Taiwanese Journal of Obstetrics & Gynecology 54 (2015) 343–349

Contents lists available at ScienceDirect

Taiwanese Journal of Obstetrics & Gynecology

journal homepage: www.tjog-online.com

Review Article

Noninvasive prenatal diagnosis

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ARTICLE INFO

Article history:
Accepted 27 May 2015

Keywords:
prenatal examination
noninvasive prenatal diagnosis
microfluidic system

ABSTRACT

Prenatal examination plays an important role in present medical diagnosis. It provides information on fetal health status as well as the diagnosis of fetal treatment feasibility. The diagnosis can provide peace of mind for the perspective mother. Timely pregnancy termination diagnosis can also be determined if required. Amniocentesis and chorionic villus sampling are two widely used invasive prenatal diagnostic procedures. To obtain complete fetal genetic information and avoid endangering the fetus, noninvasive prenatal diagnosis has become the vital goal of prenatal diagnosis. However, the development of a high-efficiency separation technology is required to obtain the scarce fetal cells from maternal circulation. In recent years, the rapid development of microfluidic systems has provided an effective method for fetal cell separation. Advantages such as rapid analysis of small samples, low cost, and various designs, greatly enhance the efficiency and convenience of using microfluidic systems for cell separation. In addition, microfluidic disks can be fully automated for high throughput of rare cell selection from blood samples. Therefore, the development of microfluidic applications in noninvasive prenatal diagnosis is unlimited.
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Introduction

Prenatal diagnosis is an important medical technology for nearly two centuries. The procedure can notify parents of hereditary diseases in the fetus such as Down syndrome, sickle cell anemia, Edwards's syndrome, cystic fibrosis, and Duchenne muscular dystrophy. These diseases may cause neonate stunted growth, intellectual disability, physical disability, and death. To date, expecting parents can select various methods to confirm fetal health. However, different diagnosis methods may be presented with different levels of risks, where invasive prenatal diagnosis procedures may induce miscarriage risks. Therefore, the development of safe and highly valuable prenatal diagnostic techniques is greatly sought after for scientists around the world.

The first prenatal diagnosis can be traced back to as early as the 20th century, after Wilhelm Röntgen's [1] discovery of X-rays. Although X-rays can be utilized to observe fetus appearance, it provides no genetic diagnosis. In 1966, Steele and Breg [2] separated fetal chromosomes from amniotic fluid for chromosome analysis which has laid the foundation of amniocentesis in modern medicine. Later, an Italian biologist, Simoni et al [3], performed the first trimester chorionic villus sampling (CVS) and risk assessment to validate this method as a reliable prenatal diagnosis tool [3,4]. These two methods are regarded as a model for prenatal diagnosis; however, their invasiveness may lead to a risk of miscarriage. Due to the potential risks of invasive prenatal genetic diagnosis, different noninvasive prenatal diagnosis (NIPD) techniques are actively being developed.

Invasive prenatal diagnosis

Although invasive prenatal diagnosis is dangerous, there exists no effective replacement among today's technologies. Table 1 indicates the most direct methods of siphoning fetal samples from the mother and their associated risk of miscarriage [5–9].

Amniocentesis

Amniocentesis was used to treat pregnant women with polyhydramnios in 1880. In 1966, American physicians Steele and Breg

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http://dx.doi.org/10.1016/j.tjog.2015.05.002
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Table 1
Comparison of invasive prenatal diagnosis.

	Execution time (wk)	Sampling location	Diagnosis	Risk of miscarriage (%)	Refs
Chorionic villous sampling	10–12	Chorionic villus	Chromosomal abnormalities	0.5–2	[5]
Amniocentesis	14–16	Amniotic sac	Chromosomal abnormalities/neural tube defects	0.06–1.3	[6,7]
Fetal blood sampling	≥17	Fetal umbilical cord	Chromosomal abnormalities/metabolic disorders/fetal infections	2–3	[8,9]

FISH = fluorescence in situ hybridization; PCR = polymerase chain reaction.

