# Circulating Tumor Cells in Point-of-Care Settings

Subjects: Medical Laboratory Technology

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Circulating tumor cells (CTCs) are cells that have been shed from tumors and circulate in the bloodstream. These cells can also be responsible for further metastases and the spread of cancer. Taking a closer look and analyzing CTCs through what has come to be known as “liquid biopsy” has immense potential to further researchers’ understanding of cancer biology.

circulating tumor cells immunoaffinity microdevices liquid biopsy

## 1. Circulating Tumor Cells (CTCs) Isolation Techniques

### 1.1. Immunoaffinity

The most widely used method of CTC isolation, immunoaffinity, works by using antibodies to bind to specific antigens present on cells in order to either target or weed out those cells from a sample. Positive immunoaffinity works by targeting antigens present on CTCs in order to create a filtered sample with more CTCs, whereas negative immunoaffinity is the converse; it discriminates by targeting antibodies present on normal blood cells present in the sample. To overcome the limitations of CellSearch, many technologies have taken its anti-EpCAM-targeting approach and conjugated it with another aspect of CTC isolation. A dual-immunopatterned microfluidic device maintained anti-EpCAM selection in addition to the use of an artificially developed anti-63B6 antibody [1]. The anti-63B6 antibody was able to target mesenchymal-stem-cell-like cancer cells and intermediate cancer cells, a population that is often omitted in strictly anti-EpCAM selection, thus increasing capture efficiency. Manipulation of device shape has often been used as well. A wavy herringbone pattern was implemented in one device that used anti-EpCAM selection with magnetic beads in order to aid the isolation process [2]. The device proved successful in increasing capture efficiency and purity but was limited by the unequal dispersion of magnetic beads and purely anti-EpCAM selection. Similarly, a spiral-shaped microfluidic channel that targeted anti-EpCAM-expressing CTCs with magnetic beads used a purposefully shaped channel in addition to magnetic force as a tool to increase the capture efficiency and flow rate, yet faced the same issue with regard to non-anti-EpCAM-expressing CTC capture [3]. To increase the purity, one method used platelet–leukocyte-membrane-coated immunomagnetic beads [4]. The beads inherited the ability of platelets to interact and assist binding to CTCs in addition to the capability of the leukocytes to reduce homologous leukocyte interaction. This added to the purity, but the applicability of any overall method used with the beads is still hindered by anti-EpCAM capture limitations. While these devices generally display increased capture efficiency as compared to purely anti-EpCAM-targeting methods, they often require greater setup or cost and still have difficulty with non-EpCAM-expressing CTCs. One

attempt to curb this problem implemented the use of beads coated with the malaria rVAR2 protein to bind with oncofetal chondroitin sulfate, which is expressed on both epithelial and mesenchymal CTCs [5]. Though a high sensitivity and capture of both epithelial and mesenchymal cells was achieved, a similar problem to anti-EpCAM selection was posed where not all CTCs have strong, or any, expression of oncofetal chondroitin sulfate. Therein lies the flaw in immunoaffinity as a sole solution.

## 1.2. Purely Physical Cell Characteristics

To avoid the restrictions of immunoaffinity, namely the inability to target cells that lack expression of a particular antigen, many recent approaches to CTC isolation have shifted towards the use of physical cell characteristics. The most common of these characteristics is cell size. Compared to normal blood cells, CTCs tend to have larger diameters, which makes size-based discrimination a useful tool for enrichment. With microfluidic technologies, these methods require the patternization and structure of the device. A so-called Labyrinth device was created that achieved a capture efficiency of greater than 90% when using blood samples from breast and pancreatic cancer patients [6]. The purity was also very high, as the device's strategically placed loops and curves with differing diameters were able to separate CTCs from leukocytes and other blood cells based on their inertial movement due to size/deformability. Another approach used the strategy of cell flow to discriminate based on size. When blood samples from prostate cancer were siphoned through, the CTCs would react differently upon reaching the vortices due to their larger size and would be separated from the rest of the sample. Though this device had an extremely high throughput, the efficiency was lower than similar technologies. A largely untapped problem of isolating CTC clusters as opposed to only single cells was resolved by a cluster-isolating device that used deterministic lateral displacement and a microfluidic panel with meticulously spaced micropillars to discriminate clusters and single cell CTCs from a spiked blood sample based on asymmetry and size [7]. This method, while displaying the rare ability to isolate clusters successfully and maintaining cell viability, has a comparatively low throughput, which limits the possibility of clinical use. Other technologies are specifically targeted towards simplicity in a clinical setting. One photosensitive polymer-based microfilter is able to be attached directly to a blood sample obtained by a conventional syringe [8]. This approach uses a polymer that degrades upon contact with UV radiation, revealing a surface with evenly spaced slits, which decrease in diameter towards the bottom of the device. The sample travels through, and the large CTCs are trapped towards the surface. The photosensitive polymer-based microfilter was able to achieve higher efficiency with tested DLD-1G cancer cells than A549G cancer cells, as the former had a much larger average diameter. The CROSS chip was also developed for easy application to normally obtained blood samples without pre-processing [9]. This device uses size and deformability in four different sections to filter larger CTCs from normal blood cells. In a comparative study using blood from metastatic colorectal cancer patients, the CROSS chip performed better in capture efficiency as compared to CellSearch. However, the system lacks the capacity for higher volumes and often needs the tested sample to be screened multiple times for ideal results.

