique ATP-Binding Cassette Transporters (ABCs). These ABCs provide cancer cells with multidrug resistance through their ability to actively transport therapeutic or toxic substances out of cells (46). The team were able to isolate CTCs whilst maintaining cell viability for further analysis, without using immune-affinity manipulations such as antibodies. Subsequent measurements could then be carried out on the isolated CTCs through inhibiting ABCs to analyse cellular affects, showing clear applicability to personalised patient centred medicine in the future (47). However, this study used mouse blood cells mixed with human prostate cancer cells to create a solution akin to CTCs and additionally the method assured no clear way of maintaining prostate cancer specificity in CTC separation. Although the study shows promise in principle, more work is clearly required.

A subsequent method of prostate cancer isolation of CTCs using microfluidics, employed in 2016, was immunofluorescence antibody staining for pancytokeratin, epithelial cell adhesion molecule and CD45. This research was performed on 50 patients undergoing radical prostatectomy for clinically localised prostate cancer only. Possible CTCs, via detection of these antigens, were detected in 50% of the patient cohort. The study did, however, acknowledge the uncertainty of whether the cellular matter detected were true CTCs or in fact malignant cells from the primary cancer. Interestingly, this study highlighted no correlation of detection of CTCs with other pathological risk parameters such as PSA. The research team recognised that monitoring through this method, even in localised cancer, may be of value to help in monitoring for metastatic potential, as well as sample analysis to help guide therapy (48).

Furthermore, a team based in Massachusetts who were also aware of the drawbacks of detection of CTCs using antigens alone created an innovative system to detect CTCs. The system created, involved the initial magnetic labelling of cells in whole blood before subsequently using a single microfluidic chip to separate, align and detect all cells appropriately. This study showed high sensitivity and specificity for detection of CTCs in prostate cancer patients, whilst allowing for a speed of 8mL per hour, with preservation of the cells for subsequent analysis. However, this system did rely on the fact that magnetic labelling was based on detection of three antibodies, so at least one form of these antigens was required to be present, for the system to positively identify a CTC (49).

Due to difficulties of the specificity of antigen detection on CTCs, a team in Wuhan attempted to create a novel solution. Armed with the knowledge that CTCs can have different metastatic stages within the blood, partly due to epithelial-mesenchymal transition, the team constructed a dual antibody functionalised microfluidic device that employed antibodies both against PSMA and epithelial cell specific markers. This method enabled enhanced capture efficiency of CTCs as compared to a single antigen capture system and CTCs were correctly identified in 83% of prostate cancer patients. Furthermore, the cell count of CTCs through this method, could be correctly correlated to the pathological stage of a patient's prostate cancer, which PSA levels alone could not do. Additionally, these isolated CTCs were not altered too much by their capture, and therefore could be further analysed to see their sensitivity to androgen deprivation therapy, immunotherapy, and metastatic potential. In this same study, it was also showed that PSMA expression was significantly higher in patients with cancer than those with benign prostatic hyperplasia and that PSMA expression was further increased in patients with a higher Gleason score or metastases (50).

A dual approach to microfluidic detection of CTCs was also adopted in 2013 to isolate prostate cancer cells. The technique used detected PSMA, whilst also using dielectrophoresis to enhance the specific immunocapture of prostate cancer cells, through use of an alternating current. The dielectrophoresis could be used on the basis that almost all cancer cell types have a dielectrophoresis frequency that is notably different from blood cells (51). In using these two means of cancer cell detection simultaneously, this technique showed significant promise in synergistically assisting cancer cell capture. This study highlighted the ability to ensure both high capture efficiency as well as purity at specific frequencies, such as 350kHz to isolate CTCs. The results were negatively influenced by the local shear stresses experienced by cells and the team did also acknowledge further work was necessary. However, this preliminary research enables further studies and research on metastasis and therapeutics (52, 53).

Microfluidic detection of circulating tumour cells using mechanical properties

Analysis of CTCs using biomarkers alone, such as antigens, can have significant drawbacks due to cellular change, thus indicating the need for other methods to detect prostate cancer CTCs. Therefore Renier et al collected CTCs that had become de-differentiated and lost epithelial characteristics, as well as some that were undergoing developmental transition, using a microfluidic vortex chip, that separated cells based on size (54). The team were also able to show significant correlation between the PSA level and number of CTCs isolated by this method. Additionally, unlike other methods available of separating cells based on size, this method kept cells viable and intact. However, this method did not isolate all CTCs and the research had a capture efficiency of approximately 24.5% of a prostate cancer specific cell group. Even considering this low capture efficiency, the team did conduct subsequent cellular analysis on the cells collected and highlighted that an antigen method for detection of CTCs is limited due to the changing nature of the cells. Thus, a method of label free isolation, or perhaps recognition of all possible antigens, may need to be employed to ensure sensitive capture of CTCs (54).