[2] successfully cultured amniotic fluid cells for chromosome analysis. Presently, it is the most widely used invasive prenatal diagnosis because of its high accuracy and low risk characteristics. Previous research has suggested that spontaneous abortion percentage after amniocentesis is 1.7% [10], which was slightly higher than the control group that underwent only ultrasonic examinations. The report in 2000 also suggested that the fetus miscarriage risk of amniocentesis is ~0.6–0.68% [11]. This method is applicable for women at 16–18 weeks of pregnancy, and ~200–300 mL of amniotic fluid in the uterus of pregnant women is required. Amniotic fluid contains ~2–3 $\times$ 10^5 cells per 10 mL, and these cells are produced by fetal movements in the amniotic sac due to swallowing, urination, and physical movements [12]. Amniocentesis is useful for the diagnoses of many single-gene diseases and congenital defects such as Down syndrome, thalassemia, Adrenoleukodystrophy, and Huntington's Disease.

Chorionic villous sampling

The advantage of CVS is that it is suitable for early screening for women at around 10–13 weeks of pregnancy and in special cases it can be performed as early as 8 weeks [13]. This approach extracts tissue and fetal placental chorionic cells transcervically or transabdominally, where the transabdominal method has been confirmed to be safer than the transcervical method in a previous report [14]. The miscarriage risk of CVS is higher than amniocentesis, which is ~0.5–2%. The fetal diseases that can be identified by CVS are similar to those of the amniocentesis, which include chromosomal abnormalities and genetic defects [5].

Percutaneous umbilical cord blood sampling

Cordocentesis, also known as percutaneous umbilical cord blood sampling (PUBS), draws blood samples directly from the fetal umbilical vein with a sampling needle. This method is suitable for women at the second trimester pregnancy stage, generally performed after 17 weeks of pregnancy [15]. The late sampling time of PUB than amniocentesis and CVS is because the early fetal umbilical vein is fragile and not suitable for puncture. Studies that investigated umbilical cord puncture procedure and fetal damage found no obvious pathological symptoms a week after PUBS [16]. Such direct sampling method has certain risks associated with gestational age, operating procedures, and sampling frequency of needle piercing, with different outcomes [8]. A statistical analysis has indicated that PUBS causes ~2–3% of miscarriages [9]. However, PUBS can be used to diagnose fetal chromosomal abnormalities, infections, and metabolic disorders that cannot be determined with CVS or amniocentesis.

NIPT

Currently, many scientists have been developing NIPT techniques for collecting fetal samples in order to reduce the risk of miscarriage from invasive procedures. Table 2 summarizes each technique and its disadvantages [17–28].

Ultrasonography

Ultrasonography is generally considered as a safe method of image rendering. It is one of the most common NIPT technologies, where images are formed when the echoes of ultrasound that penetrated the uterus tissue is received. The sound waves are reflected to the receiving probe with various interfaces, and after concussion, the signals are transformed to electrical signals to render two-dimensional, three-dimensional, or higher images. Ultrasound is often used for examining fetal congenital disability or abnormal development, by observing fetus nuchal translucency and nose bone development, which can predict the possibility of a fetus suffering from Down syndrome. However, in clinical practice, Down syndrome diagnosed with ultrasonography has a high false positives rate of ~5% [17]. Although ultrasonography examination cannot detect most of the inherited diseases or genetic defects, it is still an indispensable NIPT tool.

First and second trimester screening

Second trimester maternal serum screening includes double, triple, and quadruple tests. It is one of the most commonly chosen examinations. This method determines specific protein concentrations in maternal serum to calculate the likelihood and risk

Table 2
Comparison of noninvasive prenatal testing.

