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Review

Current and emerging techniques of fetal cell separation from maternal blood

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ABSTRACT

Intense research has been carried out in recent years into methods that aim to harvest fetal genetic material from maternal blood as substitutes to amniocentesis and chorionic villus sampling. Just over 30 years have past since the first fetal cells were separated from maternal blood using flow cytometry highlighting the prospect of non-invasive prenatal diagnosis of fetal abnormalities. The aim of this review paper is to describe the most commonly used cell separation methods with emphasis on the isolation of fetal cells from maternal blood. The most significant breakthroughs and advances in fetal cell separation are reviewed and critically analyzed. Although much has been accomplished using well established techniques, a rapid and inexpensive method to separate fetal cells with great accuracy, sensitivity and efficiency to maximize cell yield is still required. In the past decade MEMS (Micro Electro Mechanical Systems) technologies have enabled the miniaturization of many biological and medical laboratory processes. Lab-on-chip systems have been developed and encompass many modules capable of processing different biological samples. Such chips contain various integrated components such as separation channels, micropumps, mixers, reaction and detection chambers. This article will also explore new emerging MEMS based separation strategies, which hope to overcome the current limitations in fetal cell separation.

Contents

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1. Introduction

Analysis of a single cell or a homogenous population of cells from a complex biological system is an essential process in clinical and research settings. Detection and separation of target cells is often the first step in sample preparation. A variety of separation methods currently exist for efficient cell sorting of various cell types these include optical, magnetic and size based strategies. However current methods are limited and rare cells such as circulating tumor cells and fetal cells in maternal blood often go undetected. For these cases, highly sensitive methods of cell separation are required. Over the past decade, MicroElectrical Mechanical Systems (MEMS) also called microsystems technology has enabled the miniaturization of many laboratory processes, especially in the fields of biology and medicine. Lab-on-chip (LOC) systems have been manufactured incorporating modules capable of processing different biological samples including blood, saliva and urine. Such chips contain various integrated components such as, separation channels, micropumps, mixers, reaction and detection chambers [1,2]. Emerging cell separation strategies based on MEMS technology hope to overcome the current challenges in Non-Invasive Prenatal Diagnosis (NIPD) by integrating the processes of fetal cell separation and analysis into a LOC system. This review article aims to describe the most commonly used cell separation methods with emphasis on the isolation of fetal cells from maternal blood for NIPD. New MEMS based developments in fetal cell separation are also described and their relative merits discussed.

2. Prenatal diagnosis and fetal cells in maternal blood

Amniocentesis and chorionic villus sampling (CVS) are today the gold standard methods used to obtain fetal genetic material for prenatal diagnosis. Both procedures are hugely invasive, as they involve the removal of fetal material from around the developing fetus. Once obtained, the material is analyzed for cytogenetic, molecular and biochemical abnormalities [3,4]. The associated risks with such procedures include bleeding, leakage and infection of the amniotic fluid and miscarriage. Amniocentesis leads to miscarriage in approximately 1% of cases and CVS in around 1–2% of cases [5,6]. These percentages are quite significant as approximately 20,000 amniocenteses and 5200 CVS are conducted every year in the UK [7]. The existence of cell free fetal DNA (cffDNA) [8], cell free fetal RNA (cffRNA) [9] and fetal cells in maternal circulation provides a unique opportunity for the development of techniques for NIPD. Rapidly cleared from maternal circulation after birth [10], cffDNA is successfully used today in clinical applications to detect sex-linked conditions [11], rhesus D status [12,13] and as an indicator of pre-eclampsia [14]. A number of technical and clinical problems currently limits the use of cffDNA; for example it is difficult to distinguish cffDNA from maternal cell free DNA as the fetus inherits half its genes from the mother, making it problematic to diagnose fetal aneuploidy and single gene defects [14]. Nonetheless ongoing research aims to overcome these issues and a recent paper by Fan et al. [15] recently highlighted the use of shotgun sequencing to successfully diagnose fetal aneuploidy.

