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Three-dimensional digital reconstruction of human placental villous architecture in normal and complicated pregnancies

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Condensation

Three-dimensional reconstruction of the human placenta offers a new strategy for investigating pathologic changes in pregnancy complications

Abstract

Objective

This study aimed to examine the use of digital technology in the three-dimensional reconstruction of human placentas.

Study Design

Placentas obtained at term elective caesarean section were sampled, formalin-fixed and embedded in paraffin. Two hundred 5 μm consecutive sections were cut from each specimen and the resultant slides stained with haematoxylin and eosin. Slides were then scanned and the digitised images reconstructed using customised software.

Results

Three-dimensional reconstructions were successfully achieved in placentas from normal pregnancies and those complicated by pre-eclampsia, growth restriction, and gestational diabetes. Marked morphological differences were readily identifiable, most clearly in the stem villus architecture.

Conclusion

This method is an emerging research tool for examining placental histoarchitecture at high resolution and gaining clinically relevant insight into the placental pathology allied to pregnancy complications such as PET, IUGR and GD.

Keywords: 3D reconstruction, placenta, stem villi, pre-eclampsia, intrauterine growth restriction.

Introduction

Complications such as pre-eclampsia (PET), gestational diabetes (GD) and intrauterine growth restriction (IUGR) affect up to 10% of pregnancies. Although their aetiology can be traced to acute and/or chronic placental dysfunction, assessment techniques have largely focused upon a two-dimensional description of the placental architecture to characterise its villous and vascular changes. More complex methods such as stereology have also been used to quantify these changes. In combination, these approaches have identified changes particular to each of these different pregnancy pathologies: Egbor and colleagues (1) noted aberrant vascular and villous morphology in both early onset PET and IUGR. Others reported narrower terminal villous diameters, reduced overall villous density and a greater prevalence of villous inflammation and infarction in IUGR pregnancies (2,3). Related histological changes including patchy necrosis, chorionic villous thinning, microvillous distortion, increased syncytial knotting and basement membrane thickening have been described in PET placentas (4). GD also impacts on placental structure, with stereological studies revealing increased terminal villous volumes as well as capillary length and volume (5). All of these changes are thought to adversely affect placental function.

There has also been interest in recreating the three-dimensional (3D) architecture of placental stem villi in an attempt to capture changes in villous and vascular morphology. Initial studies using electron microscopy (6) provided a detailed ultrastructural description of first trimester human placentas. This was subsequently repeated in normal, full-term placentas by Rhodin and Terzakis (7), while Leiser and co-workers (8) later carried out 3D reconstructions of the peripheral stem villous tree based on serial semi-thin sections which were photographed, magnified and converted to drawings. More recently, representation of the vascular architecture

has been achieved by corrosion casts (9), micro-computed tomography (10), 3D power Doppler ultrasound (11) and confocal microscopy (12).

A corresponding model of the 3D arrangement of the placental stem *villous* network remains wanting. Histological approaches aimed at characterising the villous architecture have largely relied upon comparatively less sophisticated 2D stereological techniques, such as the creation of simplified diagrams produced from confocal microscopy-based image processing (13). Analogous approaches were used with some success by Castelucci and co-workers who created 2D spatial reconstructions of stem villous histoarchitecture based on serial section photographs (14).

Recent developments in imaging software and computational power have proved amenable to being harnessed for the creation of high-resolution 3D tissue images (15). This method offers the advantage of combining whole slide image scanning, registration and visualisation in one package wherein serial sections are used to reconstruct 3D models based on a 'slice-to-slice' approach using automatic registration algorithms customised for virtual slides. The aim of this pilot study was therefore to examine the applicability of this technology to the reconstruction of human placental 3D placental villous microanatomy in healthy pregnancies, and those complicated by PET, IUGR and GD.