	Execution time (wk)	Target	Diagnosis	Detection rate for Down's syndrome (%)	Disadvantage	Ref.
Ultrasonography	<20	(1) Nuchal translucency (2) Nose bone	Down's syndrome Deformity	80–90	High false positive (4.5–6.0%)	[17–19]
Triple test	16	(1) α-fetoprotein (2) Oestriol (3) hCG	Down's syndrome Neural tube defects	60–70	No definitive diagnosis	[20,21]
Cell-free fetal DNA (mRNA) in maternal blood	>12	(1) Fetal mRNA (2) Fetal DNA	Single-gene disorders Aneuploidy	>99	Large sample Cost effectiveness	[22–25]
Fetal cell in maternal blood	4–14	(1) Fetal lymphocytes (2) Trophoblasts (3) Nucleated red blood cells	The disease can be detected by PCR or FISH	75	Very rare	[26–28]

FISH = fluorescence in situ hybridization; PCR = polymerase chain reaction.

assessment of trisomy 21. Maternal serum screening utilizes indicators such as alpha-fetoprotein, oestriol, and human chorionic gonadotropin [29,30]. The execution time is during the second trimester of pregnancy, which is equivalent to ~15–20 weeks. The attractive advantage of maternal serum screening is that the serum is taken directly from the mother, with no invasion into the uterus. However, previous studies have indicated that this method of testing for Down syndrome is associated with a 5% false positive rate and the sensitivity is ~57–60% [31,32]. The current most effective noninvasive screening for trisomy 21 is a combination of maternal age, measurement of fetal nuchal translucency, maternal serum free β-human chorionic gonadotropin, and pregnancy-associated plasma protein-A concentration at 11^{+0} weeks to 13^{+6} weeks of gestation. This combinatory method provided around 90% detection rate of trisomy 21 with the false-positive rate of 5% [33]. In different strategies, all of the high risk assessments are recommended to be performed in combination with amniocentesis or CVS for definitive confirmation.

Cell-free fetal DNA/mRNA in maternal blood

In 1996, two teams observed cell-free DNA in patient serums for small-cell lung cancer and head and neck cancer [34,35]. The identification of cell-free DNA in serum has prompted the development of other related diagnostic technologies. Lo et al [36], was successful in identifying a male fetus cell-free DNA from the mother's serum and plasma. Three years later, fetus cell-free mRNA was also found from the mother's plasma [37].

A previous study has used real-time polymerase chain reaction (PCR) to quantify the cell-free DNA found in maternal plasma, and observed 3–6% of Y-chromosomal sequences [38]. This result suggests that maternal plasma contains a certain amount of fetal cell-free DNA for prenatal analysis. Generally cell-free DNA is commonly used for sex determination, fetal Rhesus D blood group identification, and the diagnosis of single-gene disorders such as achondroplasia and β-thalassemia [39–42]. However, due to the fragmented nature of cell-free DNA, the identification of fetus DNA is often difficult, where the distinctions are often made by fetal DNA markers such as male fetus Y chromosomal markers, single-nucleotide polymorphisms, and unique fetal DNA methylation markers [43]. Because of fragment information, cell-free DNA analysis has limitations for identifying fetal aneuploidy diseases such as Down syndrome, Edward's syndrome, and Patau syndrome [38,44]. Next generation sequencing can sequence a large amount of DNA sequences in a short time with upgraded computing speed and decoding algorithms. With massive parallel sequencing and powerful bioinformatics processing, maternal fetal cell-free DNA diagnosis of fetal aneuploidy has been demonstrated recently [45], with a 99% detection rate of Down syndrome and false positive rates of <1% [46–49]. In August 2011, a technology called Noninvasive Fetal Trisomy NIPD of Down syndrome was made available in a private prenatal diagnosis center in Hong Kong [45]. This method uses GC-correlation methodology with massively parallel sequencing for large-scale sequencing of maternal serum cell-free DNA [50]. The DNA fragments were obtained through computer analysis of sequencing results, and the analysis of aneuploidy was performed by comparing the DNA fragments to those found with normal levels in pregnant women in the control sample pool [23]. However, the acceptance of such exorbitant novel technology from the general public is very low.