Detection of fetal mRNA in maternal plasma was first achieved by Poon et al. [9] using a Y chromosome specific gene. Analysis of mRNA could possibly allow prenatal prediction of aneuploidies, by monitoring fetal gene expression for example Oudejans et al. [16] demonstrated the presence of chromosome 21-encoded mRNA, LOC 90625 of placental origin in maternal plasma. This gene has two key factors to make it an ideal marker for trisomy 21, firstly LOC90625 is upregulated in trisomy 21 placentas and secondly, the gene is located within the Down syndrome critical region (DSCR) on chromosome 21.

The use of fetal cells for NIPD has the main advantage of providing a complete genetic make up of the fetus free from maternal contamination [17]. Most research in NIPD has been focused today on three types of fetal cells: trophoblasts, leukocytes, and nucleated red blood cells (NRBCs), also called erythroblasts [18]. Trophoblasts are large epithelial cells and play a vital role in the development and function of the placenta. The first noted record of trophoblasts crossing the placental barrier was made in 1893 by Schmorl [19], who found them in the lungs of women who had died from pre-eclampsia. The use of trophoblasts for NIPD has the major drawback that they can sometimes be multinucleated or anucleated and also have a 1% risk of placental mosaicism, which could lead to misdiagnosis [19,20]. The existence of fetal leukocytes in maternal circulation was first demonstrated by Walknoska et al. in 1969 [21] through the detection of a Y chromosome signal in maternal blood. Since then fetal leukocytes have been shown to persist in maternal blood and are believed to play a role in some autoimmune diseases [22–24]. NRBCs are one of the earliest cellular stages in erythropoiesis they are mononuclear with a small round condensed nucleus, have a big nucleus to cytoplasm ratio and a limited lifespan of 90 days once in maternal circulation [18,25].

The rarity of fetal cells in maternal blood makes their separation a formidable challenge. Normal human whole blood consists of red blood cells (RBCs) ($5–9 \times 10^9$ ml^{-1}), white blood cells (WBCs) ($5–10 \times 10^6$ ml^{-1}) and platelets ($2.5–4 \times 10^8$ ml^{-1}). The absolute number of fetal cells has yet to be established but their frequency has been estimated to be between one to two fetal cells per ml of maternal blood [26,27], or 1 in 10^5–10^7 maternal cells [28]. The number of fetal cells has also been shown to increase in abnormal pregnancies which is believed to be due to an impaired placenta, leading to an increase in maternal-fetal transfusion [29,30]. The one major concern when isolating NRBCs is the possibility that some of these cells could be of maternal origin; therefore confirmation of fetal origin must be achieved with 100% confidence before diagnosis [31–33]. Morphological properties of fetal NRBCs have been used to differentiate them from other cells. Fetal hemoglobin (HbF) and Y chromatin staining by fluorescent in situ hybridization (FISH) has also been used to identify fetal NRBCs. However the use of the Y chromatin markers limits the detection to only male fetuses [26]. One recent development is the use of light-scattering spectroscopy, Lim at al., used a Confocal Light Absorption and Scattering Spectroscopic (CLASS) microscopy system to differentiate between cord blood NRBCs and adult NRBCs.

3. Techniques in fetal cell separation

The most commonly used techniques for fetal cell enrichment are step density gradient centrifugation, fluorescent activated cell sorting (FACS) and magnetic activated cell sorting (MACS). This section describes the current methods of cell separation as well the latest advances in LOC devices with emphasis on fetal cell separation (Table 1).

3.1. Cell size and density based separation techniques

3.1.1. Centrifugation

One of the most commonly used methods of cell separation based on cell size and density is centrifugation. During this process centrifugal forces created cause particles within a centrifugation tube to move away from the axis, thereby allowing their separation from the suspending fluid. In more complex biological mixtures such as blood, density gradients have been applied to purify a particular type of cell. In this method solutions are used which increase in density from top to bottom of the centrifugation tube, either continuously or in steps. An example of a commercially available

Table 1
Advantages and limitations of methods for separation of fetal cells from maternal blood.