Materials and methods

Sample collection and tissue preparation

Term placentas were sampled at elective caesarean section for the purposes of this pilot study from a healthy uncomplicated pregnancy and others complicated by PET, GD and IUGR (*n*=1 for each). Ethical approval had previously been obtained from the Yorkshire & The Humber (Bradford/Leeds) Research Ethics Committee (13/YH/0344). Written informed consent was obtained from all participants prior to delivery. Each placenta was trimmed, weighed, assessed for the presence of gross abnormalities and full depth placental segments (circa $3cm^3$) were excised using a scalpel and immersed in PBS. These were transported to the laboratory where each segment was washed three times in PBS to remove maternal blood and placed in 10% formalin in PBS (containing 4% paraformaldehyde) for 48 h (<1 h of delivery) to ensure effective fixation. Thereafter, individual placental specimens were dissected into three to four smaller full depth segments and returned to formalin for a further 72 h. Tissue samples were placed in a Leica ASP 200 tissue processor (Leica Microsystems, Milton Keynes, UK) for 48 h and embedded in paraffin wax. Two hundred serial 5 μm sections from each specimen were cut using a Leica RM2235 microtome, placed on Superfrost Plus slides (Fisher Scientific, Loughborough, UK), stained using haematoxylin and eosin, mounted in DPX mounting medium (Sigma-Aldrich, Gillingham, UK), and scanned on Aperio high-throughput T2/T3 systems using a 20x objective (0.23 μm/pixel final resolution) (Aperio Technologies Inc., San Diego, USA).

Sequential image based registration and image transformation

Scanned slides were uploaded and registered on customised software (15), using a sequential slice-to-slice image-based registration method to create a virtual stack of slides. An image towards the centre of the virtual stack, containing the most tissue, defined the volume size and

was selected as a reference for automated sequential alignment of other slides within the stack. This alignment was carried out using rigid and non-rigid techniques, respectively. Additionally, relevant areas of interest were manually selected for the creation of sub-volumes from slide sets previously described (15). These subareas underwent further registration at higher resolution, to allow us to visualise the microarchitecture of such areas in more detail.

3D rendering, segmentation and visualisation

Upon completion of alignment, a volume was generated for each virtual slide stack at a 1/32 zoom resolution, whereby axial, coronal and sagittal views could be studied using *Volume Viewer*. The software was used to 3D volume render the dataset volume, which in turn, allowed interactive viewing and rotation in all axes. Clip planes controlled by sliders used with a zooming functionality enabled the visualisation of small structures internal to each 3D volume (e.g. following stem villus branching). *Volume Viewer* was subsequently used to perform manual segmentation which allowed bespoke colour-coding of specific structures (15). This software is based on ray tracing and code from the *Visualisation Toolkit,* which supports 3D volume colour rendering of raw data and segmentation iso-surfacing. The latter feature was applied to different structures within the dataset to create the final images, and allow the selective viewing of different parts of the reconstructed volumes independently of one another.

Results

Each dataset produced from scanning the histological slides successfully underwent both rigid and non-rigid registration. Qualitative performance was evaluated by reviewing coronal and sagittal views where inaccuracies occurring during registration would result in apparent discontinuity between constituent image rows within the dataset. Minimal misalignment errors were observed in all cases, indicating that the registration accuracy was within the typical 1.2% error quoted for this software (15). The successful registration (coronal view), volume render and segmentation (3D visualization) workflow for a normal placenta is given in Figure 1.

Stem villus segmentation indicated that larger calibre villi mostly concentrated in the fetal third of the placenta, running parallel and just inferior to the chorionic membrane, and decreased in both calibre and density in the middle and basal thirds where their distribution was more even. By contrast, the overall density of stem villi throughout the entire segment was greater in the placenta from a pregnancy complicated by PET (Figure 2). Moreover, the proximal stem villi in the fetal third were of a lower calibre compared to those in the normal placenta. Elsewhere in the middle and basal thirds, stem villi were densely concentrated, branching in a more extensive and spatially complex manner than their normal placenta counterparts. In the case of the IUGR placenta, the stem villi appeared to be concentrated within the fetal third, where proximal stem villi were wider than those observed in both normal and PET placentas. Interestingly, the thinning of the villous network was an abrupt phenomenon, with larger stem villi immediately giving rise to much thinner branches throughout the sample. Moreover, this specimen was characterised by a relative paucity of stem villi located in the middle third of the placenta. Finally, in the placenta from the pregnancy complicated by GD, the distribution and calibre of stem villi was uniform throughout the thickness of the placenta. The stem villi were

relatively elongated, of small calibre and tended to curve downwards. In addition, their branching network appeared simpler than that noted in the PET case.

