A Comparison of 2D and 3D Imaging Tools to Quantify Structure of the Human Placenta

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Introduction

The vascular-rich villous trees of the human placenta form complex three dimensional (3D) structures critical for materno-fetal exchange (*Fig. 1*). 3D MicroCT imaging of the branching villous and vascular networks may deepen understanding of their 3D spatial relationships, integral for informing blood flow haemodynamics. However, how the metrics obtained from 3D imaging compare to those from traditional stereology has not yet been evaluated.

This research aimed to compare 2D and 3D imaging/analysis approaches of normal villous and vascular architecture to improve measures of placental structure.

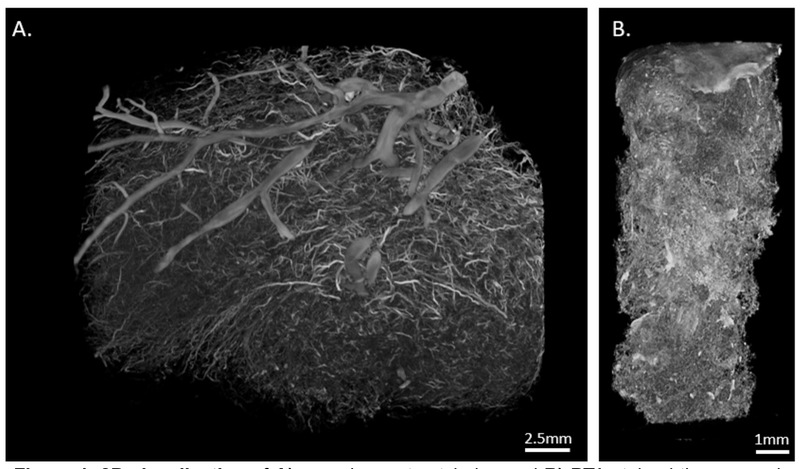

Figure 1. 3D visualisation of **A)** vascular cast cotyledon and **B)** PTA-stained tissue punch microCT datasets within CT-Vox software.

Methods

Tissue processing and image reconstruction: Normal term (n=4) placentae were collected, and the feto-placental vasculature was cast with BriteVu® (*Fig. 1A*). Tissue punches were taken from each placenta and PTA-stained (*Fig. 1B*). Samples were dehydrated in an ethanol series and imaged with a Skyscan 1272 microCT scanner (*Fig. 2*) . Following scanning, NRecon software was used to reconstruct 3D image stacks.

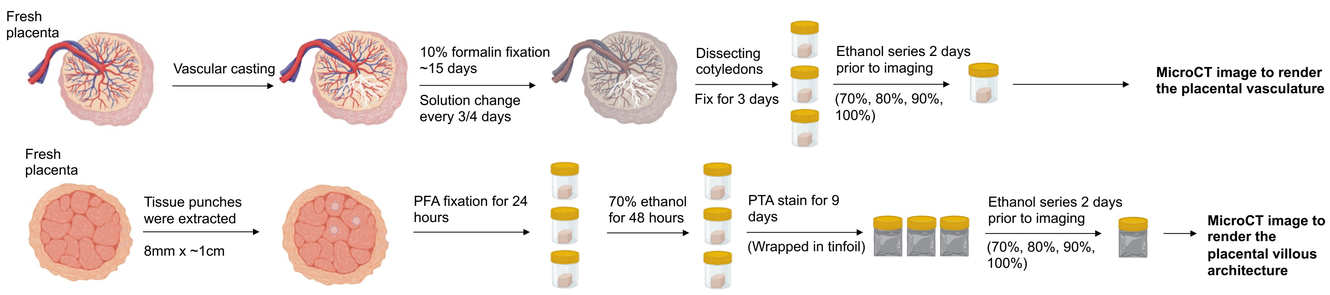

Figure 2. MicroCT tissue preparation workflow for vascular casting (top) and tissue punch PTA staining (bottom).

