Establishment of the Full Term Placental Chorionic Villous Proteome by Free Flow Electrophoresis and 2D SDS-PAGE

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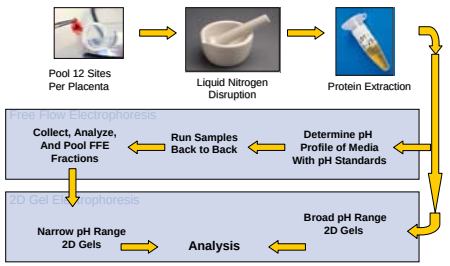
Abstract

Preeclampsia (PE) is a pregnancy-specific disease that affects 5-8% of all pregnancies worldwide and is the leading cause of maternal and neonatal mortality. While the cause of PE is unknown, clinical evidence unequivocally points to the placenta as the site of pathophysiology. It is thought that impaired placental development results in chorionic villous (CV) hypoperfusion and the release of endothelial toxicity factors into the maternal circulation. We hypothesize that comparison of CV proteomes between normal and PE pregnancies will provide clues to the pathophysiology of the disease. It is accepted that inter-patient variability exists in clinical proteomics. Therefore, prior to differential display analyses, it is essential that the proteomic baseline and its variance in a normal patient population be established. In this study, we present and show the efficacy of free flow electrophoresis (FFE) coupled with 2D SDS-PAGE in the analysis of full term CV.

High reproducibility, protein separation rates of up to 100mg/hr and high tolerance for endogenous salts make FFE a robust and effective tool of protein pre-fractionation. Here, we compare three full-term CV samples and the quality of separation across a 60-minute 60-fraction protocol. Furthermore, we show that FFE is an effective method for pre-fractionation of protein samples prior to narrow-range 2D gel electrophoretic analysis and protein identification by MALDI-R and LC-MS/MS.

Experimental Approach



Free Flow Electrophoresis

The use of free flow electrophoresis in the pre-fractionation of proteins, organelles and whole cells is well established and has been in use for decades (1). FFE consists of two fundamental forces: 1) an applied voltage in the x-axis that establishes a stable pH gradient via the prolytes in the separation media and 2) the laminar flow of separation media in the y-axis that moves focused sample through the separation chamber (Figure 1).

Through slow application of sample into the pre-focused liquid media, application of an external voltage and continuous laminar flow it is possible to obtain reproducible isoelectric focusing and separation of samples in a rapid and robust manner.

Figure 1: FFE, A Stable Balance Between pH Gradient And Laminar Flow of Liquid Separation Media.

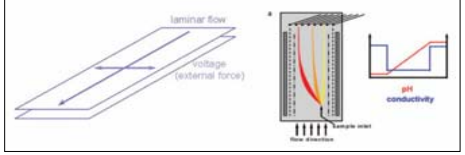
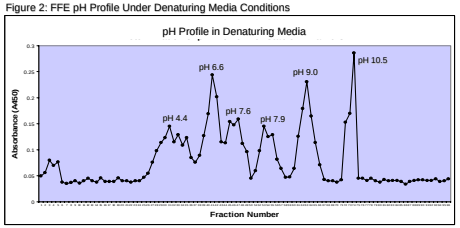
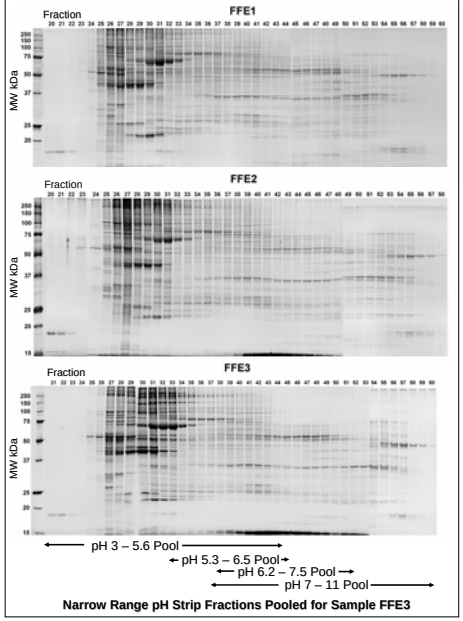


Figure 2: FFE pH Profile Under Denaturing Media Conditions



Due to slight variations in the media composition between runs, it is necessary to perform a pH calibration run with chromophoric pI markers (Figure 2). Data collected from this run may be applied to concurrent sample separations as long as the voltage and laminar flow of media stay constant (3). By running samples back to back, with only brief resting periods between samples, it was possible to obtain reproducible sample separations across a known pH range (Figure 3). As shown below, three CV protein samples run on this instrument all exhibit the same separation profiles. Although single protein species were not confined to single fractions, it should be noted that high abundance proteins were successfully separated in the samples allowing for the enrichment of lower abundance proteins in adjacent fractions. By pooling fractions within known pH zones (Figure 3) the pre-fractionated protein pools can then be subjected to narrow-range 2D gel electrophoresis.

