CEREBRA
Working wonders for children with brain conditions

Cerebra in Leeds

Preventing neonatal brain injury and childhood disability

Programme Grant Final Report, 2024

Cerebra in Leeds 2020-2024: The placental origins of brain injury

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Introduction

Brain injury occurring around the time of birth can have lifelong, irreversible, adverse impacts and commonly results from inadequate fetal development *in utero* or premature birth. Unfortunately, up to a fifth of pregnancies are complicated by fetal growth restriction (FGR), pre-eclampsia (PET) and/or preterm birth (PTB). Whilst the pathophysiology of these conditions is multifactorial, they have one common element: placental dysfunction. As the key transient organ which supports fetal growth and development during pregnancy, any degree of placental failure (attributable to architectural and/or functional issues) has the potential to have protracted adverse *sequelae* both during pregnancy and in the postpartum period. As such, the Leeds programme of research has focused on tracing pregnancy complications to one or more root causes at a placental level along four, interrelated workstreams.

(i) Creation of the Cerebra Placenta Biobank

A lynchpin of the current programme has been the creation of an extensive Cerebra placental tissue bank given the relative dearth of tissue available for research, especially for rarer indications such as FGR, PET and PTB. Our original target of 500 biobank cases has now been exceeded and we have been able to extend this endeavour through an amendment to the Research Ethics Committee which approved the original study. To compensate for some of the problems posed by the Covid-19 pandemic at the beginning of the programme, we adopted a two-pronged approach to collecting tissue. We initially focused on collecting placenta specimens surplus to diagnostic requirements from the Leeds Teaching Hospitals histopathology archive, which increased our holdings for rare presentations and that we would otherwise have difficulty recruiting to the study. This material proved excellent for technique optimisation as well as for applications such as immunohistochemistry and for the work outlined in Sections (ii) and (iii). Upon the reopening of routine clinical services, these holdings were bolstered by a resumption in the collection of fresh placenta specimens from delivery suite and elective caesarean sections. These, by contrast, offer material particularly suitable for transcriptomic and, to a lesser degree, proteomic analyses. Given that Leeds Teaching Hospitals has two maternity units, we teamed up with colleagues at Leeds General Infirmary to double recruitment. The Cerebra placental tissue bank is now being made available for collaborative research programmes with other groups nationally.

(ii) Characterising placental morphometric aberrations

The current pathological evaluation of placental pathology centres on the assessment of haematoxylin and eosin-stained tissue sections of placenta and umbilical cord. Whilst this strategy enables the identification of patchy necrosis, chorionic villus thinning, microvillus distortion and syncytial knotting, it fails to account for interrelated three-dimensional (3D) features that would provide a better assessment of both structural and putative functional abnormalities. The creation of representative models has proved elusive over the years, with stereology, reconstructed serial sections, corrosion casts, micro-computed tomography, confocal microscopy and 3D power Doppler ultrasound affording only semi-quantitative glimpses into 3D placental pathology. We therefore decided to apply state-of-the-art technology to address this challenge: light sheet microscopy. The appeal of this fluorescence microscopy method combines the benefits of high optical resolution with fast, high contrast image acquisition (and, crucially, not field-limited) of clarified 3D tissue specimens. Moreover, this can be used in association with software which enables quantitative metrics to be extracted from the reconstructed structures – to this end, Arivis Vision4d, a modular software for multi-channel 2-4D images was used as a platform for the quantification of 3D image data.

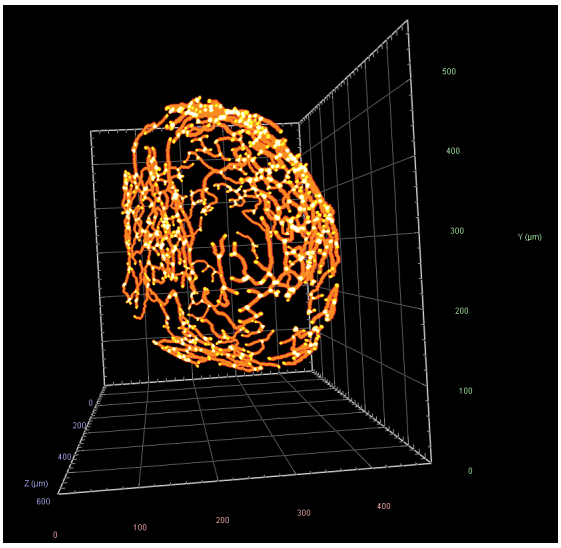

Figure 1: Analysed skeleton highlighting vascular networks branch points (in yellow).