In order to determine whether this approach was capable of producing accurate microanatomical reconstructions, a small area of the original registration devoid of stem villi and containing only lower order villi was selected for further registration at higher resolution to generate a 3D model of mature intermediate and terminal villi (Figure 3). In the normal placenta, both were distributed densely and uniformly throughout the sub-volume. By contrast, in PET, villi were more diffusely distributed and the mature intermediate villi appeared to be of a higher calibre.

Discussion

The underlying pathogenesis and microanatomical basis of placental dysfunction remains ill-defined, principally due to the fact that a reliable high-resolution method to visualise the architecture of the placenta remains elusive. We have shown that the methodology described above offers a novel approach to studying 3D reconstructions of stem villi in healthy and diseased placentas which could shed more light on the abnormal placentation and developmental patterns associated with PET, IUGR and GD.

Following earlier validation in the reconstruction of representative 3D models of solid organs, such as human liver and colon, rat kidney and the murine fetus (15), this method was successfully applied to the creation of 3D iso-surfaced volumes of placental tissue enabling visualization of the stem villi and their successive branches in fine detail. The segmentation of stem villi revealed marked differences across normal and pathological pregnancies in terms of size, distribution and branching patterns, although we recognise that this study only considered a limited number of specimens and was intended to outline what the technique could achieve, rather than characterise what was typical within each disease state. Further iterations of the technology may allow for the qualitative and semi-quantitative assessment of the complex spatial arrangement of stem villi, lower order villi and fetal/maternal membranes.

The merits of the present approach are best appreciated in its historical context. Early attempts to reconstruct stem villi from histological sections were led by Leiser and co-workers (9) who produced simplified drawings of the peripheral portion of the stem villo-vascular tree based on stem villi with diameters of 80-400 μm. Semi-thin sections were photographed and magnified to recreate 2D drawings of the branching pattern to the peripheral 3mm of one particular villous tree. By comparison, our method enabled the reconstruction of all the branching stem villi from

the truncus chorii down to the lowest order ramuli chorii in full depth, placental sections. Furthermore, our computer-based approach could now use algorithms to generate volumes directly from the placental sections, obviating the possibility of human error associated with a 2D depiction of a 3D specimen which has not been drawn to scale. Using voxel analysis in this way could enable a more accurate estimation of volume in both vascular and stromal compartments. 3D reconstructions may also enable computerised branching analysis to quantify vascular and villous networks in the normal and diseased state.

Subsequent groups have used a combination of confocal microscopy and image processing to create 3D reconstructions of peripheral villous capillaries (12). Confocal microscopy offers the advantage that the placenta can be sectioned with minimal cutting-associated distortions. However, the drawback of this method lies with the fact that automated segmentation could not be performed due to the low contrast of the images. In turn, this meant that chorionic villous volumes could not be directly rendered from 2D optical sections such that they had to be hand-drawn and filled in using computer software.

Langheinrich and co-workers (10) tackled the issue of trying to reconstruct more representative 3D models of placental villous vascular trees by using X-ray micro-computed tomography (micro-CT). Micro-CT has the great advantage of being non-destructive, although it can produce imaging artefacts including shading, ring or cone beam and presents a vascular bias during reconstructions. A more common approach used vascular network corrosion casts, an approach based on the infusion of a casting compound, which solidifies prior to the removal of surrounding tissue (8,9). However, infusing a contrast agent or casting compound has its limitations insofar as complete and uniform filling of the villous vasculature post partum is difficult to ensure, particularly given that injection of these agents may cause reactive

constriction of vascular walls which could potentially compromise the resulting reconstructions. Moreover, structural details of the perivascular tissue may be lost such that areas affected by thrombus or necrosis will not be apparent in the final reconstruction. The technique described in this paper offers the opportunity to characterise both the vascular *and* villous network whilst preserving the tissue for further analysis.

As such, existing methodologies have their limitations and, notably, lack convenient software capable of performing multilevel registration to produce high-resolution reconstructions. The novel approach presented herein is advantageous in this regard as it combines whole slide imaging/scanning, image serving, registration and visualization into one user-friendly package. Minimal user interaction is required downstream of basic, high-throughput histological techniques, and input from computational scientists is not required. The time commitment for each 3D reconstruction case can be further reduced by using automated sectioning, staining and mounting facilities. Moreover, the slide arrangements chosen allow reconstructions to be made with the inclusion of one or more serial immunohistochemical stains which allows a functional dimension to be added to morphostructural investigations (e.g. profiling local angiogenic growth factor networks relative to neoangiogenesis).

