Public Abstract
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CHAPTER 1

IN VITRO NON-INVASIVE PRENATAL DIAGNOSIS WITH MAGNETIC NANOMATERIALS

Prenatal Diagnosis is a useful tool in the modern-day era to obtain information about the genetic health and makeup of a child (progeny) before its birth. This method helps parents gain prior knowledge about any genetic mutations that are present in the fetus (baby), which helps them and the doctors to plan the possible treatment and health management options after birth.
There are a few different techniques for prenatal diagnosis, namely Amniocentesis and Chorionic Villus Sampling (CVS). Albeit having a high sensitivity and accuracy, this procedure poses considerable risk to the fetus and is conducted only in the second or third trimester of pregnancy. CVS also poses potential risk to the health of the fetus. It is crucial to develop a more non-invasive technique, where fetal cells or fetal DNA can be obtained from the mother without directly affecting the amniotic sac or the placental tissue. Trophoblast cells play an important role in the fetal implantation into the endometrium wall and hence can be found into the uterine region of the mother. The trophoblast cells are in a pool of mother's epithelial cells and are sparsely populated. Hence it is crucial to enrich the trophoblast cells from the mother's cells and extract genetic information from them. To this effect, we made a magnetic nanoparticle based enrichment platform to isolate trophoblast cells from a vaginal swab sample collected from pregnant women who are in the first trimester of pregnancy. We created a library of magnetic nanoparticles for this purpose.
We conducted preliminary studies with human choricarcinoma (Jeg3) and breast cancer (SKBr3) cells to determine the sensitivity and selectivity of the magnetic nanoparticles. The magnetic nanoparticles demonstrated a capture efficiency of 75%. Encouraged by the results we then conducted clinical studies to test whether magnetic nanoparticles can selectively isolate trophoblast cells from a pool of epithelial cells. Trophoblast cells were extracted from placental tissues of male fetus and spiked in epithelial cells obtained from the vaginal swab obtained from non-pregnant women. Two different extraction methods were used, direct conjugation and HLA-G saturation. The isolated cells were imaged under fluorescence and DNA was extracted to amplify them using PCR and were analyzed using STR sequencing. The capture efficiency of direct conjugation method was around 65-70% according to fluorescence images. DNA analysis also demonstrated a 50-65% enrichment of the trophoblast cells. Capture efficiency of HLA-G saturation method was around 75-80% according to fluorescent images, DNA analysis demonstrated a 75% enrichment of the trophoblast cells. We then conducted clinical studies on samples on swab samples collected from pregnant women in the first trimester of pregnancy. Cells were isolated using direct conjugation and HLA-G saturation methods. The cells were stained with ?HCG-Cy5 and analyzed under fluorescent microscope. IHC analysis with CD10 and CK7 antibodies was conducted on the extracted trophoblast cells. The cells were enriched after magnetic separation. DNA was extracted from the isolated cells and the amplified and quantified using PCR. The amount of DNA extracted corroborated with the theoretical analysis. A male profile was obtained from the extracted DNA from some of the samples suggesting that the DNA could be from the male fetus. Thus in conclusion, we have demonstrated that magnetic nanoparticles can extract trophoblast cells from a pregnant swab samples (less than 12 weeks

GA) with around 75% efficiency.

CHAPTER 2
NANOPARTICLES FOR SCREENING OF RETINOPATHY OF PREMATURITY (ROP)

Retinopathy of Prematurity (ROP) is an abnormal retinal vascular developmental disease which primarily affects premature infants born before 32 weeks of gestation or born with birthweight under 1500gm. ROP inhibits the retinal neuronal and vascular development in the preterm infant that results in aberrant vascularization of the retina. ROP is the leading cause of blindness among infants and children. ROP does not present any visible symptoms in the infant in the earlier stages. Severe ROP leads to blindness. The current diagnostic procedure for ROP involves a physical fundus exam of the premature infants by an ophthalmologist by dilating the eye of the infant. This analysis method is very subjective, and the results are prone to changing based on the doctor examining the eye. In addition, the procedure is very discomforting for the infant and parents would not be amenable to consenting to this method more than once or to be used as a screening technique.

An imbalance of ROS in the body is created which leads to oxidative stress. During the embryonic growth, the production of antioxidants takes place at the later gestational stage (after 30 weeks). Infants who are born premature (before 32 weeks) have much weakened or poorly developed antioxidant production which leads to increased oxidative stress in their body. An intracellular ROS causes strand breaks and modifications in the DNA. 8-OHDG is formed as a result of oxidative DNA damage as result of an enzymatic cleavage followed by 8-hydroxylation of the guanine base.

We hypothesized that levels of 8-OHDG in patients with ROP will be higher than in patients without ROP. We collected urine samples from infants who were prematurely born and under 1500 gm weight using cotton balls with the parents' consent. The urine samples were stored in -20C till they were analyzed (n=32.We had collected 209 samples from the patients. The OHDG levels were determined by competitive ELISA technique with a Gelatin-BSA-OHG substrate and by Lateral Flow Immunoassay (LFIA) based paper assay technique with Gold nanoparticle and antibody conjugate and BSA-OHG as a substrate.