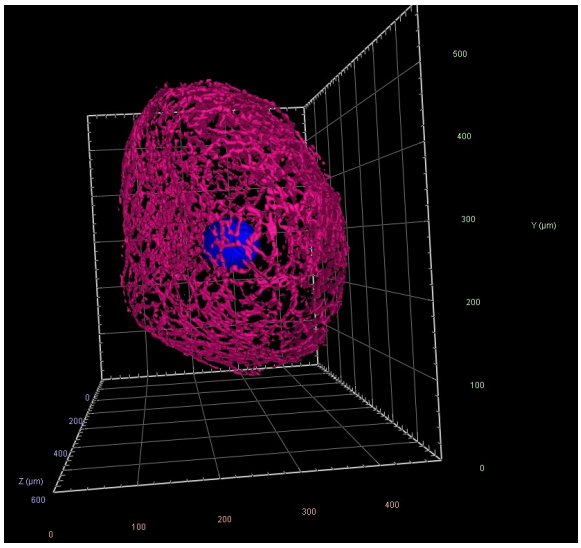

Figure 2: Segmentation objects (metrics collection). At this point, the perifollicular vascular networks are artificially reconstructed in pink and enable metrics collection (oocyte in blue).

Given the non-trivial challenges involved in applying this methodology to placentas, we decided to develop it first on small, discrete and self-contained vascular networks, in this instance mouse ovarian perifollicular vascular networks. This was achieved successfully using a combination of lectin (Wheat Germ Agglutinin (WGA) lectin) and antibody (CD34)-based staining approaches to visualise the oocyte zona pellucida and vasculature, respectively (Figures 1 and 2).

Following the successful development of this quantitative protocol which is more flexible and economical (now due for publication), we have progressed to deploying it for the characterisation of the placental bed across a range of normal and pathological placentas. These are more challenging given the greater complexity of villovascular branching and density (Figure 3). Notwithstanding, we are perfecting code in Arivis Vision4d to allow us to extract meaningful quantitative data (villovascular volumes, density and branching) from placental specimens.

