Placenta 43 (2016) 54–60

Contents lists available at ScienceDirect

Placenta

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

Three-dimensional modeling of human placental terminal villi

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ARTICLE INFO

Article history:
Received 29 January 2016
Received in revised form
28 April 2016
Accepted 4 May 2016

Keywords:
Placenta
Terminal villi
CLSM
Modeling
FEA

ABSTRACT

Introduction: Placental transport is the main factor affecting the health and development of the fetus. Due to the placenta's geometrical and mathematical complexity, the structure-function relations of placental terminal villi have not been successfully modeled. Hence, a novel modeling approach is proposed.

Methods: Computational models of four different specimens were generated from the three-dimensional reconstruction of confocal laser scanning microscopic image stacks. To evaluate the capabilities of the proposed methodology, stationary oxygen diffusion transport was calculated in the terminal villus volumes.

Results: The reconstructions automatically provided the spatial arrangement of the fetal capillaries inside the terminal villi. The surface and volume ratios between the fetal capillaries and the villus were also calculated, and the effects of model parameters on the placental diffusive capacity were assessed by parametric analysis.

Discussion: The potential of three-dimensional reconstructions combined with finite element analysis as a research tool for the human placenta was tested. The methodology herein could serve in the future as a simulation platform for complicated *in vivo* and *in vitro* scenarios.

1. Introduction

Placental oxygen transport is believed to be the most important function of the human placenta. At the smallest branches of placental villous trees, diffusive transport takes place due to concentration gradients that arise between the outer surface of the trophoblast layer and the inner surface of the fetal capillary endothelium [1]. These terminal villi are believed to be the most important sites for gas exchange due to their thin barriers between the maternal and fetal bloodstreams [2]. Therefore, their structure-function relations are of great interest to placental physiologists.

Histological methods have been very popular for the assessment of terminal villous geometry since they provide an approximation of the complex three-dimensional (3D) structure [3–5]. However, the published data are sparse and inconsistent, mainly due to the different investigation and fixation procedures employed. The 3D architecture of the villus was first visualized and quantified using scanning electron microscopy and vascular corrosion casts [6,7], which allowed for easier and more exact approaches than histology. Currently, 3D imaging techniques such as X-ray micro-computed tomography (microCT) and confocal laser scanning microscopy (CLSM) are providing new methods for studying vessel alterations in humans and animals. The 3D fetoplacental vascular bed of normal and pathological human placentae has been investigated using microCT [8,9]; however, its resolution is not enough for the acquisition of a detailed structure of the terminal villi. CLSM has provided unique information regarding the 3D spatial arrangement of microvascular beds in normal and pathological placentae, during the first [10] and third [11,12] trimesters. Volumetric reconstructions of CLSM images could provide useful insights if combined with mathematical modeling.

Mathematical models of placental exchange function were initially described more than 40 years ago [2,13]. The different modeling approaches can be classified into two broad groups: based on experimentally obtained geometry and on parametrized geometry. The first group is mostly composed by studies that

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http://dx.doi.org/10.1016/j.placenta.2016.05.001
0143-4004/

adapted the two-dimensional (2D) morphometric model developed for the gas exchange in the lungs [14] to the placenta [3,5,15–18]. A different methodology was employed by Gill et al. [2], who used digital photomicrographs to extract the 2D geometry of the villi and their respective capillaries. The extent of diffusional screening in placental villi was then numerically calculated. Their work is pioneering and has the advantage that it can be performed in multiple villi simultaneously. Similarly, Chernyavsky et al. [19] estimated the distribution of villous branches in the intervillous space, and solute transport was modeled. However, when experimental data are not available, such as for the branching villus trees, the only way of solving a more comprehensive mathematical model is by simplifying the geometry to known geometrical shapes - *e. g.* tubes and ovals. These type of studies form the second and smallest group of placental modeling [20–22]. Thus, most of the studies performed on the oxygen transport in the human placenta have successfully modeled it in 1D and 2D, but those who attempted to do so in 3D were required to simplify the geometrical structure. For a detailed review on models of oxygen transport in the placenta the reader is referred to Serov et al. [23].

In the current study, three-dimensional reconstructions of terminal villi and their capillary beds are combined with finite element (FE) analysis to test their potential as an investigative tool. The technique is evaluated by solving stationary oxygen diffusive transport. This approach accounts for specimen-specific geometry where the contribution of the villous membrane and fetal capillary variability is directly incorporated, while their effect in 3D is quantified and visualized. It also provides the opportunity to investigate in detail the structure-function relation in the terminal villi.

