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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