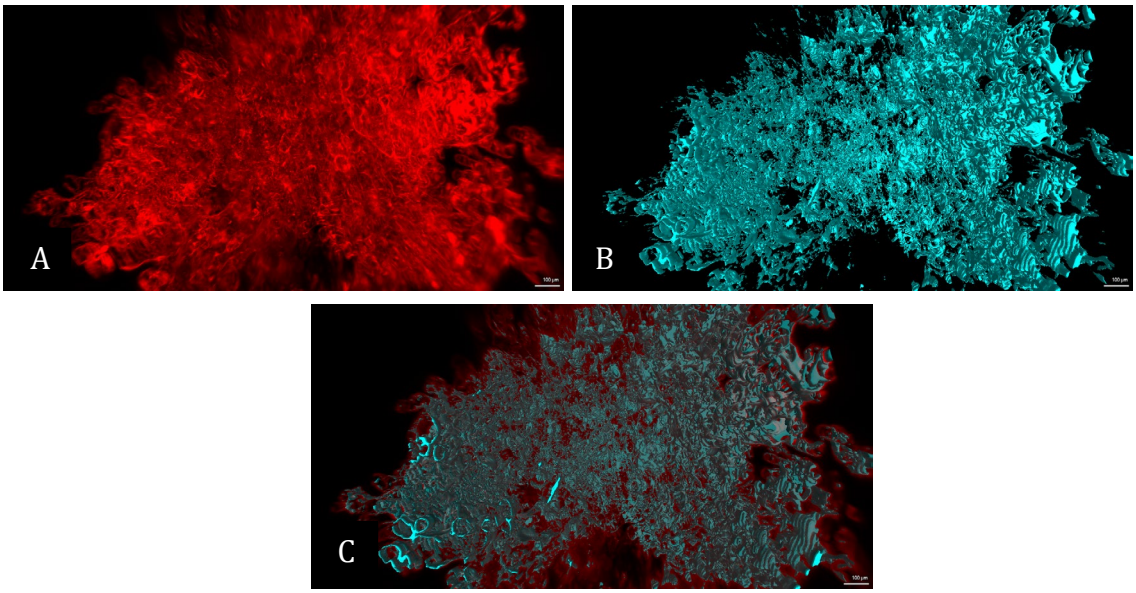

Figure 3: Placental reconstructions with fluorescent signal intensity (A) and binary mask (B) with the two overlaid (C).

(iii) Biomarker investigations in placental dysfunction in pregnancy complications

One area of investigation covered by our programme focused on identifying the mechanisms responsible for the development of placental pathology. Our initial work was based on the reported potential role for amyloid in this process i.e., the pathological buildup of toxic misfolded proteins. It is understood that normal pregnancy is associated with the upregulation of so-called *pregnancy zone protein* (PZP), whose role is proposed to be key to maintaining placental

extracellular proteostasis. PZP's activity is thus purported to prevent the pathological buildup of amyloid and its deleterious effects on placental function. In PET, for example, inadequate levels of PZP have been blamed for placental cytotoxicity, in turn leading to placental dysfunction. The challenge to date has been that evaluating – and more specifically, *quantifying* – these putative amyloid deposits has been hampered by a lack of sufficiently sensitive techniques and equipment. To tackle this problem, the team has spent considerable time and effort concentrating on developing a robust working protocol and in-house equipment for quantitative polarisation microscopy imaging of histopathology placenta specimens in partnership with collaborators at the University of Salford (Dr Tiehan Shen, Dr Pika Miklavc). This novel quantitative approach offers a unique opportunity to measure anisotropic molecule (i.e., those exhibiting properties with different values when measured in different directions such as amyloid) birefringency. This is achieved by measuring Stokes parameters, which vary according to changes in the phase of light as they pass through placentas and their constituent molecules. These advances have been detailed in previous reports but have largely focused on establishing whether the signals measured were due to the presence of amyloid or other proteins, such as collagen, by using orthogonal techniques including immunohistochemistry and special histological stains.

Our work to date has shown that whilst amyloid can be detected very focally and at very low levels in placenta, we believe that it is unlikely to be a very significant contributor to placental dysfunction. Instead, the strongest signals that we have identified could be traced to collagen, a natural constituent of the placenta whose role is to provide a structural scaffold for placental villi (Figure 4).

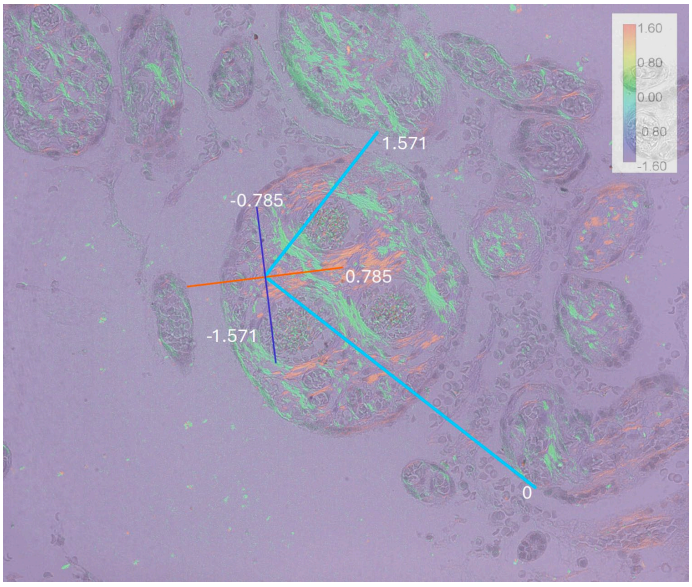

Figure 4: Polarisation microscopy image of a placental villus. Quantification of Stokes' parameters has enabled a more detailed evaluation of placental histological architecture in health and disease. The colour coding is in birefringent retardation (δ_s, in nm) and highlight collagen fibrils in green and red (the colours reflect the perpendicular arrangement relative to each other around the villous vessels).

