TIME DEPENDENT FETAL RAMAN SPECTROSCOPY FINGERPRINTS OF PLACENTAL HYPOXIA

TEXAS TECH UNIVERSITY HEALTH SCIENCES CENTER

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INTRODUCTION

The *ex-vivo* dual placental perfusion technique is currently used to study not only organ functions but also transfer profile and metabolic pathways of different compounds[3]. Placental hypoxia is associated with impaired uterine arteries invasion, poor villous development, loss of vascularity, reduced spiral artery remodeling as well as complications of pregnancy including hypertension, preeclampsia, oxidative stress, and intrauterine growth restriction, among others pathologies[1, 4] (Figure 1).

Raman spectroscopy (RS) is the methodology which allows for an investigation of physiology at cellular and tissue levels using photon scattering. It is a non-destructive and non-invasive method. Each peak in a Raman spectrum is associated with a unique part of the molecule and can be used for identification and confirmation. Raster Orbital Scanning (ROS) is a novel Raman spectroscopy sampling technique that helps to drive more accurate measurements and comprehensive analysis. ROS increases the sensitivity of Raman measurements up to 10 fold by rastering a tightly focused laser beam over a larger area. RS could be used as the specific metabolic fingerprint and therefore represents a unique diagnostic tool (Figure-2, Monograph, Introduction of Raman spectroscopy, Metrohm, USA).

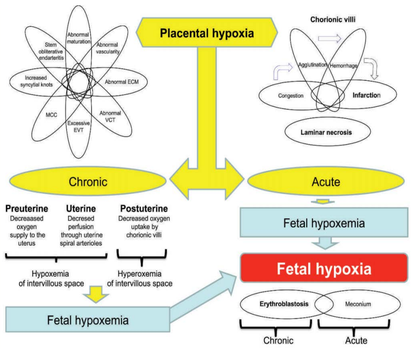

Figure 1: Diagram of acute and chronic placental lesions with relation to fetal hypoxia (ECM-extracellular matrix of chorionic villi; VCT-villous cytotrophoblasts; EVT-extravillous trophoblasts; MCC-microscopic chorionic (pseudo)cysts[6].

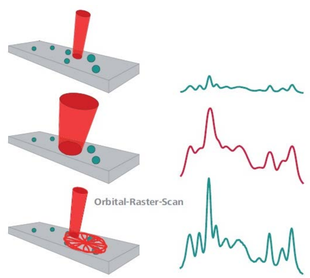

Figure 2: Dispersive spectrometers use tightly-focused beam (top), resulting in a high spectral resolution, but components in heterogeneous samples can be missed completely. Simple broadening of the beam would result in a loss of special resolution (center). The ORS technique (bottom) scans a larger sample area and maintains high spectral resolution that is required for analyte identification. [Monograph, Introduction of Raman spectroscopy, Metrohm]

OBJECTIVES

1) To analyze fetal perfusates using Raman spectroscopy (Mira M-1) in the *in-vitro* model of maternoplacental hypoxia *ex-vivo* human dual placental perfusion. 2) To compare Raman spectra of hypoxic and normoxic perfusates at different time intervals.

MATERIALS AND METHODS

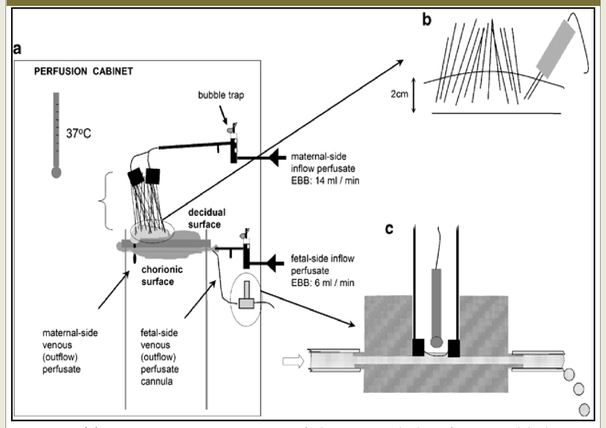

Figure 3: (a) Diagrammatic representation of the *in-vitro* dual perfusion model showing maternal and fetal-side perfusion, featuring delivery tubing for fetal and maternal-side perfusate and the collection of maternal and fetal-side venous perfusate. **(b)** A cross-sectional representation of 22 maternal cannulae inserted to alternate depths of approximately 1-2 cm below the decidua into the intervillous space. **(c)** A cross-sectional illustration of fetal venous perfusate oxygen electrode housed within a flow-chamber. Both oxygen electrodes were coupled to a two-channel oxygen monitor, from which soluble oxygen concentration were read[3].

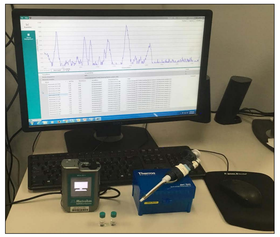

Figure 4: Experimental setup for Raman spectroscopy instrument (Mira M-1, Metrohm, USA).

- Earle's bicarbonate buffer containing 5.6mM glucose, 0.5mM dextran 70, 0.017mM bovine serum albumin and 5000 IU/l heparin was used as perfusate and equilibrated to the appropriate oxygen concentration[2, 5].
- Human placentas were obtained from normal term pregnancies within 30 min of vaginal delivery or cesarean section. *In-vitro* dual perfusion model was set up according to Figure 3. Perfusate was delivered to the cannulae from reservoirs via tubing sections composed of silicone and tygon materials[3].
- Perfusates were gassed at the appropriate oxygen concentrations in the maternal and fetal reservoirs. In normoxic condition, an average physiological oxygen tension was around 5-7% during perfusion, but in the hypoxic condition, the physiological oxygen tension was less than 3%[3].
- In this perfusion system, fetal perfusion was commenced at a rate of 6 ml/min followed by maternal-side perfusion at 14 ml/min. The perfusates from both conditions were collected at 30 min time interval for up to 360 min[3] (Figure 3).
- The collected hypoxic and normoxic perfusates at different time interval were scanned using Raman spectroscopy (Figure 4).