## 1.3. Other Isolation Techniques

While purely immunoaffinity or size-based technology tend to be more common, some researchers have attempted to use a combination of these or entirely different factors for the process of isolation. The SDI (size-dictated immunocapture) chip, combines these two methods in a joint manner [10]. On the surface of the chip, a size-based micropillar approach is implemented, but the micropillars are coated with anti-EpCAM antibodies to direct the flow and capture of the CTCs. This method achieved a 92% capture efficiency and higher sensitivity than CellSearch but faced difficulty in protecting the cells from shear stress during the process. The Monolithic CTC iChip was able to use negative immunoaffinity and size-based capture to discriminate a spiked blood sample with one experiment yielding a median recovery of ~99% [11]. This chip held 128 multiplexed deterministic lateral displacement devices containing ~1.5 million microfabricated features to deplete red blood cells and platelets and used coated magnetic beads to target white blood cells with CD45, CD16, and CD66B surface antigens. However, the device's inability to filter smaller cells and struggle to remove white blood cells with low expression of the CD45, CD16, and CD66B surface antigens detracts from its extremely high capture rate in previous tests. A lateral filter array was created as well, and used with positive anti-EpCAM discrimination [12]. This device isolated CTCs by using hydrodynamic force with immunoaffinity. If the binding force of the cells (with anti-EpCAM antibodies) was stronger than the experienced hydrodynamic force, the cells would be filtered for isolation. This method achieved a maximum efficiency of 98%, which decreased as the throughput was increased. Though estimates show that the addition of the hydrodynamic filter to normal immunoaffinity techniques added about 10% efficiency, the device still struggles to isolate non-anti-EpCAM-expressing CTCs. The CaTCh FISH chip is another device that uses negative immunoaffinity [13]. The first step of the two-step process includes tagging CD45-expressing white blood cells with magnetic nanoparticles. Specially placed magnetic micropores then attract these cells into traps before the blood sample is subjected to a size-based filter. A spiked sample was sorted with 90% efficiency using this method, but once again, the difficulty of removing white blood cells with low CD45 expression rates is a significant issue. LFFF-DEP (lateral fluid flow fractionation-dielectrophoresis) technology is a method that uses the difference in conductivity between CTCs and normal blood cells to discriminate a sample [14]. The CTCs will experience positive dielectrophoresis and migrate towards one electrode, while the rest of the sample will typically experience negative dielectrophoresis. Though no statistical analysis was conducted with this technology, a test trial with breast cancer patient blood was described as "successful". Another innovative approach uses optofluidic technology to enrich a sample [15]. This approach uses folic acid to facilitate the binding of multiple homologous red blood cells with CTCs. This increases the refractive index of the much larger CTC-RBC combination cells and allows for laser illumination to be used as a separation tool before the red blood cells are lysed. This approach demonstrated a highly successful recovery rate of ~90% and a purity of 92%, although the cost of such a process is unspecified. Acoustic separation has been another area of study with regard to CTC isolation. One recent acoustic separation technique used tilted angle standing surface acoustic waves in part with a polydimethylsiloxane-glass channel to form an acoustic enclosure, thus boosting the energy density of the acoustic waves [16]. Once a sample was filtered through the chamber, the acoustic waves would change the trajectory of the cells dependent on their size/shape and separate the leukocytes from the CTCs. Though this process achieved 86% capture efficiency and a high throughput of 7.5 mL/h, it requires a pre-processing RBC lysing step, which increases the operational time and may mistakenly remove CTCs from the sample.