Our ongoing work is now focusing on whether placental pathology associated with pregnancy complications leading to preterm birth are affected by collagen deposition which, if aberrant, could potentially affect the diffusion of gases, nutrients and waste products in the placenta, in turn affecting the developing fetus. This work is currently being completed and is being prepared for publication as a manuscript.

(iv) Spatial transcriptomics

The original, final element of our programme of work was to develop and strengthen collaborative research ties with other Cerebra-funded centres in Barcelona and Birmingham through a shared, interdisciplinary postgraduate student scheme. Unfortunately, in the wake of the Covid-19 pandemic, these original plans had to be revised given the difficulties in supporting a laboratory student exchange programme. We instead focused on working with a new technology: spatial transcriptomics. This technique is typically used to characterise the pattern (i.e., localisation) and regulation of gene transcriptional activity within tissues. Combined with large field-of-view imaging, this can reveal new structural features in tissues, with specific signatures being associated with a disease and/or its allied immunological correlates. The appeal of spatial transcriptomics is that it provides a high granularity evaluation of tissue-based functional changes which may or may not be independent from pathological architectural changes (Figure 5). Most importantly, this method preserves the spatial context of cells and can highlight, *inter alia*, cellular injury associated with pathological processes (e.g., amyloid deposition) or abnormalities in localised immunosuppression at the fetomaternal interface, which is essential to normal placental and tolerance of the fetal allograft. Other studies have shown that detailed expression profiles for thousands of genes align very well with standard pathologist annotations in different contexts (e.g., oncology) which provides reassurance of the technique's robustness and applicability.