Similarly, an international team in 2012, attempted to refine a mechanical method of recovering CTCs using three different free fluorescently immunolabelled prostate cancer cell

lines within a mixture of white blood cells. The team were able to separate the CTCs based on size through ultrasonic acoustophoresis in rigid microfluidic channels whilst maintaining cell viability. The technique used three fundamental characteristics of size, density, and compressibility to aid cell separation. Their experiment was also enhanced by a novel pre-alignment procedure that improved separation efficiency, and an optimal channel width in the microfluidic system. The team found that there was increased ability to retrieve cancer cells at higher acoustic energies. However, the team also found that recovery of cells varies based on the prostate cancer cell line used, and not all white blood cells could be removed entirely from the tumour cells, indicating that other cellular attributes such as mass density may also have a role in separation, which acoustophoresis alone could not separate. The team noted that refinement was required to enable detection of all CTCs at clinically relevant low concentrations of cells (55).

Ren et al also attempted to separate CTCs based on specific biophysical properties, after noting the issue of antigen separation alone. Their study specifically looked at isolation of CTCs based on size and deformability. They used a microfluidic multiple row-based device that could trap cells with 96% efficiency, whilst retaining the cell shape. However, in this case, a specific prostate cancer cell line mixed with rodent whole blood was used, so further investigation is needed prior to patient tests. (56)

In conclusion, with regard to CTCs, more work is required to ensure detection that is sensitive and specific to allow for effective reproducibility and marketability. For a microfluidic system to be safely used for screening, there would need to be a low false negative rate. With some CTCs undergoing transition and not displaying certain biomarkers, a more significant study on patients would be required using one of the methods employed above, or otherwise, to ensure patient safety. CTCs also have a significant drawback, in that unless they are being produced by a prostate cancer (i.e. it has metastatic potential) it may be of limited use. However, it is important to remember that once isolated these CTCs would have potentially limitless potential in enabling patient specific therapeutic tests and analysis.

Microfluidics to aid with prostate cancer physiology and management

Aside from detection of prostate cancer through means such as PSA and CTCs, vast amounts of research have been undertaken into using microfluidics to assess some specific aspects of prostate cancer, namely its physiology and management.

Microfluidics and prostate cancer management

In 2014, microfluidics was used to assess the effects of combination chemotherapy on a prostate cancer cell line in Korea. A microfluidic system was designed to both assist in drug screening against patient cells whilst helping in optimisation of drug concentrations, to not affect non-cancerous cells. A microfluidic high throughput drug screening program was created through a platform consisting of eight different concentrations of two chemo-therapeutic agents to create 64 chambers with 64 varying concentrations of the two agents. This study utilised the two agents, curcumin (a natural component thought to enhance chemotherapy cell responsiveness) and tumour necrosis factor related apoptosis inducing ligand. The system had the benefits of low cost, not requiring continuous perfusion after the wells were appropriately filled, and reduced shear stress on the cells. This method was shown

to produce valuable results on effective therapeutic concentrations and was proven to be more effective than using a well plate method to find optimal therapeutic concentrations for prostate cancer cell effect. The system also held significant advantages in the frequent replenishment of fresh media and drug solution. (57)

Within the same year a team in Michigan used microfluidics to create a prostate cancer cell line clonal expansion before characterising sub-phenotypes to assess response to drug treatments. The technique offered advantages in comparison to the conventional cell assays which allowed only an average representation of cell lineage cell types at high cost and labour, whilst analysis on a smaller scale avoids this and the lineage of even a single cell from microwell in a microfluidic chip could be traced. The team were able to isolate single cells in the microfluidic chip, before studying them and the phenotypes of their progeny prior to assess their chemotherapeutic responsiveness. To maintain cell growth, a continuous flow at a rate of 2 microlitres/hour was maintained. The ability to double was seen to be like that of petri-dish culture and migration between microwells was prevented. As had previously been seen in cancer cell development, three different sub phenotypic clones were isolated in this method from the single cells, holoclones, meroclones and paraclones, which each exhibited its own morphology and growth rate (58). Each phenotype was then tested against different chemotherapeutic agents such as docetaxel with varying response, thus highlighting the clinical use of this method to isolate and track the response of a single patient's cancer cells to therapeutic agents, even as the cells may develop (59) .

Furthermore, with regards to therapeutics and novel treatment analysis, a team in 2011 isolated prostate non-coding serum microRNAs (miRNAs) from humans using a multiplex quantitative reverse transcription PCR method. Similar to proteins found in disease and normal physiology, miRNAs are thought to be significant biomarkers of pathology, whilst allowing analysis of prostate cancer genotypes (32). After isolation, the miRNAs underwent uniplex analysis on a microfluidic chip to analyse the miRNAs further, highlighting oncogenic and tumour suppressive miRNAs in untreated prostate cancer patients. This method helped to both distinguish healthy patients from those at high risk but also those patients at curable stages of prostate cancer. The purpose of this was to allow for a more prostate cancer specific biomarker and to permit better individualised patient care based on the analysis (33).

Microfluidics and prostate cancer physiology

Microfluidics has also been proven to play a key role in understanding high propensity to metastasise to bone, which occurs in up to 90% of those patients with metastases (60).