Method	Advantage	Limitation	Source
Cell size and density based			
Centrifugation	Large volumes can be processed. No cell labels required. Some capable of temperature controlled environment. Rapid separation.	Cell loss and damage. Generation of aerosols. High-energy consumption. Have to be carefully balanced. Expensive to purchase and maintain.	[25,26,35–37]
Filtration	Ease of operation. Label free method.	Slow separation. Clogging. Non-continuous method. Disposable, membranes can be costly.	[38,39]
Filtration on chip	Inexpensive. Disposable, chips are relatively low cost. Can be multiplexed.	Slow flow rates. Cell adhesion and clogging. Non-continuous method. Not easy to integrate.	[41]
Lateral displacement	Cells are not retained by posts. Integration. No cell labels required. Can be multiplexed.	Cell adhesion.	[42]
Optical based			
Flow cytometry	Multiple parameters can be measured simultaneously. Cells can be kept sterile if required. Analysis done on individual cells. Rapid analysis and sorting. Cells recovered with high purity and yield.	Expensive equipment and reagents. Requires specially trained personnel to operate. Intrinsic cell fluorescence. Clogging. FACS requires cell labels. Fetal cell loss.	[18,32,43,45–50]
LMPC	Allows capture of a single cell. Non-contact separation. No cell damage.	Expensive equipment. Cells have to be identified by operator or cell scanning software.	[25,34]
Magnetic based			
MACS	Easy and quick to use. Cheaper when compared to FACS. Bench top.	Cells have to be labeled. Only one cell parameter at a time. Target cells can sometimes still have beads bound to them. Bead-to-bead interaction can lead to non-specific entrapment. Large shear forces may cause bound cells to be damaged.	[17,52–59]
Magnetophoresis	Label free. Ease of use. Non-contact nature. Low cost.	Magnetic fluxes generated can sometimes be too small to have an effect on non-labeled biological cells.	[42,51,60]
Adhesion			
Adhesion	High recovery of NRBCs.	Low purity. Labour intensive.	[61,62]
Electrical			
CFS	Large number of viable NRBCs recovered.	Multiple steps required, time consuming.	[63,64]
DEP	Label free. High Sensitivity	Electrode fouling. Complicated fabrication procedures. Overheating.	[65,66]

density gradient medium is Percoll™ used for the centrifugation of cells, viruses and sub-cellular particles. During centrifugation, particles will travel in the gradient and be retained in the medium that has a density equal to their buoyant density; this position is known as the isopycnic position of a particle [35]. Most researchers involved in fetal cell separation use step density gradient centrifugation as the initial separation step to reduce the amount of maternal blood cells, especially mature red blood cells (RBCs), whilst enriching the mononuclear cell layer [25]. Typically maternal blood is layered on top of the gradient(s) and then identified by their morphological characteristics by staining with May-Giemsa [26,36]. Problems with centrifugation include the generation of aerosols which can be a potential risk of infection, cell damage and cell loss of up to $40 \pm 10\%$ [37]. This figure is quite consider-

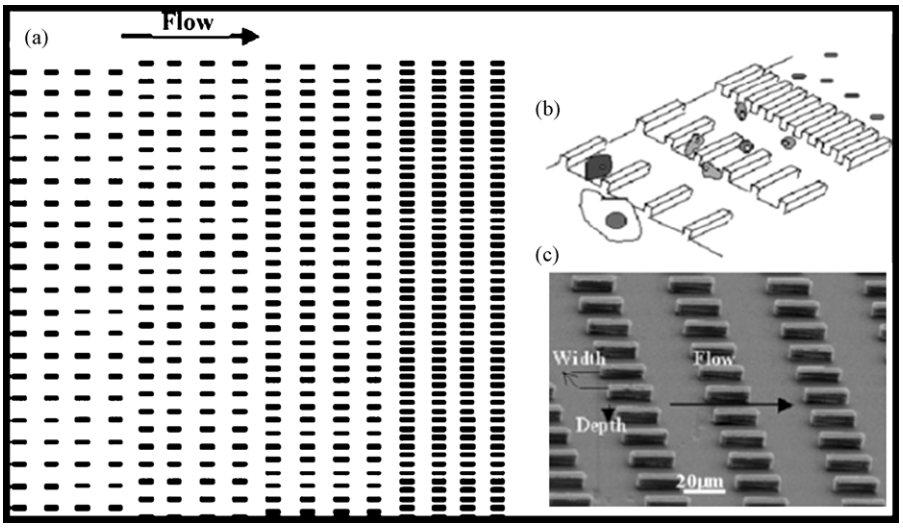

Fig. 1. (a) Layout of device designed by Mohammed et al. [41] for the separation of NRBCs based on cell size. Image shows fours row of successively narrowing channels made by pillars (b) working concept of the device, smaller more deformable cells can pass through all four segments, while larger cells are trapped by pillars and retained (c) SEM image of pillars forming filter fabricated in PDMS.

able when compared to the rarity of fetal cells in maternal blood. Centrifugation can also be labour intensive and time consuming, as samples have to be balanced carefully during loading.