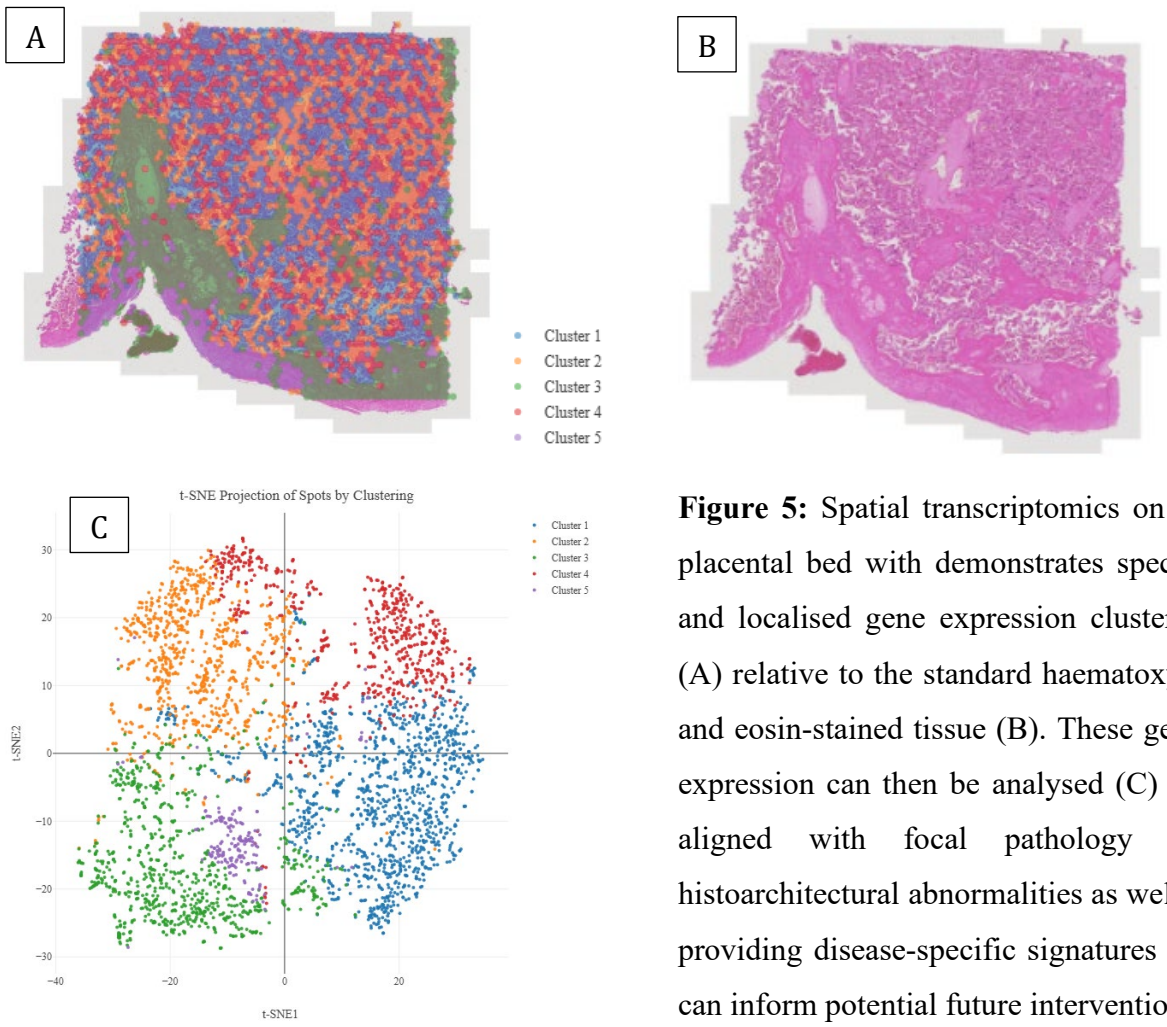

Figure 5: Spatial transcriptomics on the placental bed with demonstrates specific and localised gene expression clustering (A) relative to the standard haematoxylin and eosin-stained tissue (B). These genes expression can then be analysed (C) and aligned with focal pathology and histoarchitectural abnormalities as well as providing disease-specific signatures that can inform potential future interventions.

Summary

This has been a very successful programme for the team, despite some of the challenges posed by the COVID-19 pandemic. Firstly, our establishment of a Cerebra placenta tissue bank has guaranteed longevity to research in this area, with teams other than our own at Leeds now accessing material for their research focused on better understanding the mechanisms underlying the pregnancy complications leading to preterm birth and perinatal brain injury. Secondly, we have been able to develop high resolution in-house light sheet microscopy approaches to better understand placental histoarchitecture and how aberrations in vascular bed modelling can contribute to common obstetric disorders – a theme that we will continue to pursue across a wide range of indications. Thirdly, we have developed entirely novel techniques in quantitative polarisation microscopy which can be used to shed light on the mechanisms responsible for placental dysfunction and poor fetal development. Finally, we have been one of the first teams to exploit the availability of spatial transcriptomics services and to apply them to placental pathology

to clarify the nature of cellular and tissue injury leading to placental dysfunction.

As a team, we are firm believers that laboratory-based research should not occur *in silo*; rather we believe that it has a key role to play in informing clinical practice. Accordingly, the work described above was conducted hand in glove with the inception, adoption and expansion of national and international research and clinical endeavours through an array of translational initiatives, including the *Futures Institutes First 1,000 Days of Life,* the *PERIPrem Neonatal Optimisation Programme,* and the latest iteration of NHSE's *Saving Babies Lives*. The latter two in particular focus on the improved delivery of ten care interventions for mothers and preterm babies which have shown to have a significant impact on both brain injury and mortality rates. As ever, the Team is very grateful for Cerebra's funding which has enabled us to make significant advances into new areas of pregnancy complications as well placing Leeds centre stage in leading new approaches in interventional obstetric interventions for the benefit of both mothers and babies.