Starting in 2009, a team in Michigan looked at the microenvironment of prostate cancer metastasis to bone using microfluid generated spheroids. Spheroids, as sphere shaped groupings of cells, act as excellent tumour models and provide reliable therapeutic information (61). Using a technique previously to form embryoid bodies, the spheroids were formed using microfluidics, to create a 3-dimensional in vivo bone microenvironment (62). The spheroid microenvironment, created using osteoblasts and endothelial cells, mimicked bone tissue that a metastatic prostate cancer cell could reside in. The prostate cancer cells were then transfected with a fluorescent protein to track their numbers within each spheroid during the experimental period, during which they were able to survive and proliferate. Though the proliferation rate of the cells was reduced as compared to the normal 2-

dimensional in vitro studies, it was thought that this was an effective mimic of what happens in vivo. Additionally, the viability of the cells was not affected, and so the behaviour of the prostate cells could effectively be studied. In fact, some of the cells lines even died comparatively when a monoculture was done in the 3D study environment, revealing that the spheroid created by the microfluidic chip has non-additive synergistic effects. The team acknowledged that this research may show utility at a later stage for testing of drugs in an in vivo like environment (63).

Furthermore, a team in 2014 used a microfluidic platform with multi-photon imaging techniques to assess prostate cancer and found that an osteotropic prostate cancer cell line, when with bone stromal cells, had increased physiological function. This was detected through an increase in fluorescence, likely due to increased cytoplasmic enzyme activity, which permitted creation of reactive oxygen species. This increase in reactive oxygen species, allowed for an overall increase in protrusive phenotype and potential invasive capacity of the prostate cancer cells in the bone environment. In addition to highlighting how bone metastases can proliferate, this technique offered multiple advantages in allowing multiple assays to be performed on one device, with relative ease and low-cost customisation. Microfluidics thus clearly offers vast possibilities for further prostate cancer model design and analysis. (64)

Building on attempts to understand prostate cancer in specific environments, a team in Arizona developed a partial microfluidic based model of the prostate gland, to create a unique 3-dimensional microenvironment. The model used two stacked microchannels separated by a porous membrane which allowed long term cell cultivation within a microfluidic chip. The membrane permitted the culture of two separate cell populations on either side (stromal and epithelial cells), which permitted paracrine signalling without cellular crossing. This environment allowed for study of the epithelial stromal interface lining the ductal systems of the human prostate gland. The system involved the flow of culture media at an appropriate rate to not disturb communication between the two sections, whilst optimising growth and differentiation. As a transparent device the microfluidic chip permitted bright field and fluorescence imaging throughout. By the end of the experiment, groups of cells within the chip appeared to begin to show features resembling prostate tissue architecture. The effects of various hormones could be studied throughout the research. Though not directly involved in prostate cancer research at this stage, the team were better able to understand paracrine and endocrine cross talk within the model which was hoped to permit further investigation. This research may therefore hold a key to better understanding morphogenesis and disease development, in appropriately recapitulating the biology and physiology of the carcinogenic human prostate (65).

Khanal et. al in 2014 undertook more specific research about the prostate cancer microenvironment and worked to analyse the effect of hypoxia on prostate cancer. They did this through using a microfluidic device to test the susceptibility of cancer cells to the chemotherapeutic agent staurosporine. Hypoxia is a known critical feature of the tumour microenvironment (66). Analysis on the microfluidic chip offered significant advantages in increasing temporal and spatial resolution, ensuring high throughput analysis, as well as the ability to sense oxygen via dyes (67). The conclusion of the study was that hypoxic preconditioning acted to increase drug resistance with statistical significance, as measured by

fluorescent staining to identify cellular apoptosis. The apoptotic cells could be sensed as early as one hour after drug treatment. The study also highlighted that even just one hour of hypoxia is enough to enhance cancer resistance to therapeutic agents. Thus, this microfluidic format can be used to assess therapeutic agents in hypoxic environments, as those studies performed at a normal oxygen level are likely not a true indicator of chemotherapy effect *in vivo* (68).

A research team in the previous year also looked at the key role of hypoxia in cancer, by analysing 8-hydroxy-2'-deoxygyanosine (8-OHdG) as a product of DNA oxidation damage and mutation in prostate cancer. 8-OHdG is a chemical product that can be detected in the urine as a marker of oxidative stress associated with prostate cancer. A small detection limit of 5pg/mL was found using their microfluidic device designed for the specific purpose to estimate oxidative DNA damage. This device used modified paramagnetic particles with monoclonal antibodies to allow for immunoextraction and immunodetection, with electrochemical detection of the 8-OHdG determined with 1-naphthol. As a patient based study it was expected that this experimental design will have future clinical application in personalised therapies (69).

Looking further at hypoxia, research has shown that the uptake of cancer associated fatty acids, such as palmitic acid is increased in hypoxic conditions and can stimulate progression of cancer (70). Subsequently, a microfluidic system was created with a Raman microscope to optimise real time measurements in variable conditions, to show that uptake of omega 3 fatty acids such as docosahexaenoic acid reduced the uptake of cancer stimulating fatty acids by prostate cancer cells. Microfluidics added to the work in enabling study of the biological system in a natural environment, with dynamic flow provided by the microfluidic system to permit in vivo physiological conditions (71). This research holds significant value for further research analysis in both highlighting the value of hypoxic studies and in understanding cancer effects at a cellular level.

