以微流體晶片技術發展非侵入式的試管嬰兒胚胎植入前基因診斷技術

Development of microfluidic chip for non-invasive preimplantation genetic screening (PGS) during in-vitro fertilization (IVF)

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The current method to detect the fetus genome during in-vitro fertilization (IVF) is highly dependent on the amount of cell free DNA (cf-DNA) derived from the embryo. Here, we have developed the first magnetic-based cell free DNA (cf-DNA) extraction with an EWOD system in IVF. We have introduced a new format for cf-DNA extraction using digital microfluidics (DMF), electrowetting-on-dielectric (EWOD) and magnetic forces to separate, suspend DNA-coated magnetic particles (Figure 1). This new methodology extracts fetal DNA without damaging the embryo development before implantation. Compared to conventional techniques, this method decreased the reagent volumes, duration of analysis and extraction quantity of DNA, while retaining a level of analytical performance required for clinical screening. We have hence provided a great prospective EWOD platform and point-of-care testing (POCT) method for IVF application. We have successfully extracted cf-DNA at an average weight of 91.47 femtograms (fg) from the mouse embryo cultured medium (2.5 days & 3.5 days). These results show that DNA extraction with EWOD appears promising, which will pave a new path towards a renowned lab-on-a-chip concept (As illustrated in Figure 2). The extraction protocol in cf-DNA extraction from mouse embryo-culture medium (KSOM medium) at 3.5 and 2.5 days has been carried out. The quantitative result analysis is shown in figure 2. For 2.5 days' sample (E 2.5), the weight of cf-DNA in samples ranges

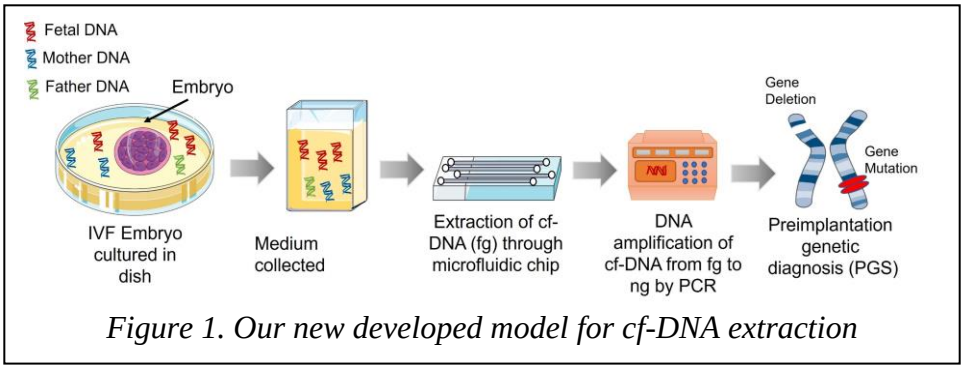

Figure 1. Our new developed model for cf-DNA extraction

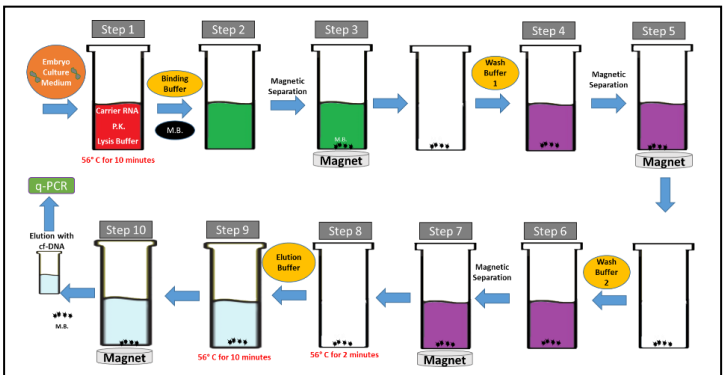

Figure 2: The typical way of cf-DNA extraction

from 7 femtograms (fg) to 243 femtograms (fg) which in turn gives an average weight of 91.47 femtograms (fg). For a 3.5 days' sample (E 3.5), the weight of cf-DNA in samples ranges from 0.14 femtograms (fg) to 5.71 femtograms (fg) which in turn gives an average weight of 3.28 femtograms (Figure 3). However, as the extraction quantity is in fg range, current biomedical technology is not matured enough to enhance/amplify the extracted cf-DNA. Hence, we tried to modified our cf-DNA extraction protocol and then used "two times qPCR" to amplify the amount of cf-DNA. We first used genomic DNA to test 'two times qPCR" and found it can increase the amount of DNA from 4.78ng to 98ng through PCR amplification (Figure 4). Currently we are working on cf-DNA extraction followed by two times PCR from the mouse embryo culture medium (2.5 days & 3.5 days). Once we can get cf-DNA at ng level, we will perform preimplantation genetic diagnosis (PGS) to verify the genetic information from cf-DNA.

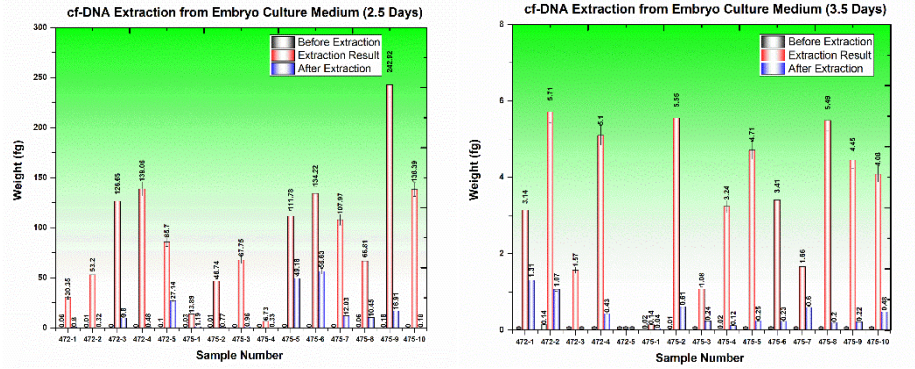

Figure 3: The quantitative result analysis of cf-DNA extraction from mouse embryo culture medium from 2.5 days and 3.5 days of culture maturity.

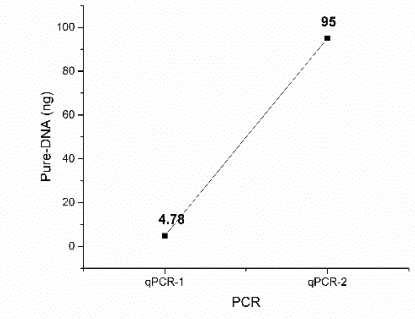

Figure 4: The amount of g-DNA after Two-times qPCR

Overall, our modified way of cf-DNA extraction will help to imcrease the amount of cf-DNA extraction. Along with this, 'two-times qPCR' protocol will further enhance the amplification quantity. We have successfully implemented in genomic DNA, and right now we are trying to do the same from the cf-DNA isolated from the mouse embryo cultured medium.