MicroCT image analysis: Vascular cast cotyledons: Sub-stacks of 20 images from three horizontal regions (near-chorionic, middle and near-maternal) and one vertical region (centre of the cotyledon) were identified using Fiji and automatically segmented using a custom algorithm in Python (*Fig. 4*). **PTA-stained tissue:** Three horizontal regions (near-chorionic, middle and near-maternal) were manually segmented using ITK-SNAP (*Fig. 3*). **Analysis:** The MorphoLibJ plugin within Fiji was used to calculate a) total vascular and villous volume and b) surface area from the regional segmented sub-stacks (*Fig. 5*), from which volume fraction and surface area:volume ratio was calculated.

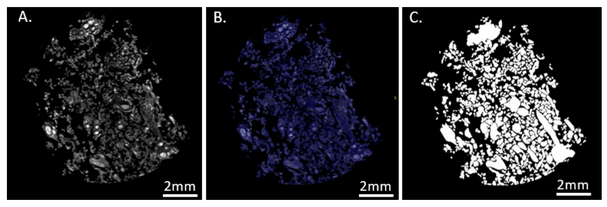

Figure 3. Tissue punch segmentation workflow. A) A 2D microCT image plane from a reconstructed, dataset of PTA-stained tissue. **B)** 'Ground-truth' manual segmentation (blue) of the villi. **C)** Segmentation converted to a binary mask for analysis of placental architecture.

Stereology: Samples were paraffin embedded and 5 µm sections were cut horizontally or vertically using a microtome following systematic random sampling (*Fig. 6*). The tissue was H&E stained and imaged using a MetaSystems Vslide slide scanner. Using Stereo Investigator® software, the 'Area Fraction Fractionator' probe was used to quantify the vascular and villous volume fraction, and the blood vessel to villous tissue volume ratio and the 'Cycloids for surface volume' probe were used to quantify vascular and villous surface density.

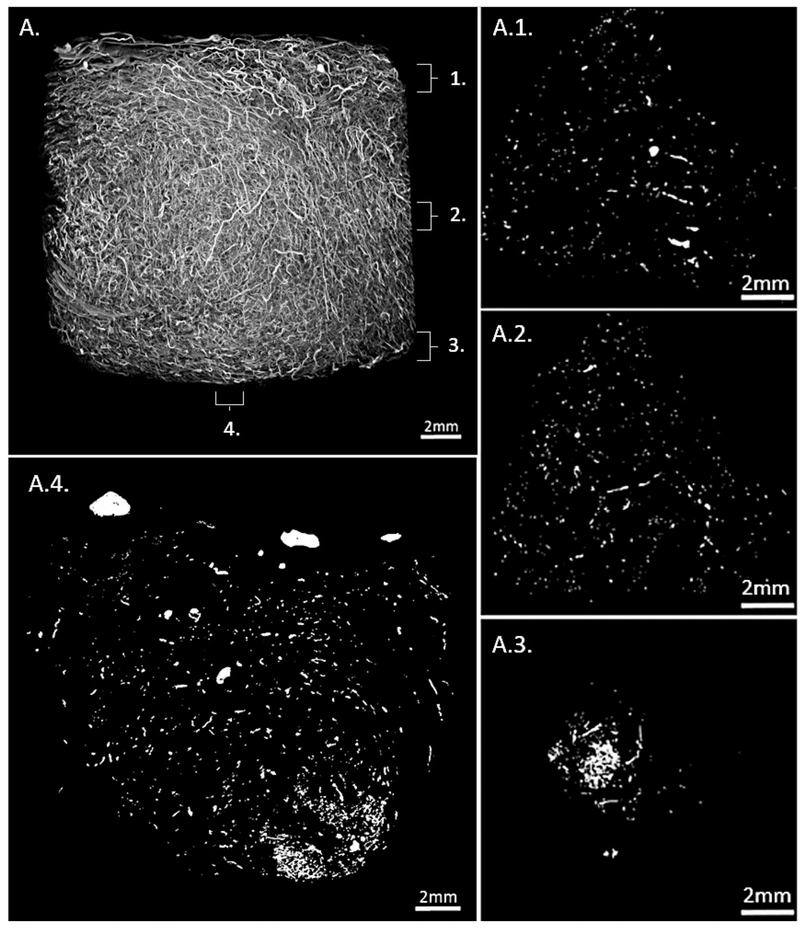

Figure 4. MicroCT image of the vascular cast cotyledon revealing the placental blood vessels. A) 3D rendering of a vascular cast cotyledon. **(1,2,3)** Horizontal sub-stack locations: **A.1)** near-chorionic, **A.2)** middle, and **A.3)** near-maternal regions. **A.4)** One vertical region.

Results

Vascular parameters: Metrics differed between analysis by microCT imaging and stereology, highlighting that microCT analysis under-quantified the microvasculature.

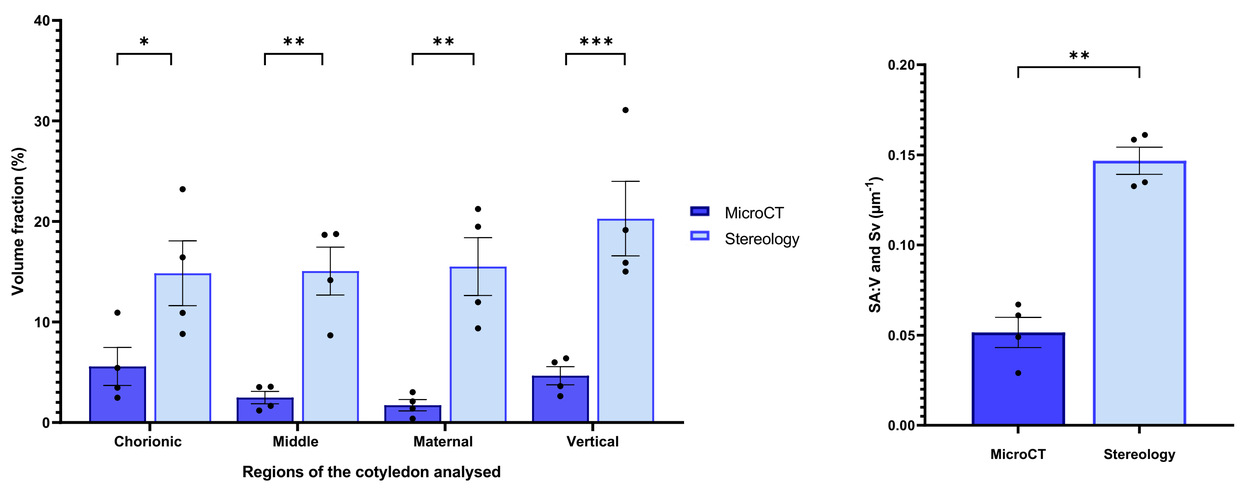

Figure 7. Bar graphs comparing the regional vascular parameters of normal term cast cotyledons quantified from both microCT and stereological analysis. **A)** Volume fraction and **B)** vascular surface area:volume ratio (SA:V).

Tissue surface analysis: Villous volume fraction is comparable between microCT imaging and stereology in near-chorionic and middle regions.

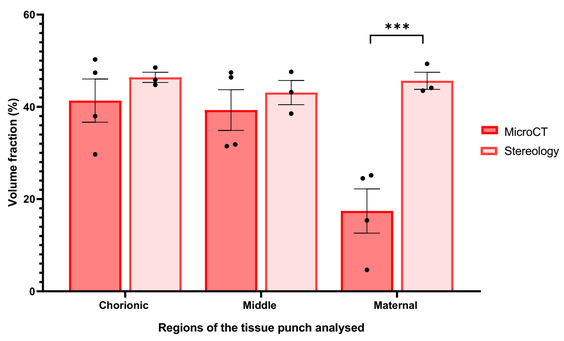

Figure 8. Bar graph comparing the regional villous volume fraction of normal term PTA-stained tissue punches quantified from both microCT and stereological analysis.