3.1.2. Filtration

Cell separation by filtration has the advantage of not requiring cell surface markers, a useful property for the isolation of cells that have no specific cell surface markers. In 2000, Vona and coworkers [38] developed a new filtration based technique called Isolation by Size of Epithelial Tumor Cells (ISET) for the separation and characterization of circulating tumor cells from peripheral blood samples. In comparison to other blood cells, epithelial tumor cells are larger in size allowing them to be filtered from blood. Various tumor cell lines, such as MCF-7 (human breast adenocarcinoma) and HeLa (human cervix epitheloid carcinoma), were used to validate the technique. Two years later, the ISET method was implemented for the separation of fetal trophoblasts from maternal blood [39]. After isolation, recovered trophoblasts were micro-dissected and their fetal origin confirmed by PCR. The main attractions of the ISET method are its high sensitivity, the possibility of separating a single fetal trophoblast, its ability to separate cells without damaging cell morphology and the avoidance of labeling cells with antibodies. Cells collected from ISET also prove suitable for downstream analyses such as FISH and PCR. A drawback of this method as mentioned earlier is the use of trophoblasts for NIPD, which can potentially lead to misdiagnosis.

3.1.3. Filtration on chip

Micro-separation devices based on cell size frequently consist of an array of pillars or obstacles within a microfluidic channel. Fluid containing a heterogeneous population of cells is usually pumped through such a device and depending on the design of the filter, target cells can be collected upon exit or are retained at certain locations in the system. The microscale dimensions of the channels ensure that the fluid flow is laminar resulting in predictable and reproducible cell movement [40]. An example of filtration on chip was demonstrated by Mohamed et al. [41] to separate fetal NRBCs based on their size and deformation characteristics as shown in Fig. 1. The device contains four segments of successively narrow channels of 15, 10, 5 and 2.5 μm spacing with a depth of 5 μm. Fabrication was carried out in polydimethylsiloxane (PDMS) using micromachined silicon as a mold. Preliminary testing was first done using goose RBCs as a model for fetal NRBCs as they are nucleated and alike in size, 12 m diameter for the former compared to 9–13 m for the latter, followed by testing with cord blood. Results showed that goose RBCs were consistently retained at the 2.5 μm channels, cord derived WBCs were also trapped at this position while the cord blood fetal NRBCs transversed all channels and could be collected in the outlet reservoir. These successful results stem from the ability of fetal NRBCs to deform and squeeze through the 2.5 μm channel unlike goose RBCs. Unfortunately this device suffered from a low flow rate of approximately 350 μl/h and cell adhesion to channel walls. To help resolve this problem, a centrifugation step was employed before testing to reduce the concentration of RBCs. Cell viability and non-specific adhesion of cells to channels walls remain a concern [40] Separation by filtration on chip has the major drawback of being a non-continuous flow method. Therefore this type of device cannot be easily integrated with other modules.

3.1.4. Deterministic lateral displacement

Recently Huang et al., used a deterministic lateral displacement (DLD) method as a preliminary step in the enrichment of fetal NRBCs from maternal blood [42]. The chip consists of a microflu-

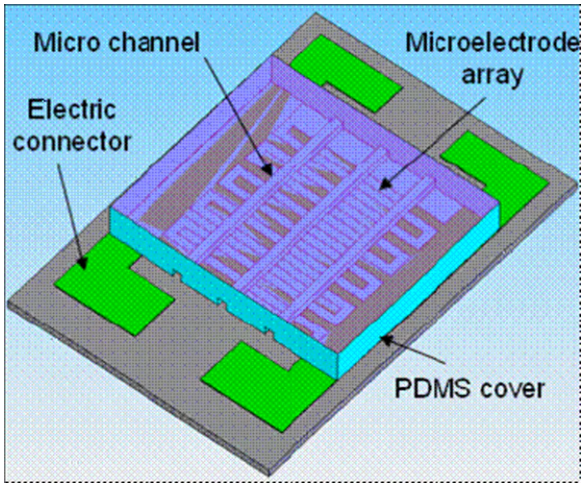

Fig. 2. Microfluidic device with microelectrode array developed by Xu et al. [67] for the dielectrophoretic characterization of NRBCs.