We observed that the levels of 8-OHdG can be correlated with onset of ROP. We did not notice a significant difference in the average values of 8-OHdG for patients with Stage 1 and Stage 3 ROP. However, there was a significant difference between the average 8-OHdG values of patients with Stage 0 (No) and Stage1 or Stage 3 ROP. This demonstrates that 8-OHdG is a marker of the onset of ROP and not necessary a tool to indicate the stage of ROP. The distribution values and average plots of the 8-OHdG values for both competitive assay and ELISA indicate that the patients with ROP have an average 8-OHdG value higher than 25-30ng/ml. Thus, for the purposes of a screening device, the cutoff value for the patients would be 25ng/ml 8-OHdG. The patients who are at risk of developing ROP, i.e. patients who were born before 32 weeks or who are born with a weight less than 1500gm would be screened for ROP. Urine samples from these patients will be collected at 24 hours after their birth and then at 72 hours and then once every week till they are discharged. If the 8-OHdG values of the patient remains under 30ng/ml, the patient is not at risk of developing ROP. However, if the value of 8-OHdG increases to 50ng/ml or above, the patient is at risk of developing ROP and the child would be checked for ROP by physical examination by an ophthalmologist.

Since 8-OHdG is an oxidative stress marker, the levels of 8-OHdG can be affected by some other exacerbations like Brochopulmonary dysplasia (BPD), Hyperglycemia, Number of days in incubator and number of days in the NICU. The status of BPD, Hyperglycemia and Number days in incubator and NICU data was collected from the clinics and the regression analysis was conducted to determine the influence of each of these factors in the values of 8-OHdG. It was observed that the status of BPD, hyperglycemia and number of days in the incubator did not have any effect on the outcome of the values of 8-OHdG. However, it was noticed that there was a correlation between 8-OHdG values and the number of weeks spent at the NICU. The 8-OHdG values were high for patients who had been in NICU for more than 8 weeks. Hence, for screening purposes, the screening should be limited to 8 weeks in the NICU. If the patient is in the NICU for a longer time, the 8-OHdG levels would be high and might not corroborate with ROP status of the patient. Future studies with a larger sample will be required to establish 8-OHdG as a robust biomarker for the screening of ROP.

CHAPTER 3

NANOPARTICLE SENSOR FOR NON-INVASIVE DETECTION OF 8-OHDG FOR SCREENING OF DIABETIC RETINOPATHY

Diabetic Retinopathy (DR) is a slow progressing microvascular complication of Diabetes Mellitus (DM) which affects the retina leading to impaired vision or complete vision loss. Advanced hyperglycemia causes substantial damage to the microvasculature of the retina which leads to the loss of its ability to convert light signals.

Diabetic Retinopathy can be classified as Non Proliferative and Proliferative DR. The first visible symptoms of DR are the appearance of micro and internal hemorrhages which can be detected by Fundus photography. These symptoms indicate that there has been persistent blood vessel damage accumulating over several years in response to the hyperglycemic environment. The ideal diagnostic technique would be the one which can allow the detection of DR at an earlier stage (when the retinal vessel damage starts to occur), using a suitable biomarker.

Several metabolic pathways have been implicated in the development of DR. The most common pathways studied for Diabetes are the Polyol Pathway, formation of advanced glycation end products, activation of protein kinase C (PKC) and the hexosamine pathway and oxidative stress. Out of all the mechanisms, the oxidative stress has been reported to be the most important reason for the damage of the retinal cells. 8-OHdG is the most prominent form of free radical induced DNA lesion. After the damage occurs, the cells have the capability to recognize and remove the oxidized lesion by base excision pair mechanism. The oxidized guanine is cleaved by enzymes such as endonuclease and glycosylase, removed from the cell into the blood which is then further excreted out through the urine

We hypothesized that the amount of urinary 8-OHdG and the 8-OHdG/Creatinine ratio will be higher in patients with Diabetic Retinopathy than in patients with no DR. To this effect, we conducted a study with a two-pronged approach. First, we developed different substrates for the accurate and fast detection of 8-OHdG. Secondly, we conducted an exploratory study to examine whether urinary 8-OHdG levels can be correlated with the status of Diabetic Retinopathy in a cohort of Caucasian population (n=80).

We developed a Gelatin-BSA-OHG substrate and gold nanoparticle-antibody against 8-OHdG substrate for Competitive ELISA and LFIA. We then tested the specificity and detection limit of the 8-OHdG for each of the sensors. Interference and proof of principle studies were conducted with artificial urine and varying concentrations of 8-OHdG. We collected urine samples from patients with or without DR from the Mason Eye Institute at University of Missouri (n=80). 8-OHdG levels were quantified with competitive ELISA and LFIA methods. The DR status of the patients was confirmed by Fundus photography. The 8-OHdG values correlated well with Fundus values. A few outliers were observed. Thus, we could conclude that 8-OHdG levels in the urinary 8-OHdG is higher in patients with DR than with no DR and 8-OHdG can be explored as screening biomarker for the onset of DR.