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Technical note

Imaging the human placental microcirculation with micro-focus computed tomography: Optimisation of tissue preparation and image acquisition

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ABSTRACT

Micro-CT provides 3D volume imaging with spatial resolution at the micrometre scale. We investigated the optimal human placenta tissue preparation (contrast agent, perfusion pressure, perfusion location and perfusion vessel) and imaging (energy, target material, exposure time and frames) parameters.

Microfil (Flow Tech, Carver, MA) produced better fill than Barium sulphate (84.1%(±11.5%) vs70.4%(±18.02%) $p = 0.01$). Perfusion via umbilical artery produced better fill than via chorionic vessels (83.8%(±17.7%)vs78.0%(±21.9%), $p < 0.05$), or via umbilical vein (83.8%(±16.4%)vs69.8%(±20.3%), $p < 0.01$). Imaging at 50 keV with a molybdenum target produced the best contrast to noise ratio. We propose this method to enable quantification and comparison of the human fetoplacental vascular tree.

1. Introduction

Fetal health and development is intricately bound with human placental circulation, yet there is no validated quantitative method with which to assess vascularisation of the human placenta. Developing a quantitative method may improve our ability to investigate, and therefore understand, normal placental function and pathologies such as fetal growth restriction, stillbirth and twin-to-twin transfusion syndrome.

Micro-focus Computed Tomography (micro-CT) provides three-dimensional volume imaging with spatial resolution at the micrometre scale. The technique has been used to investigate the branching structure and tortuosity of the fetoplacental circulation of mouse placentae [1] [2], and shown differences in vascular density of the human placenta between normally grown and growth restricted pregnancies [3], [4].

This study was designed to develop optimised tissue-specific preparation and micro-CT imaging parameters, in order to provide a validated approach to human placenta micro-CT.

2. Method

This series of experiments is divided into two sections; investigating tissue preparation techniques, and then micro-CT imaging parameters. The full experimental methodology is described in supplementary data.

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2.1. Tissue acquisition

Experimental procedures were approved by Bloomsbury National Research Ethics Service Committee and by University College London Hospital Research and Development (REC Reference number 133888). Placentas delivered by elective term caesarean section were taken directly to the laboratory, had the membranes trimmed, and the amnion removed.

occluded and the contrast agent was left to set. The placenta was dissected into 2 × 2cm full thickness blocks, which were fixed in 4% formalin for a minimum of 48 h. One full thickness section stained with hematoxylin and eosin (H&E) was cut for every block and 6 micrographs at x100 magnification taken (see supplementary material).

Histological analysis was done in FIJI (ImageJ Version 2.0.0-rc-54/1.51f) [8]. Vascular fill was calculated for each micrograph as shown in equation one.

$$\textit{Vascular Fill}\ (\%) = \left(\frac{\textit{Total Perfused Vessel Area}}{\textit{Total Perfused Vessel Area} + \textit{Total Unperfused Vessel Area}}\right) \times 100 \quad (1)$$

2.2. Tissue preparation comparators

We designed experiments to compare (Table 1):

- Contrast agent – comparing Barium sulphate with Microfil (Flow Tech, Carver, MA.).
- Perfusion pressure – comparing manual pressure with no quantification of perfusion pressure, with controlled pressure of 60 mmHg, physiologically relevant to fetal life [5–7].
- Cannulation location – comparing perfusion via the umbilical artery with perfusion via a chorionic artery.
- Arterial or Venous Cannulation – comparing perfusion via cannulation of the umbilical artery with perfusion via the umbilical vein.

The fetal vessel of interest was cannulated, and a cut made in the main draining vessel close to the point of cannulation, to create a fluid exit vent. 0.9% sodium chloride solution with 5IU heparin/ml was perfused until the outflow ran clear, then contrast agent was perfused until the chorionic vasculature was fully perfused and contrast agent was seen in the draining vessel. The vessel was

2.3. Micro-CT imaging comparators

We designed experiments to compare (Table 1).

- Energy level – from 30 to 100 keV in 10 keV increments.
- Target material – comparing Tungsten, Copper and Molybdenum.
- Exposure time–500 and 1000 ms
- Averaged frames per projection–1 and 2

A 2 × 2cm full thickness block of human placenta was repeatedly imaged (XT H 225 ST Micro-CT, Nikon Metrology, Tring, UK) adjacent to a 3 mm internal diameter tube filled with Microfil. Scans were reconstructed using a modified Feldkamp filtered back projection algorithm with proprietary software (CTPro3D; Nikon Meterology), and the average greyscale values of recorded areas of interest drawn over placenta, Microfil and air were calculated. The contrast to noise ratio was calculated as shown in equation two.

Table 1
Comparison of placental tissue preparation and micro-CT imaging parameters used in this study and in two previous studies, and optimised protocol as determined by the results of this study. SNR = signal to noise ratio.

	Langheinrich [4] (Human)	Rennie et al.[10] (Mouse)	Assessment Parameters	Optimised Protocol
Tissue Preparation				
Contrast Agent	Microfil and BaSO4 in gelatin	Microfil	Microfil and BaSO4 in gelatin	Microfil
Perfusion Pressure (mmHg)	74	Not reported	Manual pressure and 60	No difference Manual and 60 mmHg give equivalent results
Perfusion Location	Chorionic (peripheral) perfusion	Umbilical (central) Perfusion	Chorionic (peripheral) and umbilical (central) perfusion	Central vessel, ideally umbilical vessel
Perfusion Vessel	Chorionic plate artery	Umbilical Artery/ Umbilical Vein	Chorionic artery/Umbilical artery/Umbilical vein	Artery
Tissue sampling technique	8 × 2 mm full thickness blocks	Whole placenta	8 × 2 cm full thickness blocks	Dependent on magnification and field of view required
Micro CT Imaging				
Cone-beam energy (keV)	60	80	30-100 in 10 keV increments	50
Target material	Not reported	Not reported	Tungsten, Molybdenum, Copper	Molybdenum
Isotropic voxel size (μm)	13 and 4	13	13	Dependent on magnification and field of view required
Radiograph exposure time (ms)	2400	Not reported	500/1000	Balance with throughput 1000 gives good SNR
Number of projections	400	720	3141/6282/12564	Balance with throughput 3141 gives good SNR