idic channel containing 24 arrays of microposts with one blood inlet and 24 buffer inlets fabricated in silicon using deep reactive ion etching (DRIE). The method works by taking advantage of the hydrodynamic size difference between nucleated cells and non-nucleated cells. Whole maternal blood is pumped through the device under laminar flow at a small angle relative to the chip. Filtration is achieved by the width of the flow stream and not by the spacing between each post. Cells that are smaller than the width of the flow stream, such as RBCs and platelets (non-nucleated cells), stay within the stream. Larger cells, such as NRBCs and WBCs, which come across a micropost are displaced out of the stream into the buffer stream, thereby allowing their separation. Following this preliminary separation the nucleated fraction containing the NRBCs and WBCs are passed through a magnetic column and separated due to the presence of deoxygenated hemoglobin, the underlying principle of this method will be discussed later. Cells collected from the magnetic column were stained and scanned using an automated microscope to identify NRBCs based on their morphological characteristics.

3.2. Optical based separation techniques

3.2.1. Flow cytometry and fluorescent activated cell sorting (FACS)

Flow cytometry, the counting, examining and sorting of cells in a fluid, is perhaps the oldest and still most popular method for cell analysis and sorting. A typical flow cytometer consists of a light source, usually a laser, light collection optics, fluidic and electrical components. Lasers used in flow cytometry can be gas (argon, neon and helium-cadmium) lasers, solid state lasers and dye lasers. The sorting capability of a flow cytometer has been adapted from inkjet graphic printing technology and involves the electrostatic deflection of droplets [43]. Many flow cytometers also have the ability to sort cells. For such a task, target cells must be labeled with fluorescently tagged antibodies for cell specific antigens. Once inside the cytometer the cells pass one at a time through a vibrating exit nozzle; the vibrations produce single droplets containing individual cells. As the droplet passes through an array of laser beams and optical detectors, a photomultiplier tube detects the fluorescence emitted by the fluorochromes. Based on the fluorescent signal the droplet is then given either a positive or negative charge. Charged droplets then pass through a pair of high voltage deflection plates, which attract the droplet of opposite charge into its appropriate collection or waste container [44]. In flow cytometry and FACS, measurements are made on each separate particle in the suspension and are not just an average of the whole cell population [43]. Cells separated by FACS can also be kept sterile if required; an essential condition if the cells are to be cultured.

Fetal cells, especially NRBCs, express many cell surface antigens, most of which have been used in different combinations for their separation by FACS. The most commonly used antigens are Cluster of Differentiation, CD71 (transferrin receptor) [45], CD36 (thrombospondin receptor), glycophorin A (GPA) [46,28]. Fetal leukocytes were the first fetal cells to be separated by FACS in 1979 by Herzenberg et al. [47], based on differences in human leukocyte antigen (HLA) expression. However this method requires the knowledge of the parental HLA type before sorting [18,48]. Bianchi et al. carried out the first detection of fetal NRBCS by flow cytometry in 1990 [45]. The separation was solely done on the expression of CD71, followed by PCR for the detection of Y specific sequences. Unwanted cells can also be depleted by selecting for markers on the surface maternal cells for example CD45, CD4, CD32 and CD19 therefore relatively pure populations of fetal cells can be obtained using a combination of cell markers with FACS [46,48,49]. Unfortunately most flow cytometers are not simple instruments and require specially trained personnel. Samples must be prepared before analysis to ensure a suspension of single particles, otherwise the fluid may not flow smoothly and the system could get blocked [50]. Other disadvantages of flow cytometry and FACS include the cost of the equipment and intrinsic cell fluorescence, which can render the distinction between positively labeled and negative cells difficult. The above disadvantages and its low sensitivity for separating fetal cells indicate FACS is not efficient enough to be used in routine clinical settings [32].

