CEE™ CELL ENRICHMENT AND EXTRACTION TECHNOLOGY

Enabling Early, Non-invasive Isolation of Rare Cells for Diagnostics

There is universal interest in enrichment and recovery of rare cells circulating in blood. The clinical benefits associated with rare cell recovery and analysis are extraordinary. Research in rare cell recovery has long been supported by the National Institutes of Health in a program to recover rare fetal cells from maternal circulation with the goal of providing a non-invasive prenatal diagnostic test. In addition, National Cancer Institute aggressively funds research for the early detection and diagnosis of solid tumors on the basis of rare tumor cell detection. Nevertheless, the technical challenges associated with enrichment and/or recovery of rare cells is significant and this is true for all investigations. For rare cell selection strategies, the number of contaminating cells (including dead and abnormal cells) can be daunting (i.e., ranging from 10^6 to 10^9 unwanted cells). Therefore, current cell enrichment methods usually involve a trade-off between loss of target cells and the purity of the processed specimen. These two characteristics are inversely related: depletion of contaminating cells will result in the loss of some percentage of target cells.

Thus, researchers from both the cancer and prenatal communities conclude that new technologies are necessary to successfully recover and enrich rare cells. Such technologies must involve minimal sample processing and permit relatively fast through-put. Current methods, including magnetic activated cell sorting (MACS) and fluorescent activated cell sorting (FACS), may be too cumbersome and unreliable to be of practical clinical utility.

BIOCEPT

ENGINEERING
NEW DIRECTIONS IN DIAGNOSTICS™

DEVELOPMENT OF CELL ENRICHMENT AND EXTRACTION TECHNOLOGY

Biocept engineers and scientists have developed novel CEE™ Cell Enrichment and Extraction technology to meet clinical goals. CEE combines advanced attachment chemistry, microfluidics, and nanomanufacturing to create a new class of diagnostic tool. This technology addresses both the extraction of rare cells and sample enrichment. CEE is a single use device that fits onto a standard microscope slide designed to either enrich cell populations or capture rare cells based on a cell-specific antibody-antigen reaction (Figure). The cells of interest may be captured by the antibody while the remaining unwanted cell population is allowed to flow through the device for collection. This system is compatible with most bodily fluids, including whole blood.

CEE is based on the principles of microelectromechanical systems (MEMS), a class of devices that integrates mechanical and electrical components. Each CEE device contains a series of posts that are precisely placed, based on mathematically modeled fluid dynamics, to maximize capture of rare cells. Each post is coated with specific antibodies that selectively attach to the target cell type. The antibody coating can be modified depending on the properties of the targeted cell. A fluid sample containing the targeted cell passes over the CEE device at a precisely controlled rate to extract rare cells, creating an enriched sample. Standard marker and FISH analysis of the cells can be completed within the device for highly accurate diagnosis.

WHAT DISTINGUISHES THE CEE TECHNOLOGY FROM OTHER MEMS DEVICES?

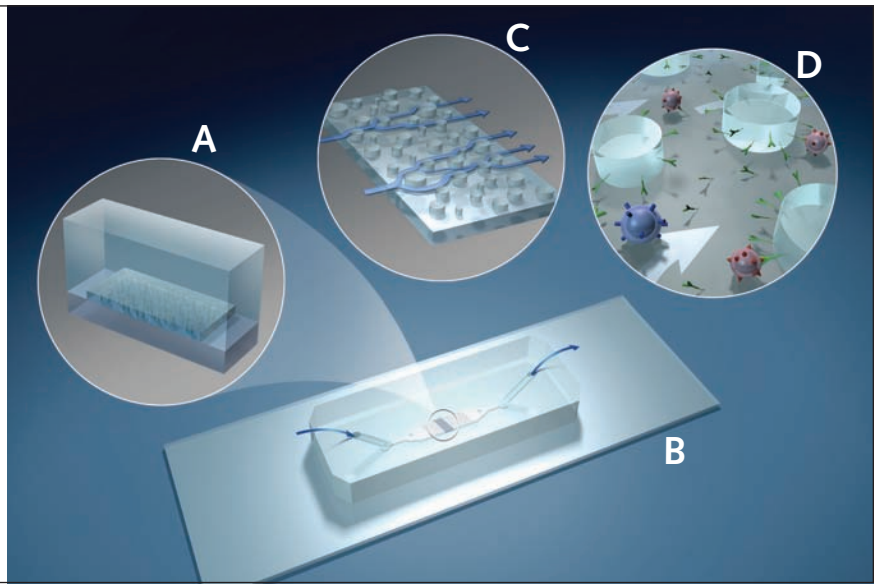
(i) Nearly all MEMS devices are developed for assays involving a small specimen volume (less than 100 microliters). However, clinical utility in the capture of rare cells requires processing of a significantly larger sample volume (up to 10-20 ml). This is overcome by the CEE device which enables recovery of rare cells following minimal sample processing with volumes ranging from 250 microliters to 10 ml per device.

(ii) With conventional methods, antibody-labeled beads pass through cells in suspension. In contrast, in CEE technology cells pass through posts (immobilized beads) within the CEE device. In addition, these immobilized beads are larger (400µm) than typical magnetic beads (2µm) used to capture cells by MACS. This increase in bead size is equivalent to the increase in surface area, thus increasing the volume searched (cm^3/hour) by nearly 500 fold.

(iii) CEE design uses nanotechnology that is well known in the engineering electronics field. The design concept is a mathematically modeled series of posts that are designed to create a randomized flow pattern. The chaotic flow increases the likelihood of cell antibody interaction by maximizing the distance traveled between the inlet and exit sample ports.

(iv) Cells captured within the device remain suitable for molecular diagnosis as either intact cells (for fluorescence in situ hybridization and immunohistochemical staining) or for lysis (for DNA, RNA, proteins).

Figure. Enrichment using the CEE Device.
The device is small and contained within PEG molding (A) which fits on a microscope slide (B). CEE technology combines microfluidics and attachment chemistry. Mathematically modeled flow rate and placement of posts (C) maximize cell capture. Targeted antibodies are suspended on a surface covering the base and posts to selectively attach to target cells, creating an enriched cell sample (D). After attachment to mechanical pumps, the sample is run through an inlet port with "un-captured" cells collected at outlet port.



FIRST APPLICATION: FETAL CELL RECOVERY FOR NON-INVASIVE PRENATAL DIAGNOSIS OF DOWN SYNDROME

The first application of CEE technology will be isolation of fetal nucleated red blood cells (nRBCs) from maternal blood.

Definitive recovery of fetal cells for genetic diagnosis currently requires chorionic villus sampling or amniocentesis. Non-invasive screening tests such as nuchal translucency are widely applied, but are not definitive, with relatively high false-positive detection. As a result, a significant number of pregnant women who screen positive for Down Syndrome undergo invasive testing. With new American College of Obstetricians and Gynecologists (ACOG) guidelines recommending that all pregnant women be offered prenatal testing for Down Syndrome[1], the need for a non-invasive diagnostic test has never been more pressing.

Fetal cells are present in maternal blood at frequencies ranging from two to four per milliliter and are likely to be present in the blood of all pregnant women as early as six weeks of gestation. Though multiple fetal cell types circulate in maternal blood, the fetal nucleated RBC (nRBC) is an ideal candidate cell for targeted recovery and analysis from blood. Fetal nRBCs represent 30% to 50% of fetal blood cells and have a unique morphology. In addition, adult nRBCs are very rare, minimizing the risk of misidentification. Reports in the literature support successful detection of most common fetal trisomies and sex chromosome aneuploidies by targeting isolation of fetal nRBCs.[2,3]

Two recent studies demonstrate the promise that the CEE device shows in its ability to isolate and analyze fetal cells. In both studies, cells that FISHed male (XY) were assumed to be fetal; female (XX) were only scored as female if they were antibody positive. The fetal gender, as determined by Biocept, was compared to a metaphase karyotype supplied to Biocept by the center.

The first sequence involved 14 samples of maternal peripheral blood, representing gestational ages ranging from 8 weeks, 0 days to 12 weeks, 0 days. Five samples were non-informative for the following reasons: two samples, females by karyotype but no antibody positive cells detected; two samples, high background; one sample, high background and cell clumping in CEE device. The informative rate was 64% (9/14) and the sensitivity and specificity rates were both 100% (6/6 and 3/3 respectively).

Study 1, Sensitivity and Specificity

		Karyotype	
		XY	XX
	XY	6	0
Biocept Test			
	XX	0	3

[6 / (6+0)] = 100% sensitivity (alternatively, detection rate)
[3 / (0+3)] = 100% specificity

The second study examined the performance of a different probe cocktail. It involved 20 samples of maternal peripheral blood, representing gestational ages ranging from 10 weeks, 4 days to 14 weeks, 1 day. Eight samples were non-informative for the following reasons: five samples, high background; three samples, processing errors. The informative rate was 60% (12/20) and the sensitivity and specificity rates were both 100% (11/11 and 1/1 respectively).

Study 2, Sensitivity and Specificity

		Karyotype	
		XY	XX
	XY	11	0
Biocept Test			
	XX	0	1

[11 / (11+0)] = 100% sensitivity (alternatively, detection rate)
[1 / (0+1)] = 100% specificity

Ongoing clinical validation studies will further evaluate the optimal gestational age and the number of fetal cells required for reliable diagnosis of trisomy 21.

FUTURE APPLICATIONS

Going forward, Biocept engineers and scientists will adapt CEE technology for numerous diagnostic applications, including oncology. By capturing rare cells from heterogeneous samples, CEE technology has the potential to enable early, accurate diagnosis of a range of conditions, providing opportunities for earlier intervention in optimal patient care.

REFERENCES

1. Screening for Fetal Chromosomal Abnormalities. ACOG Practice Bulletin: Clinical Management Guidelines for Obstetrician-Gynecologists. Number 77, January 2007.
2. Bianchi, D.W., Simpson J.L., et al. Fetal Gender and Aneuploidy Detection Using Fetal Cells in Maternal Blood: Analysis of NIFTY I Data. Prenatal Diagnosis 2002; 22: 609-615.
3. Bischoff FZ, Sinacori MK, et al. Cell-free fetal DNA and intact fetal cells in maternal blood circulation: implications for first and second trimester non-invasive prenatal diagnosis. Human Reproduction Update 2002; 8: 493-500.

BIOCEPT

ENGINEERING
NEW DIRECTIONS IN DIAGNOSTICS™