Placenta 60 (2017) 21–27

Contents lists available at ScienceDirect

Placenta

journal homepage: www.elsevier.com/locate/placenta

Modelling the effect of intervillous flow on solute transfer based on 3D imaging of the human placental microstructure

S. Perazzolo [a, b], R.M. Lewis [b, c], B.G. Sengers [a, b, *]

[a] Bioengineering Research Group, Faculty of Engineering and the Environment, University of Southampton, Southampton, SO17 1BJ, UK
[b] Institute for Life Science, University of Southampton, Southampton, SO17 1BJ, UK
[c] University of Southampton, Faculty of Medicine, Southampton, SO16 6YD, UK

ARTICLE INFO

Article history:
Received 4 August 2017
Received in revised form
29 September 2017
Accepted 10 October 2017

Keywords:
Computational modelling
3D reconstruction
Placenta

ABSTRACT

Introduction: A healthy pregnancy depends on placental transfer from mother to fetus. Placental transfer takes place at the micro scale across the placental villi. Solutes from the maternal blood are taken up by placental villi and enter the fetal capillaries. This study investigated the effect of maternal blood flow on solute uptake at the micro scale.

Methods: A 3D image based modelling approach of the placental microstructures was undertaken. Solute transport in the intervillous space was modelled explicitly and solute uptake with respect to different maternal blood flow rates was estimated. Fetal capillary flow was not modelled and treated as a perfect sink.

Results: For a freely diffusing small solute, the flow of maternal blood through the intervillous space was found to be limiting the transfer. Ignoring the effects of maternal flow resulted in a 2.4 ± 0.4 fold over-prediction of transfer by simple diffusion, in absence of binding. Villous morphology affected the efficiency of solute transfer due to concentration depleted zones. Interestingly, less dense microvilli had lower surface area available for uptake which was compensated by increased flow due to their higher permeability. At super-physiological pressures, maternal flow was not limiting, however the efficiency of uptake decreased.

Conclusions: This study suggests that the interplay between maternal flow and villous structure affects the efficiency of placental transfer but predicted that flow rate will be the major determinant of transfer.

1. Introduction

The placenta is the interface between the mother and the fetus and adequate placental function is necessary for fetal growth. Placental structure has been shown to be altered in babies who grow poorly in the womb and in response to maternal disease and lifestyle factors [1]. Understanding whether these changes in placental structure help or impair placental function, particularly in terms of gas and nutrient transfer, is an important question. Improved imaging techniques are allowing detailed 3D imaging of the placental villi over wider areas which now allow detailed mathematical modelling of these processes to predict how changes in placental structure may affect placental function.

The human placenta is heamomonochorial, meaning that maternal blood containing the solutes and gases required by the fetus are in direct contact with the fetal tissue [2]. Maternal blood enters the placenta through spiral arteries and percolates through the placental villi for solute exchange to occur. The placental villi form tree like structures, which contain fetal blood vessels surrounded by the placental syncytiotrophoblast, which forms the primary barrier and exchange surface in the placenta. The syncytiotrophoblast forms a selective barrier between the maternal and fetal circulations, transfer of gases occurs by flow limited diffusion, while nutrients and waste products must be transported across the placental barrier. Terminal villi, at the tips of the villous trees, are believed to be the primary site of solute exchange as the diffusion distance between the two circulations is least [3].

Mathematical modelling approaches allow testing of hypotheses in a way which compliments experimental findings, or where experimental approaches are challenging. Previous models of placental transfer have represented very useful 1D, 2D or

* Corresponding author. Bioengineering Research Group, Faculty of Engineering and the Environment, University of Southampton, Southampton, SO17 1BJ, UK.
E-mail address: b.g.sengers@soton.ac.uk (B.G. Sengers).

https://doi.org/10.1016/j.placenta.2017.10.003
0143-4004/

representative 3D approximations [4–11]. However, the specific 3D morphology of the placenta is likely to play a key role in the overall transfer, both locally at the microscale as well as at the macroscale. Recent advances in imaging technology have allowed 3D image based models to be developed. For example, maternal flow has been modelled to study the shear stress exerted by the maternal blood on the villous surface [12,13]. However, these studies did not include solute transport. On the other hand, the villous barrier and fetal flow were modelled to determine the relationship between fetal capillary structure and transfer in the terminal villi [14–16]. However, these studies did not incorporate the maternal flow and instead assumed a fixed solute concentration on the maternal facing surface of the villi, leaving a gap in our knowledge that needs to be investigated further.

The objective of this study is to evaluate the effects of maternal blood flow in the intervillous space on the placental transfer of solutes, modelling both maternal flow as well as solute transport, using 3D image based models of the placental microstructure. With the term solute, we will refer to any small molecular species whose transport mechanism can be described by simple diffusion, *e.g.* anaesthetics and other gases.

The hydraulic permeability of the maternal circulation associated with the placental microstructure was estimated and the placental uptake was calculated as a measure of transfer for a generic solute. In particular, the effect on uptake was assessed of different maternal blood flow rates below and above the normal physiological range.