Whilst hypoxia can induce significant change in cancer cells, androgens can also induce significant change in prostate cancer so that it becomes refractory to further androgen therapy, and becomes androgen independent (72). Therefore, research was conducted into a comparison of androgen sensitive and insensitive human prostate cancer cells to analyse their mechanical properties. In this study, a microfluidic chip was designed to deform tumour cells so that they could subsequently be analysed. The team found that androgen insensitive prostate cancer cell lines are mechanically stiffer than their androgen sensitive counterparts, and these properties can be used as biomarkers for early androgen independent prostate cancer identification. Results also suggested that cell size and deformation degree were also potential parameters for classifying cell type. It was hypothesised that this change in mechanical durability was due to the androgen therapy causing change in the mechanical properties of the cell. Though the technique was primarily used to understand how androgen deprivation therapy may alter cells so the therapy is no longer effective, it may have further applications in suggesting when the therapy is no longer as effective at a cellular level (73).

It can therefore be seen that the use of microfluidics is not limited to detecting prostate cancer, but it is also being used to understand how the cancer functions at a cellular level.

Ultimately, microfluidics will help ensure patients receive optimal treatment based on the physiology and genetics of their own specific cancer.

Future directions

Looking into the future, microfluidics will likely aid detection of many new-found prostate cancer biomarkers. Examples of these include exosomes, found in various body fluids including urine, and deemed important cancer biomarkers in liquid biopsy (57, 74). Exosomes are endosomal-derived vesicles released from cells, including cancer cells, that contain bioactive material, such as RNA, actively extruded from cells (75). In one study, exosomes were found to contain non-coding RNA, miR-409, which was found at high levels in prostate cancer cells. Upon release into the tissue environment, miR-409 is able to promote intracellular and extracellular changes in normal prostate cells to promote tumour induction (76). Furthermore, a study in 2009, analysing the RNA contents of exosomes from prostate cancer patients, found that the biomarker PCA3 was overexpressed in prostate cancer urinary exosomes (77). Additionally, the research showed that the mere presence of other biomarkers such as PSA mRNA could indicate the patients status of treatment (78). These two studies highlight the significant potential of exosomal content analysis in acting as a potential cancer biomarker for prostate cancer diagnosis.

As mentioned above, exosomes, likely shed from the urinary tract, have been found in urine of patients with prostate cancer. These exosomes from prostate cancer patients have even been shown to have a unique lipid composition, so further suggesting their potential as cancer biomarkers (79). Work in 2016 highlighted that the lipid content of urinary exosomes could be analysed, and significant differences could be seen in the exosomes isolated from prostate cancer patients as compared to a normal population. Through use of high throughput mass spectrometry, exosomes from prostate cancer could be identified with 93% sensitivity and 100% specificity. As this involves urinary exosomes, if such a test were to become validated, it would be ideal as a point-of-care test and it is possible that a microfluidic chip could be designed to carry out the process (80).

Aside from their lipid content, the protein content of exosomes in prostate cancer patients has also been shown to be unique. A research team based in Norway, compared the protein content of urinary exosomes of 16 prostate cancer patients with that of 15 control patients. With a high level of sensitivity, this team found that multiple proteins on the exosomes could be used as prostate cancer biomarkers, as they were either upregulated or downregulated. The team hypothesised that combination testing for these proteins in a multi-panel test had the potential for clear identification of prostate cancer patients from control subjects (81). We have therefore seen how the RNA, lipid, and protein content of exosomes from prostate cancer patients is notably unique and may thus constitute novel prostate cancer biomarkers if they can be appropriately detected.

One of the challenges of working with exosomes and other Extracellular Vesicle (EV) subtypes is the heterogeneity of the vesicles. Although this may not be as marked for exosomes as with microvesicles (or medium-sized EVs) there are still variations in terms of size, morphology, lipid composition, even refractive index that need to be considered for isolation and enumeration (82). Biofluids may also contain non-EV particulate matter such as

lipoproteins and protein aggregates. With many avenues now available for isolation of cancer specific exosomes however, work has already begun into microfluidic separation of exosomes from whole blood samples using acoustic waves, in what is referred to as an acoustofluidic platform. This platform was able to achieve high yield results of exosomal separation of 99%. However, in this study the team did note that more work was required to ensure exosomes obtained have the highest purity (83). Unfortunately, this study was neither a cancer study nor did it highlight differentiation between exosomes isolated, so more work would be required prior to use of this platform in prostate cancer testing.

Moving on from acoustic separation, an Australian team created a microfluidic device for exosome detection using a tuneable alternating current electrohydrodynamic method that relied on shear forces. This technique generated flow on the microfluidic chip that enhanced capture specificity. Using this method, the team were able to isolate exosomes from cells expressing PSA with high specificity and selectivity. Though most of the investigations in this study resolved around breast cancer, PSA was also tested for, highlighting the applicability of the separation method to prostate cancer (84). It is likely not long before this technique could be used to further prostate cancer diagnosis and therapy.

A non-microfluidic mean of exosomal isolation includes the use of the previously mentioned surface enhanced Raman Scattering technique for rapid and label free detection of exosomes. This technique was able to discriminate between exosomes originating from cancer cells and normal cells, based on their SERS spectral patterns, with a sensitivity greater than 95%. Combining SERS with chemometrics and machine learning algorithms could facilitate the rapid differential diagnostics, treatment-response monitoring and personalised medication of the disease. Though the method did not analyse prostate cancer cells specifically, it is not far reaching to suggest that microfluidic techniques could be combined with SERS to permit prostate cancer exosome detection, given the success of the aforementioned methods (85).

For isolation of specific exosome subsets derived from particular cell types, specificity and sensitivity can be increased by use of immuno-affinity selection using antibodies conjugated to magnetic beads or with an antibody-conjugated surface (86).

The possibility of isolating specific urinary exosomes and therefore of using liquid biopsy (with a reduced number of isolation steps compared to conventional methodologies) as a way of detecting the presence of malignant cells, will bypass the need for invasive tissue biopsies in prostate cancer and simplify screening of those at risk of developing disease. As research continues into exosomes and their utility as cancer biomarkers, it is likely that microfluidics, with all the benefits it can provide, will become increasing used in detection and analysis.

Conclusion

Based on the vast number of research studies undertaken in the last few years, applications of microfluidics in prostate cancer are greatly increasing, most likely driven by an international drive to prevent mortality and morbidity. The cost-effective nature of microfluidics, together with the easily modifiable simple device design makes it an ideal candidate for a variety of uses in treating and researching prostate cancer.

This review has demonstrated multiple routes of prostate cancer detection in the form of CTCs, demonstrated that point-of-care testing will aid future rapid diagnosis of many conditions, and that microfluidics should enable this to happen at an affordable price. Furthermore, microfluidic testing on cancer cells to highlight their chemotherapeutic susceptibility and physiology will also play a crucial role moving forward.

Unfortunately, prostate cancer morbidity and mortality is increasing internationally. However, through ongoing microfluidic research, it is hoped that patient diagnosis and personalised treatment can be vastly improved to help ensure better outcomes.

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Conflicts Interests

The authors declare no conflicts of interests

Key Points

- Use of microfluidic systems in the diagnosis and management of prostate cancer is rapidly increasing to enhance the capacity for point-of-care testing.
- Prostate specific antigen can be detected by microfluidic techniques but problems remain with cancer specificity of detected samples, though multiple avenues are available to circumvent the challenge posed.
- Circulating tumour cells can also be detected by microfluidic techniques to appropriately detect cells based on physical properties as well as antigen markers.
- Once isolated, cells can be analysed to assist in better understanding the physiology and management of prostate cancer in a wide variety of ways.
- Future directions for prostate cancer detection with microfluidics include exosomal detection which will also aid in understanding of the cancer.

Table 1. TRL (Technology Ready Level) and definitions of each level.

TRL	Definition
1	Scientific research is beginning, and those results are being translated into future research and development.
2	The basic principles have been studied and practical applications can be applied to those initial findings.
3	Generally, both analytical and laboratory studies are required at this level to see if a technology is viable and ready to proceed further through the development process.
4	Once the proof-of-concept technology is ready, the technology advances to TRL 4. During TRL 4, multiple component pieces are tested with one another.
5	A technology that is at TRL 5 is identified as a breadboard technology and must undergo more rigorous testing than technology that is only at TRL 4.
6	The technology has a fully functional prototype or representational model.
7	TRL 7 technology requires that the working model or prototype be demonstrated in a clinical environment.
8	TRL 8 technology has been tested and "clinically proven" and it is ready for implementation into an already existing technology or technology system.
9	When a technology has been "clinically proven" and widely implemented, it can be called TRL 9.

Table 2. Review of the available microfluidic platforms for PCa investigations.

Target biomarker(s) / LoD	Sample	Integrated LUOs	Time	TRL	Detection Strategy	Reference
PSA/140, VEGF/90, ERG/15, IGF-I/13, IGFBP-3/130, CD-14/150, PEDF/90 and GOLM-1/15 pg/mL	5x diluted calf serum spiked with target proteins	Incubation, washing and detection	30 min	4	Electrochemical Sandwich Immunoassay with magnetic beads (Ab2-HRP-MB)	Mercer et al. 2019 (87)
small RNAs (<30 nt)	10 ml of sera from volunteers	cluster amplification with bridge PCR (Solexa, Illumina)	N/a	9	Identity of each base of a cluster is read off from sequential images	Chen et al. 2008 (32)
miR-19a, -20b, -24, -26b, -30c, -93, -106a, -223, -451, -874, -1207-5p, and -1274a were shortlisted a/r expression screening	human serum from 38 donors: 9 healthy, 9 low, 11 intermediate, 9 high CARPA scores	dRT-PCR: thermal cycling, image acquisition, analysis	N/a	9	TaqManProbe using Fluidigm Biomark platform 96 x 96 arrays	Moltzahn et al. 2011 (33)
PSA/0.5 ng/mL	human serum	Incubation and detection	30 min	4	Amperometric Sandwich Immunoassay, secondary Ab coupled to HRP	Panini et al. 2008 (20)
PSA/10 pg/mL	PSA-spiked buffer	immobilisation, detection	7 s	3	Cyclic voltammetry, competitive displacement of GOx-PSA from immonopatterned nanoelec.	Triroj et al. 2010 (9)
PSA/0.84 pg/mL	human serum	immobilisation, detection	20 s	4	Chronamperometry, immunoassay magnetic beads PSA-specific Ab1 (M-Ab1), and Ab2-HRP	Chen et al. 2019 (21)
PSA/10 ng/mL	human serum	immobilisation, detection	< 1 hr	4	Love-wave Inter Degitated Sensor using aptamers as capture probe for PSA	Zhang et al. 2015 (22)
PSA/0.31 pg/mL	human serum from 7 donors	immobilisation, detection	20 min	4	rGO-BiFeO3-based photo electrochemical sensing magnetic controlled system	Zhou et al. 2018 (23)
PSA/0.04 ng/mL	whole blood from 3 patients	manual reagent priming of capillaries (MCF), detection phone	13 min	4	Fluorescent assay using ELISA inside surface modified capillaries fluorocarbon (MCF)	Barbosa et al. 2015 (25)

fPSA, tPSA / <0.1 ng/mL	human serum from 5 clinical samples	droplet generation & immunoreaction, magnetic immunocomplexes form., magnetic isolation immunocomp., SERS detection	N/a	5	SERS-based immunoassay technique using parallel microdroplet channels	Gao et al. 2018 (29)
PSA/0.01 ng/mL	human serum	formation, isolation, and purification of immunocomplexes, and SERS detection	5 min	6	Sandwich Immunoassay based on SERS nanotags, PSA biomarkers and magnetic beads	Gao et al. 2019 (31)
PSMA on PCTCs/ LoD not reported	20 peripheral blood samples from patients with castrate-resistant prostate cancer	Geometrically enhanced differential immunocapture (GEDI)	n/a	4	FITC-conjugated J591 Ab, specific to PSMA+ cells, fluorescence immunoassay	Gleghorn et al. 2010 (41)
PSMA/ enrichment factor 2.5e8	1 mL of blood spiked with LNCaP cells	capture of PCTCs through aptamers on high throughput micro-sampling unit	29 min	5	Trypsin, followed by conductivity cell count on-chip	Dharmasiri et al. 2009 (42)
PSMA in sln. or PSMA+ cells	serum from mice	Capture on amino functionalised gold nanoparticles with Abs onto ITO electrodes	10 min	4	Differential Pulse Voltammetry with a redox couple $[Fe(CN)_6]^{3-}$/$[Fe(CN)_6]^{4-}$	Seenivasan et al. 2017 (44)
PCTCs expressing PSMA	whole blood	geometrically enhanced differential immunocapture (GEDI) with PSMA-Ab	24 hr	3	Staining on-chip, fluorescent analysis, off-chip enumeration	Kirby et al. 2012 (45)
CTCs	human PCa cells spiked in mouse blood	isolation and drug testing on a single cell, size-based separation, e-trap	> 1 hr	3	Fluorescence m-nt of drug accumulation (DNR ± FITC)	Khamenehfar et al. 2015 (47)
CTCs	2 mL blood from 50 patients, localised PCa	Separation based on size, deformability using microfluidic ratchet for enrichment	n/a	5	Off-chip immunostaining analysis, enumeration	Todenhöfer et al. 2016 (48)
LNCaPs (PSMA+ cell line)	mixture of LNCaPs and PBMCs (from blood)	dielectrophoresis for selective CTC isolation capture onto J591 Ab	n/a	3	DEP characterisation of cells	Huang et al. 2013 (52)
CTCs	whole blood (7.5 mL) from 22 patients with stage IV PCa	size-based separation and recovery of cells using inertial microfluidics	< 1 hr	4	Off-chip immunochemistry, enumeration and mutation analysis	Renier et al. 2017 (54)
CTCs / 72.5 to 93.9% recovery	erythrocyte-lysed blood from healthy volunteers spiked with DU145, PC3, LNCaP PCa-cell lines	continuous, size-based separation using acoustofluidics	< 1 hr	4	Off-chip gene expression profiling with RT-PCR	Augustsson et al. 2012 (55)
CTCs	murine whole blood spiked	early stage CTC enrichment through trapping into	30 min for 1.2	3	On-chip microscopy and enumeration of	Ren et al. 2018 (56)

	with LNCaP-C4-2 prostate cancer cells	microchannels	mL sample		the cancer cells that express GFP	
CTCs / 86% capture efficiency	blood from healthy donors MCF-7 and LNCaP diluted in PBS (1:9, vol/vol)	capture using dead-end filtration via microfabricated Parylene device	< 5 min	4	Immunofluorescent analysis on-chip, confocal microscopy, and SEM	Zhang et al. 2011 (22)
Drug development	Chemotherapy experiments on PC3 cell grown onto 8x8 array	concentration gradient formation, integrated pumps, valves for cell proliferation and drug testing	7 days culture	5	Immunostaining and microscopy on-chip	An et al. 2014 (57)
Heterogeneity study	PC3 cells cultured onto microfluidic chip 8x8 array	gravitational cell seeding and flow, cell migration ctrl geometries, drug screening	6 days culture	5	Imaging of the growth chambers	Chung et al. 2014 (59)
Understanding PCa metastasis to the bone	PC3 cells co-cultured with osteoblasts, endothelial cells (28 wells / chip)	perfusion 3D cell culture system for seeding, proliferation, and microscopy	7 days culture	4	Imaging phase-contrast and fluorescent microscopy from each well	Hsiao et al. 2009 (63)
Paracrine & endocrine cross talk between basal and luminal cells	PrECs and BHPrS1s grown separated by a membrane	prefusion 2D cell culture proliferation differentiation, immunohistochemistry for morphological, biochemical aspects	21 days	5	Immunostaining for androgen receptor expression bright-field and florescence imaging	Jiang et al. 2019 (65)
Hypoxia-induced Staurosporine Resistance	PC3	cell proliferation and hypoxia	7 days culture	5		Khanal et al. 2014 (68)
Uptake of deuterated fatty acids	PC3	cell proliferation under normoxic and hypoxic conditions	10 days	7	BioFlux chip coupled with a Raman microscope	Tang et al. 2020 (71)
Mechanical phenotyping of PCa cells	LNCaP, DU145, and PC3	real-time deformability, imaging	N/a	4	High-speed imaging & Image analysis	Liu et al. 2019 (73)

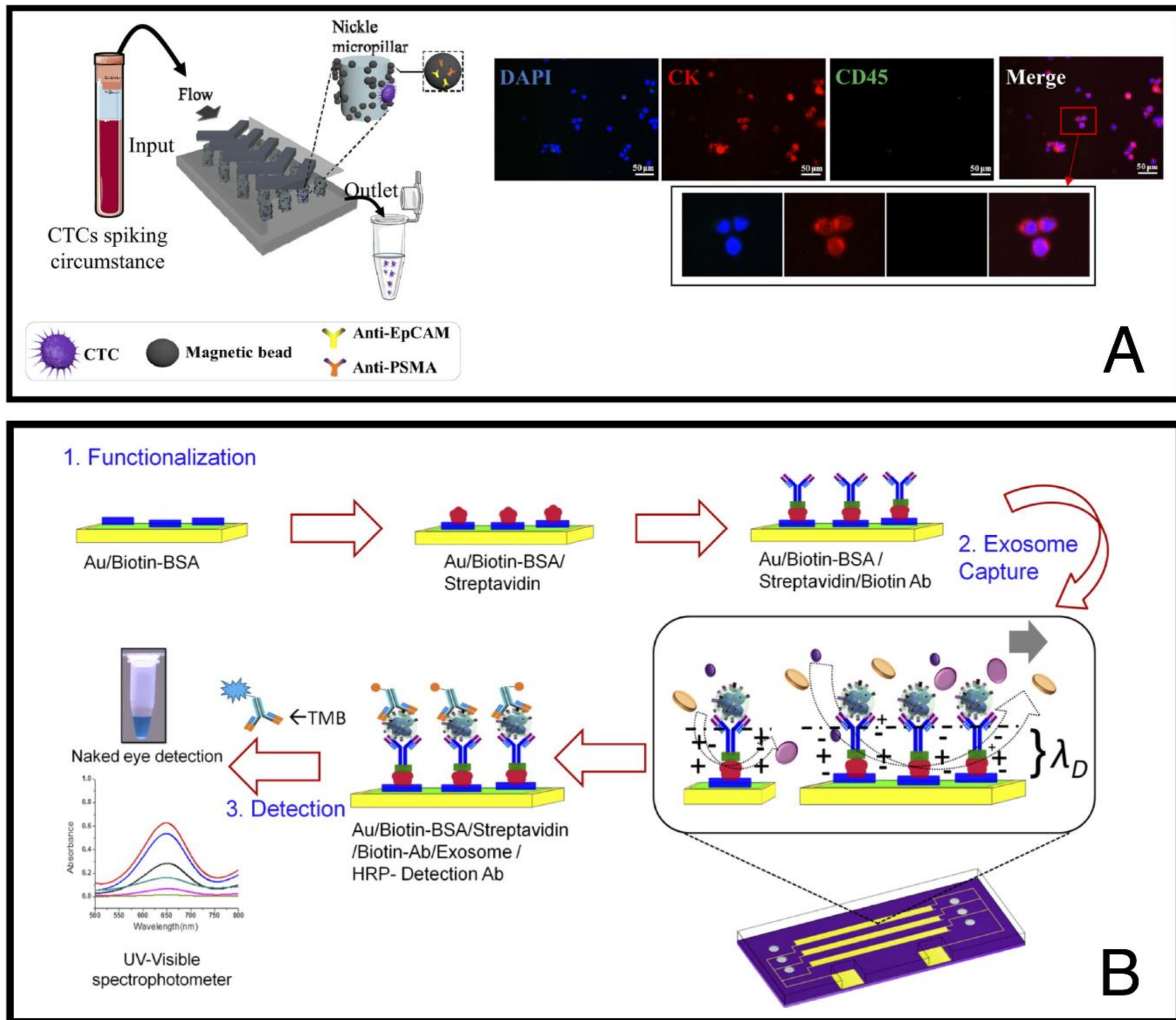

Figure 1. Antibody capture on microfluidic device.

(A) Schematic illustration of the dual-antibody-functionalized microfluidic device where antibodies against prostate-specific membrane antigen (anti-PSMA) and epithelial cell adhesion molecule (anti-EpCAM) are used to capture CTCs. Shown are fluorescent micrographs of CTCs captured from the LnCAP cell spiked normal blood samples (50). Reprinted with permission from Yin C, Wang Y, Ji J, Cai B, Chen H, Yang Z, et al. Molecular Profiling of Pooled Circulating Tumor Cells from Prostate Cancer Patients Using a Dual-Antibody-Functionalized Microfluidic Device. Anal Chem. 2018;90(6):3744-51. Copyright 2018 American Chemical Society.

(B) Schematic representation of device functionalization whereby gold (Au) microelectrodes are modified with biotinylated bovine serum albumin (Biotin-BSA), streptavidin and capture antibody (eg: anti-PSA). Following exosome capture a horseradish peroxidise conjugated detection antibody (HRP-Detection Ab) is introduced followed by a chromogenic substrate (TMB). The resultant colorimetric reaction is visible with the naked eye and can be measured with a spectrophotometer (84). Reprinted with permission from Vaidyanathan R, Naghibosadat M, Rauf S, Korbie D, Carrascosa LG, Shiddiky MJ, et al. Detecting exosomes specifically: a multiplexed device based on alternating current electrohydrodynamic induced nanoshearing. Anal Chem. 2014;86(22):11125-32. Copyright 2014 American Chemical Society.

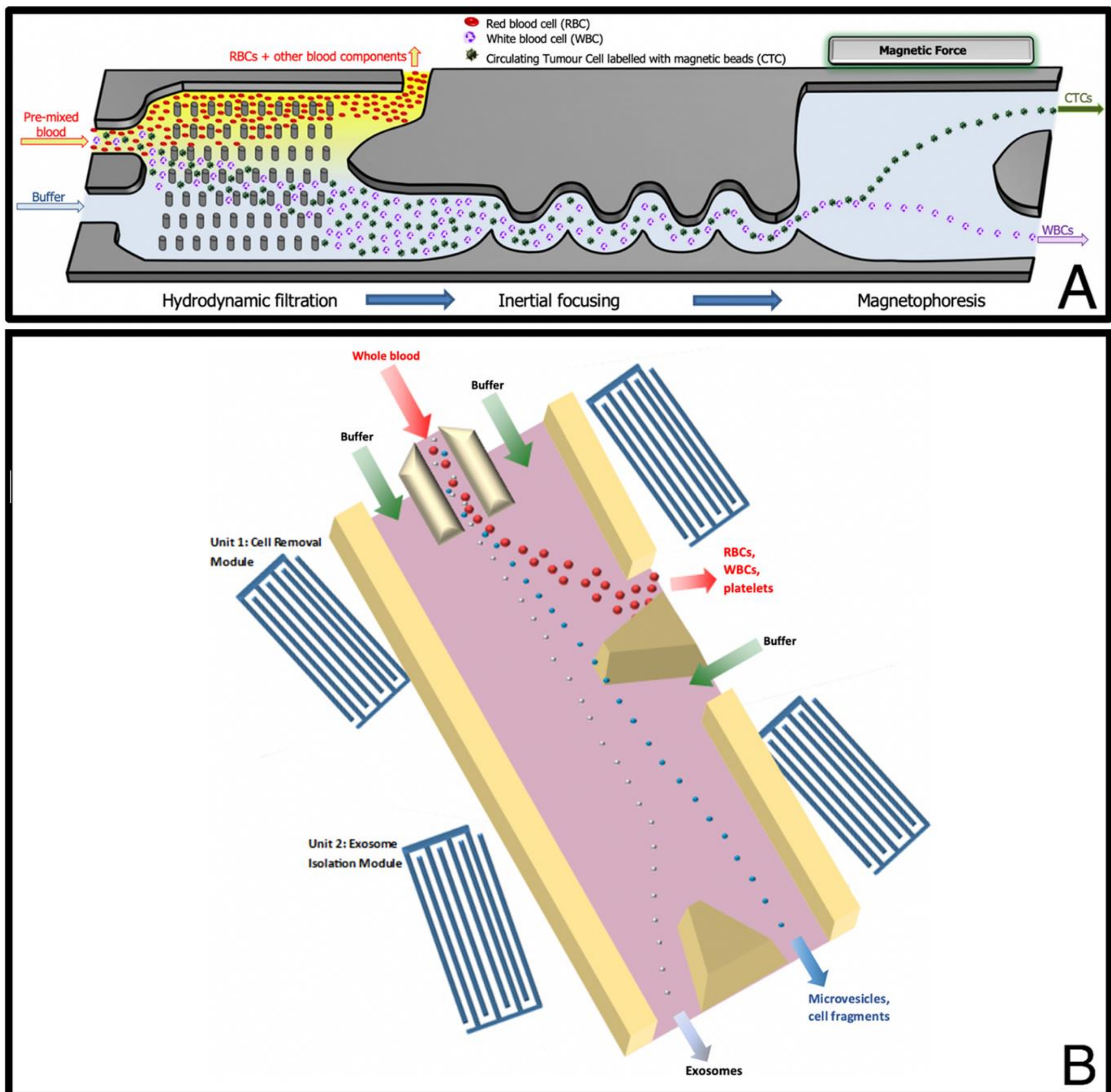

Figure 2. Continuous flow separation microfluidic devices.

(A) Schematic representation of three microfluidic components of the CTC-iChip. Input of blood premixed with magnetic beads is introduced alongside buffer. Hydrodynamic filtration occurs by deterministic lateral displacement. Inertial sorting and magnetophoresis results in CTC separation and isolation (49).

(B) Schematic illustration of integrated acoustofluidic platform for direct isolation of exosomes from whole (unlabelled) blood. The tilt of the modules allows size-dependant lateral deflection of particles by acoustic forces. Two surface acoustic wave modules act in sequence. Unit 1 separates larger cells including red blood cells (RBCs), white blood cells (WBCs) and platelets from whole blood. Unit 2 isolates smaller exosomes from remaining extracellular vesicles (83).