RESULTS

Placental Hypoxia

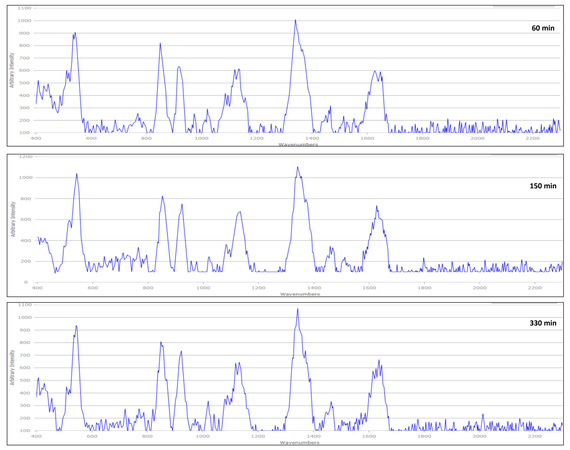

Figure 5: Representative Raman spectra of placental perfusate in hypoxic condition.

Placental Normoxia

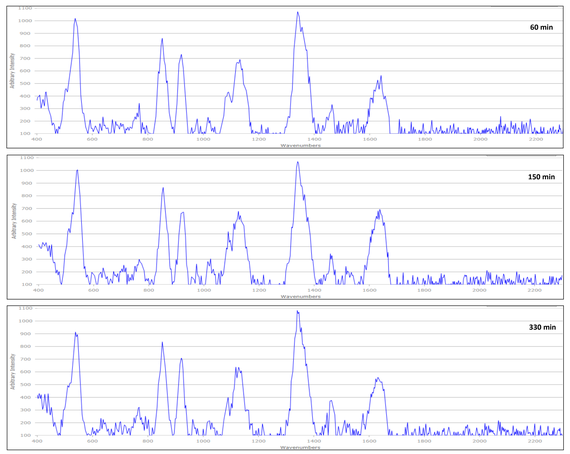

Figure 6: Representative Raman spectra of placental perfusate in normoxic condition.

Experimental Conditions / Intensity (Arbitrary unit)	Hypoxia (n=6)	Normoxia (n=5)
Mean	214.525	235.954
STDEV	158.7	195.6
SEM	3.64	4.486
P*	<0.0001	<0.0001

*Independent sample *t*-test, significance set at P<0.05

Table 1: Intensity of Raman spectra signal in fetal perfusates, obtained from *in-vitro* dual placental perfusion model of hypoxia[3] [STDEV-Standard Deviation, SEM-Standard Error of the Mean].

Fetal Raman spectra fingerprints of in vitro perfused human placenta (hypoxia (n=6) vs normoxia (n=5))

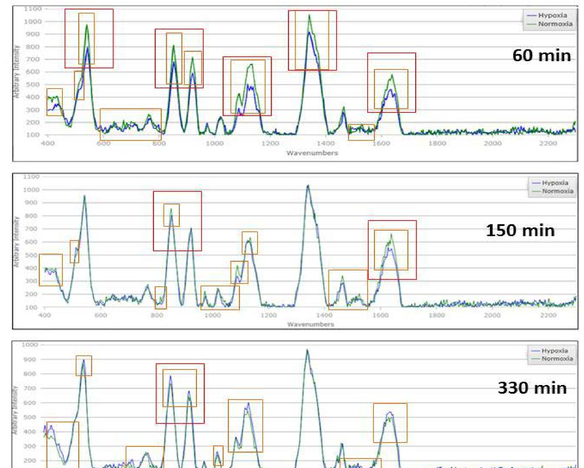

Figure 7: Raman spectra of placental hypoxia (n=6) and normoxia (n=5) perfusates.

When we compared Raman spectra of placental hypoxia (Figure 5) and normoxia (Figure 6) fetal fingerprints at different time intervals, we discovered two different patterns: i) Time independent (Figure 7, yellow boxes) and ii) Time dependent (Figure 7, red boxes). One pattern at the wave lengths 24390 nm, 19230 nm, 16666 nm, 11900 nm, 8700 nm, 7400 nm and 6050 nm showed the differences in the form and the other pattern at the wavelengths 18200 nm, 11400 nm, 10800 nm, 9090 nm and 6150 nm showed the differences in the amplitude.

CONCLUSIONS

The two RS fingerprint patterns represent unique preliminary data, utilizing a potentially new obstetric technology which could help diagnose the duration of placental hypoxia, ultimately providing novel targets for the treatment and prognosis of placental related disorders.

REFERENCES

1. Arjun J, Henning S, Eldar A, Fatimah S, *et al.* 2014
2. Brownbill P, Sibley CP, 2006
3. Soydemir F, Sitara K, Marie B, Warwick D, *et al.* 2011
4. Martha C Tissot van Patot, German E, *et al.* 2012
5. Schneider H, Panigel M, Dancis J, 1972
6. Stanek J, 2013

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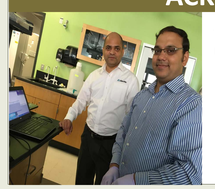

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