This pilot study has demonstrated that software-based reconstruction can be successfully used to create accurate 3D models of placentas from an array of gestational disorders. Further work needs to focus on a more thorough characterisation of the histoarchitecture of healthy and pathological placentas in a much larger number of specimens to determine whether the observations reported herein are characteristic of the different pathologies investigated.

Preliminary work has shown that our software can visualise the placental circulatory system. Future work could focus upon reconstructing the vasculature in both stem, intermediate and terminal villi. Furthermore, future work should incorporate immunohistochemical stains of 2D specimens. This antibody-based adjunct would provide greater functional insights ranging from the localisation of nuclear transporters and areas of apoptosis/necrosis through to a vascular endothelium stain-based topographical reconstruction of vascular beds. This study highlights our method as an emerging research tool for examining the placental histoarchitecture at high resolution, thereby gaining clinically relevant insight into the placental pathology allied to pregnancy complications such as PET, IUGR and GD. We believe that it has the potential to supersede conventional microscopy and allow investigators to characterise the placental microanatomical structural and functional anomalies allied to obstetric disorders more fully, contribute to understanding their pathogenesis and relate them to gestational outcome.

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Figure legends

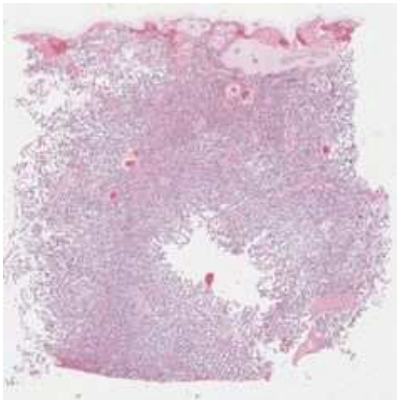
Figure 1: Image reconstruction workflow displaying axial and coronal views with 3D volume rendition and 3D visualisation of placental normal anatomy histoarchitecture. Interactive segmentation has been used to highlight the chorionic plate (red), basal plate (or decidua basalis - orange), stem villi (white) and lower order villi (yellow).

Figure 2: 3D reconstructions of (A) normal, (B) PET, (C) IUGR and (D) GD pregnancies.

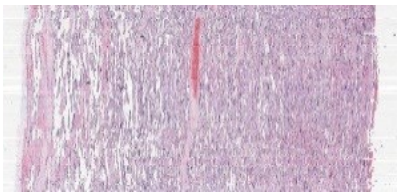
Figure 3: Workflow and generation of sub-volumes from the original rendered volumes using normal (A) and PET (B) placentas as examples. Subvolumes enabled clear visualisation of mature intermediate and terminal villi.

Figure 1

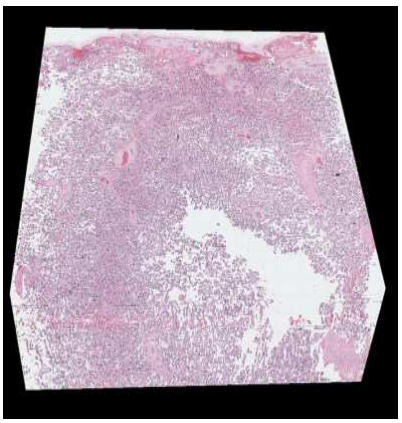
Axial view



Coronal view



Volume render



3D visualisation

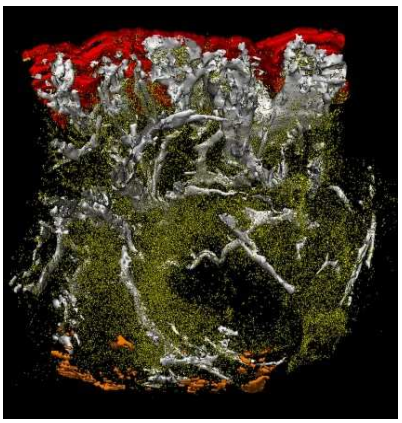
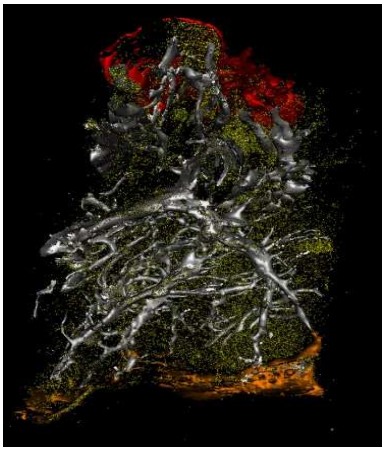