# 2. CTC Detachment/Release Techniques

## 2.1. Aptamers

The use of aptamers has been one of the most common methods in CTC detachment as of late. These oligonucleotide or peptide molecules have impressive binding capability, which can be manipulated for cell release by altering their tertiary structures. Aptamers are known to be highly selective and sensitive while these withstand unfavorable surroundings/conditions [17][18]. This process has been used in many devices such as the previously developed NanoVelcro chip, where 85% of CTCs were released, and around 80% maintained viability after surface-grafted aptamers were cleaved using Benzonase Nuclease [19]. Other devices have built upon this aptamer-based approach and received more successful results. Aptamer-modified gold nanowires used aptamer-sgc8c. After isolated CTCs bonded to the surface, electrochemical reduction desorption at −1.2 volts for 30 s was used to cleave gold–sulfur bonds (which are only stable under “normal” conditions), thus releasing the CTCs. This method achieved a 96.2% release efficiency in addition to a 90% post-release viability rate [20].

## 2.2. Microdevices/Nanodevices

Nanotechnology and microtechnology have also been prominent within the realm of CTC detachment. Nanoparticles in particular have been commonly used to facilitate successful and easy release. One study used gold nanoparticles conjugated with a mixed monolayer of 11-mercaptoundecanoic acid (MUA) and 12-mercaptododecanoic acid N-hydroxysuccinimide ester (NHS). The NHS ligands bound an amine moiety to NeutrAvidin, which held the CTCs in place on the surface of a microchip. Glutathione was then used to cleave the bonds, as it is easy to come by and use. This process resulted in release efficiencies of 92% and 91% and cell viabilities of 87% and 78% for the isolated PC3 and MDA-MB-231 cancer cells, respectively [21]. Another device used polymeric microfibers implemented with an anti-EpCAM cell isolation approach. A base of polystyrene microfibers was constructed, and specifically selected peptides with an anti-EpCAM antibody on one end were bound to the base. These peptides were cleavable by collagenase type IV, which resulted in a cell release efficiency of over 90% and a viability of 83% [22]. Though successful, the technology is nevertheless restricted by the same hindrances as all anti-EpCAM selection techniques. One process with an exceedingly high release rate of about 95% is the use of biodegradable nano-films. These nano-films are presented layer-by-layer with anionic and cationic polymer solutions and are conjugated with antibodies for cell capture (most commonly anti-EpCAM). An enzymatic solution is used to induce degradation of the nano-films, resulting in cell release. In an experimental study with spiked prostate cancer cell samples, the cells retained 90% viability after release [23].

## 2.3. Light/Electrochemical

Additional detachment techniques that have gained interest involve light and electrochemical release. These methods are favorable due to their minimally invasive nature yet may involve higher costs or an increased setup time. One tried detachment process is photoelectrochemical single cell release, which is beneficial in that it is able to target single cells, as opposed to the only detachment option being mass release. An experiment with this method utilizes carefully placed semi-conducting electrodes; when light is shined, electrons in the conduction band

of the silicon surface are excited, increasing conductivity which prompts cleavage and single cell release. At −1.2 volts for 240 s, the release was somewhat successful at around 82%, while the viability was also higher at around 90% [24]. A second light-based approach used a light-responsive hydrogel. Artificial anti-EpCAM receptors were imprinted on the gel base, which was also embedded with gold nanorods. Once CTCs bind to the artificial receptors, selected locations on the gel are exposed to near-infrared radiation (NIR). The gold nanorods heat up as a result of this process (dubbed photothermal activation), causing the gelatin to dissolve, releasing roughly 92% of all captured MCF-7 cells, which maintain 90% viability. Though this process can target specific cell release, a bulk-release approach in which the gel was simply heated to 37 °C maintained higher release and viability rates of 95% each [25].