Fetal cell in maternal blood

The first observation of fetal cells in maternal blood circulation was made by Christian Georg Schmorl, a German pathologist, in 1893. He had found multi-nucleated syncytial trophoblasts in lung tissue of a pregnant woman that died from eclampsia [51]. This discovery indicated that fetal cells are drawn into the mother's body through some mechanisms and thereby enabling NIPD by separation of fetal cells from maternal blood. Compared with the maternal fetal free DNA/mRNA, maternal circulating fetal cells have complete genetic information in their nucleus or cytoplasm that can be useful for advanced complex genetic diagnosis. This is especially useful when identifying fetal genetic defects that the mother or father also possess, since the fetal cell is not easily mistaken as a parent natively cell. However, the frequency of fetal cells is very low. Therefore, effective isolation techniques for fetal cells have been the subject of constant research and development. The types of fetal cells in maternal blood can be broadly classified as trophoblasts, fetal lymphocytes, fetal nucleated red blood cells (fnRBCs), and hematopoietic stem cells (HMCs) [52]. Due to the different characteristics of each type of cell, these cells may have a different difficulty of separation and can affect the accuracy of the analyses.

Trophoblasts

Trophoblasts have been isolated as a target in earlier cell fetus research with specific antitrophoblast antibody H315 [53–55]. However, a later report has reported that this antibody is not sufficient to separate trophoblasts for prenatal diagnosis [56]. Furthermore, trophoblasts should not appear in normal pregnant women. Some reports have indicated that ~1% of placental cells with placental mosaicism can lead to discrepancies in the true state of cell nucleus type [57,58], which can greatly affect the accuracy of trophoblast based diagnosis. Therefore, NIDP based on trophoblasts is limited.

Fetal lymphocytes

Previous studies have found cells with XY chromosomes in pregnant women from conceived male fetuses [59,60]. These cells are fetal lymphocytes that have crossed the placenta into maternal circulation. Some groups have used fetal lymphocytes for fetal sex prediction [59,61], and have indicated by microscopic observation that one in every 1000–5000 mother lymphocytes is a Y-chromosome lymphocyte [62]. In addition, fetal lymphocytes can be amplified with *in vitro* culture [43], where the rare cell population can be amplified for additional analysis. The fatal flaw of using fetal lymphocytes for NIPT is that fetal cells can retain in the mother for 27 years [63]. Therefore, the test results are not reliable for second pregnancies.

HMCs

Fetal cells are very rare; therefore many researchers have increased the number of target cells to include other potentially useful fetal materials. In recent years, some groups included $CD34^+$ fetal stem cells to the target list [64]. $CD34^+$ HMCs have the ability for *in vitro* culture like fetal lymphocytes, however, maternal and fetal $CD34^+$ HMCs are not easily distinguishable. The HMC culture, while easily enlarged, the separation of fetal cell from maternal cell contamination is an important issue. It is also worth noting that residual $CD34^+$ HMCs are also found in women after pregnancy [52].

FnRBCs

The NIPD suitable target cells should have several properties such as a short half-life, appears in the early stages of pregnancy, and have specific cell markers. The fnRBCs are a good candidate with all three properties [52], which are also the most studied and isolated fetal cells from maternal blood [64–71]. The biggest drawback of this type of cell is that they are very rare and some of

the nucleated red blood cell (RBC) is maternal [72]. Previous reports have pointed out that each milliliter of maternal blood contain one to two fnRBCs [67,68], while another group has also reported hundreds of fnRBCs/mm of maternal blood [65]. The fnRBCs are approximately between 9μm and 13 μm in diameter, which is bigger than adult RBCs (6–8 μm) [73] and more similar to white blood cells (8–15 μm). The density of fnRBCs ranges between 1.077 g/mL and 1.130 g/mL [74], similar to those of the adult RBCs (1.090–1.110 g/mL). Nucleated RBCs are RBCs that have not denucleated or are mid-erythroblasts in erythropoiesis, where the erythroblast discharges the cell nuclear and becomes a reticulocyte before entering into circulation. The discharged cell nucleus is called a pyrenocyte and are scavenged by microphages [75].