Figure 3: Three Individual Chorionic Villi Samples Separated by FFE



Narrow Range pH Strip Fractions Pooled for Sample FFE3

While broad-range 2D gel separation can be used for high resolution separations for samples with low complexity and limited dynamic range, most real-world applications of this methodology are limited by these two factors. Figure 4 demonstrates that the use of broad-range pH gradients is adequate for a fast, global look at the entire range of proteins present in the CV sample. However, when the FFE pH pools are run on narrow range pH gradients (Figure 5), it is evident that a much higher resolution survey of the CV proteome is revealed. In addition, several benefits over direct loading of sample onto narrow range pH gradients are observed. First, the pre-fractionation aspect of the FFE dimension allows the retention of valuable protein sample that lies outside of the pH range of the strip. Second, the resolution is increased and narrow range pH gradient focusing time is shortened due to the reduced load of proteins focused outside the range of the strip at the anode and cathode (data not shown). Figure 5 clearly illustrates the resolving power of FFE coupled to narrow-range 2D SDS-PAGE.

Figure 4: Broad pH Range Separation of Full Term CV

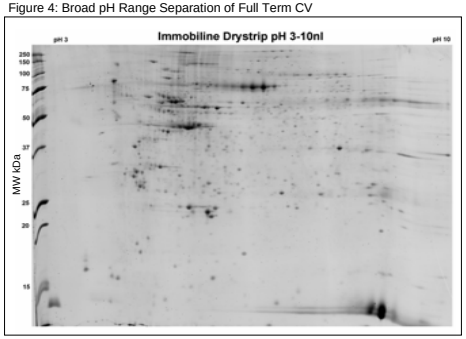
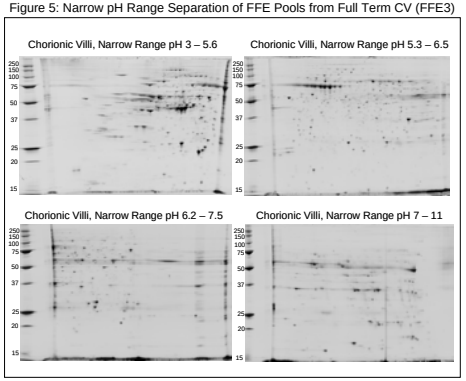


Figure 5: Narrow pH Range Separation of FFE Pools from Full Term CV (FFE3)



Hundreds of spots were selected for mass spectrometric analysis after meeting the statistical thresholds for reproducibility between gel sets. The majority of these spots were identified by MALDI-R with unidentified gel spots being subsequently analyzed by LC-MS/MS. Figures 7, 8 and 9 are representative MALDI spectra of three placental proteins identified in this study.

Figure 7: MALDI Spectrum of Estradiol 17 beta dehydrogenase

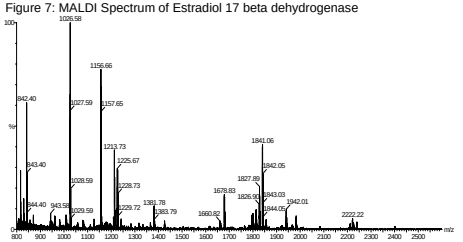


Figure 8: MALDI Spectrum of Chorionic somatomammotropin hormone precursor

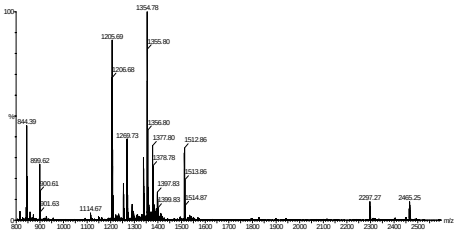
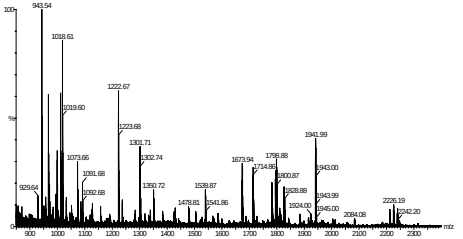
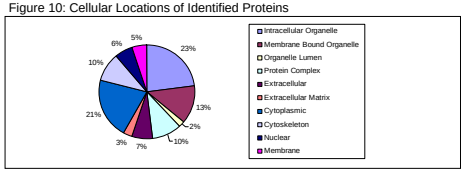


Figure 9: MALDI Spectrum of Placental anticoagulant protein III



Hundreds of proteins were identified in this study. As expected, some of the proteins identified were serum proteins. However, a large proportion of the proteins identified were solely of placental origin. Figure 10 diagrams proteins identified to date by cellular location.

Figure 10: Cellular Locations of Identified Proteins



Conclusions

- As a means to pre-fractionate Chorionic Villous samples, FFE is an effective approach.
- Tolerance to high concentrations of salt in the sample and sample separation rates of 5-10mg/hr allow this methodology to be used as a robust first dimension before narrow range 2D electrophoresis with minimal sample handling.
- The increased resolution and quality of the narrow range 2D SDS-PAGE gels post FFE allowed for the rapid identification proteins by MALDI.
- Ongoing analysis of the FFE fractions by 2D LC MS will allow for an even deeper analysis of CV samples.

References and Acknowledgements

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