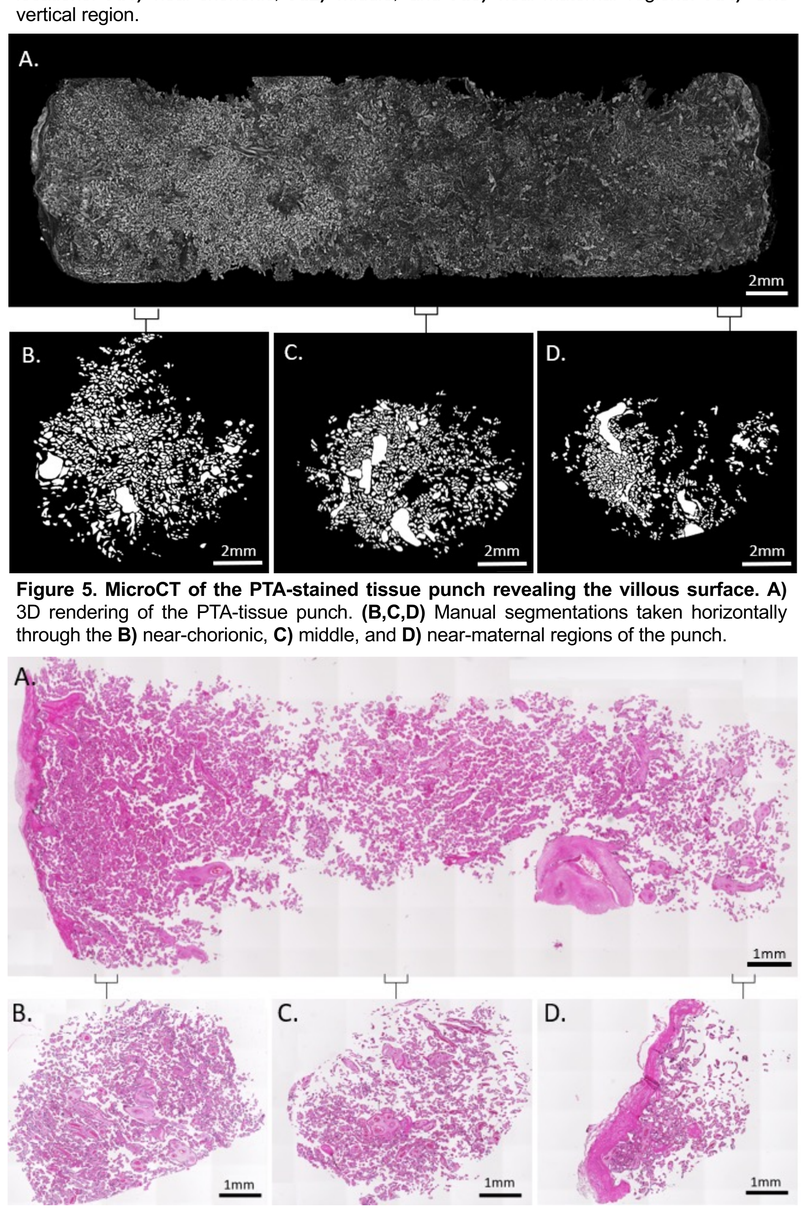

Figure 5. MicroCT of the PTA-stained tissue punch revealing the villous surface. A) 3D rendering of the PTA-tissue punch. **(B,C,D)** Manual segmentations taken horizontally through the **B)** near-chorionic, **C)** middle, and **D)** near-maternal regions of the punch.

Figure 6. Photomicrographs of H&E stained, placental tissue punch sections imaged by slide scanning for stereology. Sections cut in **A)** the vertical orientation and **(B,C,D)** the horizontal orientation from **B)** the near-chorionic **C)** middle and **D)** near-maternal regions.

Conclusions

The imaging and analysis approaches used displayed comparable ability to assess the villous volume fraction (tissue surface analysis). However, limitations in tissue preparation and MicroCT post-processing may have lead to under-prediction of the true morphology of vascular networks.

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