The inter- and extracellular cell markers of fnRBCs change following the development process. For example, burst-forming unit erythroid during early hematopoiesis express CD71 in bone marrow, but CD71 disappears when cells differentiate into mature RBCs. Hemoglobin appears later, gradually formed in the late erythroblast. CD45 has not been observed in full development process [76].

The biggest difference between fnRBCs and adult RBCs is the hemoglobin structure. Generally, a globin chain constructs of hemoglobin tetramers in RBCs and nRBCs changes at different stages of fetal development. The currently known fetal hemoglobin contains ζ2ε2, ζ2γ2, α2ε2, and α2γ2 [73], unlike adult RBCs tetramers of α2β2. The hemoglobin in fetal hematopoiesis is also called embryonic hemoglobin, and ζ and ε globin chain will be replaced by adult hemoglobin at ~9–18 weeks of gestation [77,78]. Hematopoiesis remains after the fetus's birth, and is transferred to the bone marrow. The γ globin chain will not completely disappear, but the vast majority of hemoglobin tetramers will be ofα2β2 [77,78] Therefore, ε and γ globin chain can be used to distinguish between maternal nRBCs and fnRBCs in maternal blood; however, γ globin chain may appear in general adult patients with beta-thalassemia [79], and ε globin chain disappears in early pregnancy. These specific markers are used by many researchers for isolating fetal cells [79–81].

FnRBCs separation from maternal blood

There are many methods such as a magnetic-activated cell sorting (MACS), charge flow separation, fluorescence-activated cell sorting (FACS), density gradient centrifugation (DGC), and immunomagnetic beads for separating fetal cells from maternal blood. The purity and recovery rate are different for each of the methods. For most experiments, a combination of different separation technology is often used to ensure sample purity. However, the increase in separation concatenation may decrease the recovery rate. However, poor separation methods will lead to low sample purity. Therefore, the design for optimal separation for the experiments will be the most important part of cell separation. Table 3 summarizes the performance of each isolation technique of fetal cells [26,64,65,68–70,82–84].

In fnRBCs separation, most research groups separate peripheral blood mononuclear cell (PBMC) from maternal blood, and remove RBCs using DGC [64,65,67,69,85]. The DGC separation buffer contains Ficoll, Percoll, and Histopaque (Sigma). Histopaque is Ficoll, with the addition of sodium diatrizoate products that enhance stratification in blood samples. The DGC separation buffers are divided into different densities, some of the commonly densities are 1.077 g/mL, 1.083 g/mL, and 1.119 g/mL. Previous studies have isolated fnRBCs from Ficoll DGC separated RBCs [64,67]. Another study has also determined that buffers with a higher density (1.119 g/mL) are better for isolating RBCs than low density buffers (1.090 g/mL) [68]. In addition, cell loss in Ficoll DGC and Percoll DGC with 1.7×10^6 CD45-mononuclear cells, observed after implementation of Ficoll DGC or Percoll DGC, the ratio of loss was 14.6% and 41.2%, respectively [86]. These results indicate that different DGC separation buffers in different cell types will have different recovery rates.

After extracting PBMC from maternal blood using DGC, the fnRBCs groups are further separated with fetal specific cell surface markers. The most common screening methods are MACS, FACS, and immunomagnetic beads. The removal the $CD45^+$ cells prior to separation can effectively enhance the results of all purification techniques [87]. The $CD45^-$ cell population can be used as fnRBCs specific cell marker for positive selection. CD71 is the most common fnRBCs surface marker [64,88,89] that is also known as transferrin receptor or p90. It is the first cell marker to be used to separate fnRBCs [90]. There are also some groups that have chosen glycophorin A as the main cell marker for separation [69]. However, glycophorin A may appear on mature RBCs [76], so unless the previous step has the ability to remove the RBC cleanly, otherwise it will seriously affect the purity of the result. The fnRBCs can also be selected by i-antigen or γ-hemoglobin [64,68]. The i-antigen is originally expressed on the fnRBCs, which is gradually replaced by I-antigen after birth [91]. However, adult RBCs will still express a small amount of i-antigen [92], which may interfere with the separation results. In terms of specificity, the fetal specific hemoglobin is the best choice for separation. However, hemoglobin is an intracellular cell marker where immunofluorescence and cell permeability and fixation will lead to cell loss. In addition, using

Table 3
Isolation of fetal cells.