3.2.2. Laser micro-dissection

Laser Micro-dissection and Pressure Catapulting (LMPC) is an extremely useful technique for the separation of specific single cells or other bio-molecules. This method is non-contact and therefore helps reduce the risk of sample contamination. Applications of LMPC include the isolation of various targets from cells to single living organisms from a wide range of starting samples such as cell smears, paraffin and cryosections, and cytospins. In this technique a pulsed UV-A laser beam is connected to a regular microscope and focused through the objective lenses to a micron size diameter. At the focal point forces are generated allowing unwanted material to be photo-fragmented into molecules and atoms. This photochemical process is known as cold ablation as no heat is transferred to the surrounding medium. Consequently, cells, bio-molecules such as DNA and proteins are not damaged and can be used in downstream processes. After separation, the cells can captured and lifted up into a collection device using the same laser, a technique called Laser Pressure Catapulting (LPC). For the isolation of fetal cells, automatic scanning software such as Metafer P could be used in combination with LMPC to improve the detection of fetal cells. Attempts have been made using micromanipulation to isolate individual fetal cells to confirm fetal origin by single cell PCR analysis [25,34]. However the collection of a single cell using micromanipulation is difficult and requires experience in cell manipulation [25].

3.3. Magnetic based separation techniques

A cell does not generally possess a magnetic dipole moment since its main components, water, proteins, phospholipids and DNA are diamagnetic in nature. Paramagnetic or ferromagnetic particles coated in cell binding molecules must be used to achieve cell separation. However some biological cells such as magnetostatic bacteria and deoxygenated red blood cells have an intrinsic magnetic moment and can be separated without modification [25]. The next section discusses the use of magnetic fields to separate fetal cells.

3.3.1. Immunomagnetic cell sorting (MACS)

As with FACS, MACS also relies on the interaction of cells with antibodies. These antibodies are attached to super-paramagnetic beads instead of fluorochromes as in the case of FACS. The diameters of these beads typically range from several nanometers to a few micrometers. Labeled cells are retained inside a column when a strong magnet is placed outside while any unlabeled cells will be washed away. The cells can then be collected for analysis by removing the magnetic field [51,52]. Magnetic cell separation can be done either by positive selection [53,54] of the targeted fetal cell population or by negative selection using antibodies that are known to be present on cells that are not of interest for example CD45 mediated depletion of maternal cells [55,56]. Although MACS has the advantage of being faster, cheaper and does not require specially trained personal to operate, the sorting method has a poorer yield and purity compared to FACS. After separation by MACS, fetal NRBCS have been found to have high maternal cell contamination [17]. This can be improved by selective enrichment of NRBCs with Ficoll gradients prior to performing MACS [53]. Furthermore only one cell parameter at a time can be measured with MACS unlike

FACS where up to eight parameters can be obtained simultaneously [57]. Also, after separation, target cells still often have labeling agents bound to their surface antigens, which can sometimes cause problems for further analysis by hindering labeling with secondary antibodies [58]. Contamination of the sample can also occur if the iron oxide leaks from the magnetic beads. MACS systems have the risk of bead-to-bead interaction or aggregation, which can lead to non-specific cell entrapment. Large shear forces can also cause the bounds cells to be damaged when they are being pulled through the solution [59,60].

3.3.2. Magnetophoresis

Magnetophoresis a separation technique based on the natural intrinsic magnetic properties of blood cells. Whereas most biological cells are diamagnetic, RBCs become paramagnetic when deoxygenated. The change in magnetic properties is due to the chemical interactions between the iron atom, the heme group and the globin domain of hemoglobin. In oxygenated hemoglobin the chemical bonds are covalent but in deoxygenated hemoglobin the bonds are ionic with 4 or 5 unpaired electrons present making it paramagnetic [60]. Therefore if a magnetic field is created, deoxyhemoglobin RBCs and other cells will move in opposite directions allowing their separation. In 2008 Huang et al. [42] used this method to separate NRBCs from WBCs in a magnetic column. Most of the mature RBCs had been removed on chip using the deterministic lateral displacement method as described earlier. Following separation NRBCs were then identified by staining with May-Giemsa using an automated scanner. Using this method 37.68 NRBC/ml and 37.20 NRBC/ml were isolated in singleton and abnormal pregnancies, respectively. However these figures do not take into account the possibility that some of these NRBCs may be of maternal origin.