# 3. CTC Detection Techniques

## 3.1. Direct Detection (Pre-Enrichment)

Direct detection techniques hold a significant advantage over post-enrichment techniques. Because enrichment is not a necessary step, the process becomes much simpler and often requires less time and effort concerning the pre-processing steps. However, these techniques also tend to be on the higher end with regard to cost and may be slightly more difficult to implement effectively. One of these direct detection strategies is SERS (surface-enhanced Raman scattering) detection using active magnetic nanoparticles [26][27]. With this strategy, magnetic nanoparticles are conjugated with folic acid, and the folate receptor on CTCs allows for binding. This allows for CTCs to display a much higher SERS intensity than normal cells, which in turn causes a greater signal within the blood that undergoes Raman scattering analysis. In one experiment with HeLa cells, this method was able to detect anywhere from 4–18 CTCs per 10 mL of blood [28]. Another direct investigation of CTCs was reported using solid-state micropores. This approach was able to detect CTCs one cell at a time, and electronic fingerprinting was recorded to identify and count the CTCs [29][30]. This highly sensitive technique reported more than 90% accuracy and parallel microchannels for high throughput [31]. Another direct detection possibility is the use of a photoacoustic cytophone. This in vivo testing method, though promising, is specific to melanoma. It works via the laser heating of light-absorbing hemoglobin in red blood cells and melanin expressed in melanoma CTCs, which causes the thermoacoustic and nanobubble-based generation of acoustic waves. These waves can be detected by a small ultrasound transducer placed over the skin, which displays a large spike when CTCs flow by, and a negative spike when white blood cells and platelets flow by. This method was able to detect individual CTCs at a concentration of ≥1 CTC per mL in 20 s and had an estimated specificity of 95% [32]. An optical method for direct detection known as confocal microscopy has also proved successful, although it is quite expensive. This method requires fluorescent labeling and uses a laser microscopy technique (originally based on inverted microscopy) to detect the fluorescently labeled CTCs. In one experiment the human hepatocellular carcinoma cell line (HCCLM3) was injected with enhanced green fluorescent protein into mice. Confocal microscopy was able to distinguish single CTCs from clusters, even at relatively early stages, and by day 30, the CTC counts numbered as high as 45.4 ± 6.2 per hour [33].

## 3.2. Post-Enrichment Detection

More common methods for detection are post-enrichment, as an enriched sample has a much higher proportion of CTCs, which makes them easier to detect. As discussed earlier, the CaTCh FISH chip is used for isolation/enrichment, but it can also be used for post-enrichment detection. The technology uses nucleic acid detection. Specifically, direct labeling occurs via RNA fluorescence in situ hybridization (RNA FISH), which involves the hybridization of 20–50 short, fluorescently labeled oligonucleotide probes to the target RNA by Watson–Crick base pairing. The fluorescence is then used as a marker, as it will appear more intense in spots with CTCs [13]. The EPISPOT (epithelial immunospot assay) is a test that in theory can be attached to any enrichment step. Membranes are coated with antibodies against specific protein markers, and secreted proteins are directly captured on the antibody-coated membrane. When samples are incubated with the membranes, only viable captured CTCs will be able to capture the proteins, which are then used to direct fluorescence. The end result will be increased fluorescence in the areas with viable CTCs, which is helpful in discriminating against apoptotic/unviable CTCs [34]. Immunonanospheres, or nanosphere detection, is one process that allows for simultaneous detection and isolation. The nanospheres are conjugated with green fluorescence and an anti-EpCAM antibody to target CTCs and red fluorescence and an anti-CD45 antibody to target white blood cells. The nanospheres' magnetic properties also allowed for enrichment with up to 96% efficiency [35]. A similar study used fluorescent magnetic beads (a combination of anti-EpCAM, anti-EGFR, and anti- VMT beads used) with a parallel flow micro-aperture chip, and they were able to detect CTCs with 89% efficiency. This device also contained a strong magnetic field and size filters to help with enrichment and detection [36]. The GILUPI CellCollector was a device built to contest the ability of CellSearch. The CellCollector device can be directly inserted through a catheter into a patient's vein. The system works by immunoaffinity fluorescent staining using both anti-CD45 to discriminate against white blood cells and anti-EpCAM to discriminate for CTCs. When studied using colorectal cancer patients, the CellCollector system showed no significant improvement over CellSearch and was deemed to have lesser "clinical relevance" [37].

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