Group	Year	Target cells	Method	Efficiency	Refs
Ponnusamy et al	2008	FNRBCs	Percoll 1118 DGC/MACS Depletion CD45/GPA positive selection/selection lysis	3.0 cells/20mL blood (confirmed with ε-Hb)	[69]
Samura et al	2000	NRBCs	Histopaque 1119 or 1090 DGC/MACS Depletion CD45/flow sorting γ-Hb^+	10.67–21.91 cells/17 mL blood	[68]
Ikeya et al	2005	NRBCs	Histopaque 1095/Lectin method	89.4 ± 92.6 cells/10mL blood (confirmed with MGG and HbF)	[82]
Prieto et al	2001	Fetal NRBCs	Double DGC 1.077 & 1.107/MACS selection	194.6 cells/8.5mL blood	[70]
D'Souza et al	2009	Fetal NRBCs	Percoll DGC/FACS ($CD71^+$ or GPA^+, CD45-, HbF^+)	31–499 cells/7mL bloods	[65]
Calabrese et al	2012	NRBCs CD34+ cells	Ficoll 1083 DGC/Dual-probe FISH ($CD71^+$/i-$antigen^+$ or $CD34^+$/i-$antigen^+$)	71000 cells/25mL blood	[64]
Pongsritasana et al	2006	FNRBCs	Ficoll DGC/FACS or MACS ($CD45^-/CD71^+/CD235A^+$)	$1.7–2.2 \times 10^{-6}$ in MNCs	[26]
Jeon et al	2010	Fetal NRBCs	Double Percoll DGC 1.077 & 1.119/MACS CD71 selection	9.85–14.88 cells/10mL blood	[83]
Bischoff et al	2003	Progenitor cells	RosetteSep progenitor enrichment cocktail/CD45 depletion	6200 cells/20–30mL blood	[84]

DGC = density gradient centrifugation; FNRBC = fetal nucleated red blood cells; MACS = magnetic activated cell sorting; NRBC = nucleated red blood cells.

immunomagnetic beads to hybridize against intracellular markers must consider if the beads are taken into the cells or conjugated through the primary antibody. Therefore, using intracellular cell marker for fnRBCs separation will encounter more difficulties.

Most of the group's fnRBCs separation countermeasures were to first remove most of the RBCs by DGC. The PBMC collected from interphase after DGC and $CD45^+$ cell separation were then subjected to MACS, FACS, immunomagnetic beads, or other selection technologies. However, novel separation procedures are still being developed. For example, the direct use of the lectin method after DGC crawl fnRBCs or size-base separation by using microfluidic chips [67,71,82]. Although the present method for fnRBCs separation has many options, the investigation of an optimum separation process is being investigated by scientists around the world.

The isolation techniques of fnRBCs using microfluidic

With the constant development of micro- and nano-technology, novel process technology can be combined with biological materials into a tiny chip. This portable biochip, also called Lab-on-a-chip or micro total analysis systems, are widely used in cell separation technologies such as microfluidic, size-dependent hydrodynamic filtration, magnetic, and electrophoresis [93]. Microfluidic has been proven to effectively combine a variety of analytical systems that operate with a small amount of the sample for ease of sampling [94]. Microfluidic or microfluidic disk, with respect to flow cytometry, express higher sensitivity for rare cell separation [95]. The advantage of using microfluidic for cell separation include specific chip flow channel design for a single cell, automated experimental processes, high throughput, and reduced use of reagents and generated chemical wastes [96].