3.4. Adhesion based method

3.4.1. Soybean lectin-based method

Another fetal cell separation method based on the binding properties of cell surface molecules is the soybean lectin-based method developed by Kitagawa et al., in 2002 [62]. Galactose molecules are highly expressed on the surface of erythroid precursor cells. In this method, slides are coated with a galactose-containing polymer via soybean agglutinin (SBA), a galactose specific lectin. After a density gradient centrifugation step, NRBCs are enriched by their absorption to the slides. On average between 7 and 8 NRBCs, half of which of fetal origin, can be isolated from 2.5 ml peripheral blood samples using this method. A comparative study to evaluate this method was performed by Babochkina et al. in 2005 [63]. Maternal blood samples were collected, half were subjected to MACs using CD71 and the other half to the soybean lectin-based method. Eight times more NRBCs per milliliter of maternal blood were recovered using the SBA-lectin method compared to MACS [62] Purity was significantly lower through contamination with a high amount of non-nucleated red blood cells and screening the slides was quite labour intensive. The use of an automated scanning method to screen the slides would improve efficiency.

3.5. Electrical based separation

3.5.1. Charge flow separation

The highest number of NRBCs recovered from maternal blood was achieved by Wachtel et al. in 1996 and again in 1998 using charge flow separation (CFS) [63,64]. In the first case over 2000 fetal NRBCs were recovered from 20 ml samples of maternal blood. Two years later using the same method, Wachtel et al. reported a figure of 345 NRBCs were separated per ml of maternal blood, however only 30% of these cells were of fetal origin, when identified by FISH (probes used for X Y and fetal hemoglobin). The instrument used consisted of a separator and computer driven pumps to control the flow of buffer and sample as well as a buffer counter-flow gradient. The main separation chamber is divided by porous screens into multiple channels. As the cells move vertically, they are subjected to an electrical field and a horizontal flow gradient. Recovered fetal NRBCs are not damaged by CFS and remain fully viable, even for culture after separation. Fetal sex and chromosome abnormalities were accurately diagnosed from the fetal NRBCs. Although this method is highly successful at isolating fetal NRBCs, it has never been repeated possibly because the process involved multiple steps including a density gradient centrifugation step and selective lysis step before charge flow separation. This method therefore is quite time consuming.

3.5.2. Dielectrophoresis

Dielectrophoresis (DEP) is the term used to describe the motion of dielectric particles caused by polarization effects in a non-uniform AC electric field. Depending on their intrinsic electrical properties, cells can be separated, moved and trapped using DEP. The various cell types in blood respond differently depending on the frequency of the applied electric field, allowing their manipulation by adjusting the field frequency or amplitude of the DEP device [65]. In 2006, Xu et al. [67] Fig. 2 demonstrated a microfluidic device for the characterization of NRBC dielectrophoretic properties. The device incorporated an interdigitated electrode system to generate a non-uniform electric field, deposited on a glass wafer, which was covered by the PDMS microchannels as shown in Fig. 2. Fetal cells were found to have a crossover frequency of 50 kHz when the suspending cell buffer is 15 mS/m.

4. Conclusion

In the light of the established techniques described above, a quick method to separate fetal cells with great accuracy, sensitivity and efficiency to maximize cell yield is still required. Non-invasive prenatal diagnostic techniques that can be done earlier and safer in pregnancy can help reduce psychological stress of involved with current invasive techniques. Currently affinity based methods of cell separation such as FACS and MACS seem to dominate the research arena of NIPD with little research focusing on physical based methods of cell separation. Using these methods fewer than 20 fetal cells per 20 ml sample of maternal blood are obtained [68], as the extreme diversity of cells in blood raises serious challenges for their separation with high purity. There is also a non-negligible possibility that a large amount of fetal cells are being lost in the various discrete steps of separation and analysis. In the future we expect microsystems technology to be exploited further for NIPD especially in the physical methods of cell separation. Exciting new possibilities for non-invasive prenatal diagnosis are on the horizon, which one day may make prenatal testing available to all women.

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