Figure 2

A

B



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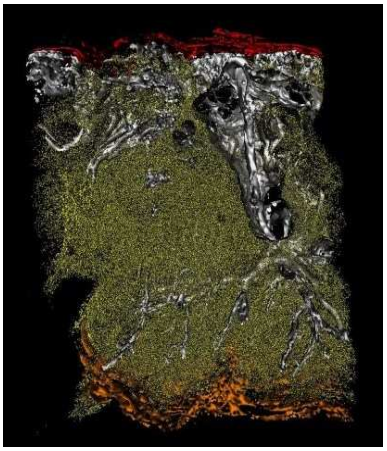
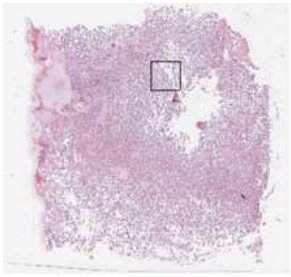
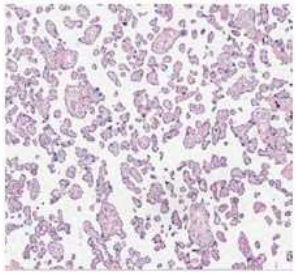


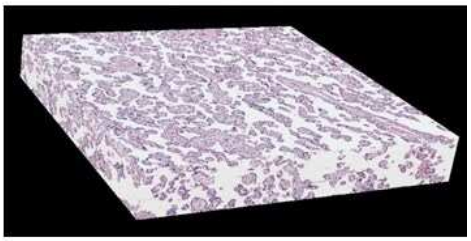
D

Figure 3

A







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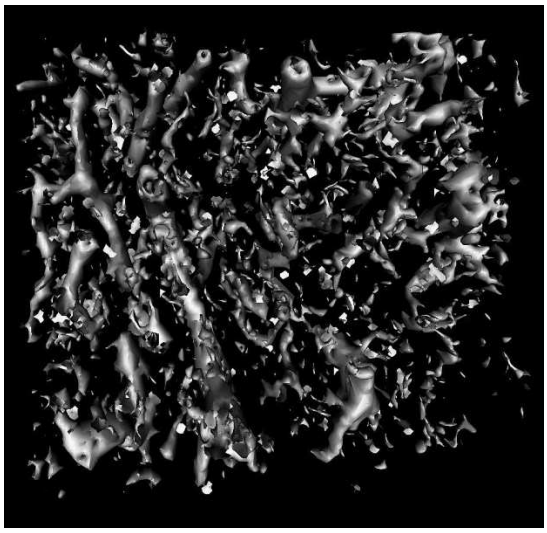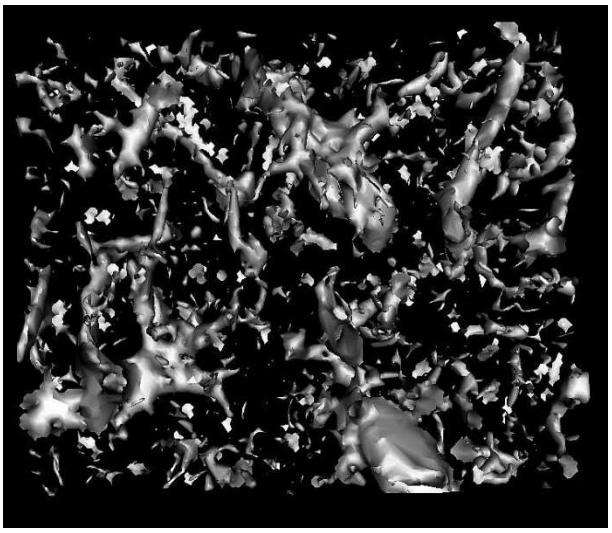
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