Microfluidic can separate fetal cells by physical properties such as size, deformability, electric, and optical properties. Antibody screening with specific cell markers can also be used with the system [96]. A previous study has used DGC followed by size-specific microfluidic chip and cell deformation to separate fnRBCs, in which it showed an average separation of 1.2 fetal cells/mm of maternal blood [67]. Another group has also used DGC, size-based microfluidic system, followed by magnetic beads for the selection of hemoglobin, which collected 274.38 nRBCs/mm of maternal blood [71]. These results indicated that the design of the microfluidic system can greatly affect the efficiency and recovery rate of separation. Currently, most practices use DGC followed by traditional methods to remove RBCs before microfluidic chip cell separation. However, DGC functional microfluidic disk has gained much attention in recent years. The microfluidic disk not only improved process automation, it was also demonstrated to separate rare cells from blood [95,97]. Despite this, there exists few reports that have utilized microfluidic disks for fetal cell separation from maternal blood. The further development of such devices still shows much promise.

Operation after fnRBCs separation

The use of fnRBCs, resulting from different separation techniques is an important topic. Known fetus common hereditary diseases such as aneuploidy, chromosome defects, and genetic defects are diagnosed by the appearance of chromosomes, gene sequencing, PCR, multiplex ligation-dependent probe amplification, and fluorescence *in situ* hybridization (FISH). However, the resulting fetal cell isolation is often contaminated by maternal cells (background interference). Therefore, the sensitivity of the methods for the subsequent genetic analysis is very important. A previous study has demonstrated the use of highly sensitive FISH for the observation of triploid chromosome 21, 13, and 18, where the sensitivity is up to 0.01% and the accuracy is >98% [85]. Techniques such as PCR and other methods may be more difficult than FISH for the observation of hereditary diseases under large background interference. Even after the amplification of specific gene fragments, the fetus signals are still easily obscured by the signals from maternal background cells. Therefore, samples with low purity are more suitable to use FISH for the direct calibration and observation of specific cells, which can avoid large interferences from the maternal background cells.

If diagnostics by PCR is required, with sequencing or other analogous molecular biology techniques, it is necessary to increase sample purity. Antibody and immunomagnetic based separation have a certain degree of limitation for improving purity, because with the use of antibodies it is difficult to avoid nonspecific calibration and adhesion. Therefore, micromanipulation may be the most effective way for improving sample purity [52]. Micromanipulation relies on the operator to identify the cell types, and a single cell is isolated from a large background of cells. Many molecular biology techniques are sufficient to support the operation of a single cell. For example, single cell PCR requires only one of the target cells for gene amplification. Capillary electrophoresis and short tandem repeat fragment analysis can then be used to distinguish the single cell from mother or fetus [64]. Many studies have also reported that mothers with abnormal fetuses have increased amount of maternal blood nRBC [24,98]. The number of normal maternal nRBCs is between one to 12 cells, however, a mother with a fetus suffering from beta-thalassemia will have increased nRBCs of about 22–158 cells; and a mother with abnormal ultrasonography fetal diagnosis also exhibits more nRBCs [98]. The reasons for this phenomenon is not yet clear, and perhaps, in the future, can be used as a noninvasive diagnostic indicator for the risk of fetal chromosomal abnormalities.

Conclusion and perspectives

Using NIPD for prenatal diagnosis of aneuploidy and genetic disease, where risk-free collection of fetal samples has become the focus of recent prenatal diagnosis developments. Although cell free DNA in maternal blood has been widely used and accepted in clinical examinations, it relies heavily on new sequencing technologies that are relatively expensive. In addition, cell free DNA for the diagnosis of aneuploid and maternally inherited diseases is difficult. Therefore, fetal cells in maternal blood are an ideal solution for this limitation. With the progressive advancement in separation technology, microfluidic cell separation has become more simple and common. The microfluidic system is easily operated through automation, and requires only a trace amount of sample, which in term saves human resources and reduces costs. Furthermore, the subsequent processing of fetal cells is not limited to large fragment gene sequencing; it is able to support various molecular biology techniques for the ease of analysis.

Conflicts of interest

The authors have no conflicts of interest relevant to this article.

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