2. Methods

2.1. 3D reconstruction of the placental microstructure

Tissue was collected from healthy term placentas after delivery with written informed consent and ethical approval from the Southampton and Southwest Hampshire Local Ethics Committee (11/SC/0529).

Samples were collected within 30 min of delivery and villous tissue was dissected and fixed in 4% paraformaldehyde in PBS at 4 °C overnight and then stored in PBS at 4 °C. Villous fragments were dissected form the fixed tissue and permeabilized in 1% triton X-100 for 2 h, washed in PBS, and incubated overnight with 10 μg/ml Biotin-Datura Starmonium Lectin (DSL, Vector laboratories) which binds specific carbohydrates on the syncytiotrophoblast; 10 μg/ml Rhodamin-Pisum Sativum Agglutinin (PSA, Vector laboratories) which binds specific carbohydrates in the stromal tissue; 10 μg/ml FITC-Aleuria Aurantia Lectinto (AAL, Vector laboratories) which binds specific carbohydrates on the fetal capillary endothelium. Samples were washed in PBS and incubated with Strptavidin-680 (Licor) and 11 nmol/l DAPI for 2 h then washed in PBS. Samples were cleared through a series of 10%, 25%, 50% and 3 × 97% 2, 2′-thiodiethanol (TDE, Fisher Scientific, UK) in PBS for at least 30 min per step. Samples were stored in 97% TDE at 4°C until imaging.

The placental villous fragments were imaged with a Leica TCS SP5 laser scanning confocal microscope. For each batch a control sample run without lectin or primary antibody (but including DAPI and any secondary antibodies) was imaged and this sample was used to determine background fluorescence levels for each channel. A series of confocal fluorescence microscopy images were collected from each of six tissue samples with overall dimensions of 0.78 × 0.78 × 0.25 mm (height x width x depth) and a voxel resolution of 0.76 × 0.76 × 1.99 μm (height x width x depth) (Fig. 1A). One additional image sample was collected with dimensions of 1.55 × 1.55 × 0.41 mm (height x width x depth) and a resolution of 1.5 × 1.5 × 4.2 μm (height x width x depth) to test the simulation of even larger sample volumes.

The six 3D image samples were used to evaluate the effects of structural variation. Voxel resolution was reduced to 5 μm in all directions to reduce computational costs in order to enable simulation of the whole image sample volume. To assess the impact of the reduced imaging resolution a sensitivity analysis was carried out in comparison with the original full resolution (Supplementary data). Images were filtered to reduce the background noise (normal Gaussian filter). From the image stack a 3D structure was reconstructed using ScanIP 2016.09-SP1 (Simpleware Ltd, UK). The 3 colour channels in Fig. 1A represent the syncytiotrophoblast (blue), stroma (red) and fetal vessel endothelium (green). Each channel was segmented separately using manual thresholding. Because the syncytiotrophoblast is only a thin layer and due to variations in staining intensity, segmentation did not produce a fully continuous layer. Therefore the syncytiotrophoblast volume was merged with the stroma, representing the overall effective barrier for a small diffusive solute. Similarly, depending on resolution and staining intensity segmentation of the capillary structure displayed local discontinuities and the lumen could not always be distinguished. Therefore, it was assumed that the endothelium was part of the volume of the placental capillaries.

2.2. Computational model

The reconstructed 3D solid consisted of three main domains: 1) The intervillous space, i.e., the volume around the villi; 2) The villous barrier, as composed of the syncytiotrophoblast and the stromal layer (DSL + PSA staining); 3) The fetal vessels, represented by the endothelium wrapping the fetal capillaries (AAL staining). Volumetric meshing was carried out by ScanIP 2016.09-SP1 (Simpleware Ltd, UK) after which the model was exported to COMSOL Multiphysics 5.2 (COMSOL Inc., USA). More detail on the model implementation, including a mesh resolution study is provided in the Supplementary data.

2.3. Intervillous space blood flow modelling

Fluid flow in the intervillous space was modelled to represent the maternal blood flow percolating around the villi. The fluid was modelled as Newtonian and incompressible. The fluid flow regime was modelled as Stokes flow (creeping flow) in first instance, described by:

$$0 = \nabla\left[-p\boldsymbol{I} + \mu\left(\nabla\boldsymbol{u} + (\nabla\boldsymbol{u})^T\right)\right] \quad (1)$$

$$\nabla\cdot\mathbf{u} = 0. \quad (2)$$

$\boldsymbol{u}$ and p were respectively the velocity (m/s) and pressure (Pa). $\mu = 3.2$ mPa s was the dynamic viscosity representative for blood [17]. A pressure difference ($P_{in} - P_{out}$) of 2.9 Pa was applied along one of the longest dimensions of the sample ($L = 0.78$ mm) (Fig. 1B), based on an estimate of the pressure gradient in the placental cotyledon of 3.7 kPa/m midway along a typical flow path [4,18]. On the remaining outer surfaces of the sample a no-flux boundary condition was imposed. No-slip boundary conditions were applied for the fluid at the villous boundary walls.

2.4. Permeability of the placental structure

The average hydraulic permeability of the placental structures κ (m^2) was estimated according to Darcy's law for porous media: