Rapid Selection of Active Circulating Tumor Cells Using Combined Electrical and Optical Detection in a "Lab on Chip" Platform

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Abstract Text:

Cancer is one of the leading cause of deaths in United States and its early diagnosis is a major challenge. According to the American cancer association an estimate of 1,735,350 new cancer cases are diagnosed and 609,640 cancer deaths are reported in 2018. The exponential rise in cancer cases makes cancer diagnosis an utmost priority in the healthcare sector. We identified Circulating Tumor Cells (CTCs) as an early biomarker to diagnose cancer which would lower the mortality burden drastically. CTCs are cells that circulated in the bloodstream after being dislodged from the primary cancer tumor and lead to a new metastasis which in turn leads to its faster progression. The present limited knowledge about metastatic cancer poses a tremendous problem, as most patients do not die from primary tumors but from CTCs which spread to other sites. With the advances in multidisciplinary studies, CTC detection has become promising for early diagnosis of cancer.

The current cancer diagnostic technique involves invasive biopsy followed by pathological analysis for detection of CTCs in the blood which is time consuming and less sensitive. Present techniques such as CellSearch (Veridex, Raritan, NJ) and Cell Collectors (GILUPI GmbH, Potsdam, Germany) are highly expensive (600 000–800 000 USD), complex and time-consuming assay. Moreover, the selectivity and sensitivity of the captured CTCs is low (i.e. high false positive and false negative rates). To overcome these major limitations, we have designed a microfluidic microchip for selective capture, enrichment and rapid detection of active CTCs using simultaneous electrical and optical method. The used of both electrical and optical methods enhances the sensitivity of the assay and avoids false positive results.

The microfluidic chip consists of a enrichment zone designed specifically to capture the CTCs in one step. For the study we selected lung cancer CTCs which are captured using specific antibody "mucin-like O-glycoprotein' (MUC1). This specific antibody is analogous to the surface protein expressed by CTCs. The antibodies are decorated to a series of electrodes, which act as capture site for the CTCs. The number of capture cells are enumerated by measuring change in the resistance of electric current which is compared with the standard graph. Further to enhance the sensitive of microchip an optical assay is incorporated which involves visualization and detection of centrosome aberration in these captures CTCs. Centrosome aberration is a hallmark event which occurs in cancer cells. The centrosome aberration is detected by fixing the captured cells in 4% paraformaldehyde, further permeabilizing them with 0.1% triton and then staining with γ-tubulin antibody tagged to a fluorescent dye. The cells with aberration in size, shape and structure confirms the presence of active CTSs. In the image (Fig 3 b) it can be clearly seen the centrosome aberration marked as red bright spots as compared to control image (Fig 3 a). The confirmation of the abnormalities was carried out by image processing software for which DMI6000 inverted Leica TCS AOBS tandem scanning microscope was used under a 100x objective. The software selects cells in at least one centrosome is present and images are captured with a maximum resolution of 75.7 nm. This dual electrical and optical method of detection enhances the specificity and sensitivity of the assay. The process time is significantly less (>45min), automated and cost effective. The final aim of the assay is to detect as low as 2-5 cells/mL of blood sample which is challenging and not accomplished by any other method presently available. In the near future the developed microchip can be modified to carry out multiplex detection of CTCs derived from various origin which will enhance the applicability of the assay.