**Novel electrochemical assay for sensitive quantification of exosomal miRNA associated with preeclampsia**

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**Introduction**

Each year, 10 million women worldwide develop preeclampsia (PE). Around 76,000 women and 500,000 babies die of PE and related hypertensive disorders per annum. PE leads to more than 20% of premature births worldwide and causes intrauterine growth restrictions in affected babies, thus posing a lifelong challenge to the wellbeing of millions of children. Disease specific circulating miRNAs, either in cell-free or exosome enclosed forms, have recently emerged as promising minimally invasive biomarkers for improved diagnostic, prognostic and streamlined therapeutic applications. Recently, we have established differences in the exosomal miRNA profile across gestation and found miRNA-486-5p to be contributing the most in differentiating between normal and PE, thus indicating its potential as a biomarker to classify women at risk of developing PE at early pregnancy (Salomon 2017). Herein we report a novel highly sensitive biosensor for PE specific miR-486-5p.

**Methods**

The assay utilizes specially designed biotinylated capture probes composed of; region complementary to miR-486-5p, and “tag sequence” corresponding to the consensus binding site for DNA binding transcription factor p53. Target miRNA is magnetically separated and purified from heterogenous pool of exosomal miRNAs using streptavidin coated magnetic beads, followed by engineering of p53 binding site via T4 DNA polymerase-based end-filling of 5`-overhang (tag sequence region in probe). The engineered target is released from capture probe by heating and is captured on to screen printed gold electrode (SPGE) surface by complementary probes immobilized on SPGE surface via thiol-gold interaction. Binding of detection probe complementary to engineered tag sequence generates double stranded binding site for HRP-labelled p53. Target miRNA concentration can then be monitored amperometrically by H2O2/HQ system. A small cohort of women at early gestation (<16 weeks) who developed PE later in pregnancy was used to validate the assay in clinical samples.

**Results**

This biosensor can detect target miRNA in clinically relevant (sub-picomolar) levels over a wide range of concentrations (100fM to 1nM). Good linearity (R2 = 0.96) and reproducibility (RSD between triplicates <5%) was observed. Assay was also able to quantify miR-486-5p in plasma samples from pregnant women before 16 weeks of gestation who developed PE later in pregnancy. Results of our assay were found to be significantly correlated (p<0.05) with the qPCR-based quantification.

**Discussion**

While the assay can be easily adapted to any miRNA target by using specific complementary capture probes, high level of specificity is ensured by using molecular engineering. We envisage that this novel assay can form the basis of highly sensitive miRNA-based diagnostics.

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