2. Material and methods

2.1. Specimen preparation

A fresh healthy placenta delivered by Cesarean section at term was obtained at the Department of Obstetrics & Gynaecology in Addenbrooke's Hospital, Cambridge (UK) for perfusion fixation [24], with ethical permission and informed written consent. Two undamaged and clot-free lobules suitable for perfusion were identified and the amnion was removed from the chorionic plate above these. The chorionic artery supplying each lobule was cannulated and the draining vein was cut to allow perfusate flow. Fetal blood was cleared from the lobules with phosphate buffered saline (PBS). The lobules were then fixed by perfusion with 10% formalin (20 min) followed by removal with PBS (an additional 20 min). The vessels were perfused with PBS containing green fluorescein isothiocyanate (FITC) conjugated Ulex lectin (FL-1061, Vector Laboratories Peterborough, Cambridgeshire, UK) that has an excitation and emission maximum wavelength of 495 nm and 515 nm respectively. Once the vessels were filled, the solution was left in place for 30 min and then removed by flushing with PBS. The two lobules were processed at 2 different perfusion pressures: 100 mmHg (specimen 1 and 2) and 30 mmHg (specimen 3 and 4) representing the extremes of physiological pressures [25]. Intermediate villi with small clumps of terminal villi attached were dissected with needles and incubated for 10 min in DiI (D-282, Life technologies, CA, USA) to stain the villous membranes (excitation maxima wavelength of 549 nm and emission maxima wavelength of 565 nm).

The specimens were scanned using a Leica SP2 CLSM (Leica Microsystems, Wetzlar, Germany) with an x25, 0.95NA objective lens. Based on the quality of the images (which depends on the quality of the perfusion) four terminal villi were selected for quantitative analysis. For each sample, image stacks with a resolution of 0.277 $\mu m^2 \times 2$ μm were automatically generated.

2.2. Geometry reconstruction

The CLSM data were imported into 3DSlicer [26], an open-source software for image analysis. The stacks were converted to grayscale images and the geometries of interest were automatically detected by the thresholding method, where each grayscale image was transformed into a binary one [27]. Thereafter, manual cleaning based on human interpretation was needed to remove image artifacts and define unclear boundaries; for example, when two close but obviously different capillary segments appear merged within the same boundary they were manually disconnected and the resulting gap equaled the pixel size (0.277 μm). For better processing, a Gaussian blur smoothing algorithm [27] was applied to further clean and reduce noise. Due to the lack of information regarding the material properties (diffusion coefficients) of the trophoblast epithelium, basement membrane, connective tissues and capillary endothelium, they were modeled together as a single entity called the villous membrane. Three-dimensional triangulated meshes were generated from the segmented stacks with the help of an interpolation algorithm [28] and converted into point clouds (a collection of points that describes the surface of a given shape) by employing an in-house code that extracts the triangle vertices. The solid bodies (volumetric geometry) which mimic the specimen specific terminal villi and fetal capillaries were automatically generated in SolidWorks-2013 software (SolidWorks Corporation, MA, USA) by employing the PointCloud function. The overall 3D reconstruction process is schematically shown in Fig. 1.

The microscopic size of the samples challenges the verification of the geometries. Therefore, a methodology that enables the estimation of the reconstruction accuracy was developed. Four $r = 90$ μm and three $r = 4$ μm spheres were scanned with equal parameters as the villous specimens, but with 3 different slice thicknesses. The spheres were reconstructed and analyzed. The data indicated that the precision of the geometrical reconstruction mostly depends on the slice thickness. For the given geometries, a 1% error is expected in each direction (x,y,z).

2.3. Computational model

The 3D solid bodies were imported and implemented using the commercial FE solver COMSOL Multiphysics 5.2 (COMSOL, Inc., Burlington, MA, USA). The villous membrane and fetal capillaries were treated as different domains to allow for different boundary conditions. These two domains were meshed separately with continuity across the boundary, by the COMSOL automesher. Due to the volume and complexity of each specimen, the size of the meshes differed greatly ranging from 430,000 to 825,000 tetrahedral elements. A typical mesh sensitivity procedure [29] in which the meshes are refined and the convergence of the results is checked was performed. Minimum element size was less than minimum thickness to reduce numerical errors.

A passive oxygen transport problem was considered to evaluate the capabilities of the presented method. The oxygen is assumed to diffuse through the villous membrane following Fick's second law of diffusion, Eq. (1), which states that the oxygen volume flow (with time t) across a membrane is related to its physical dimensions (x, y, z), the driving concentration gradient, $\nabla\mathbf{C}$, and the diffusion coefficient, D.

$$\frac{\partial \mathbf{C}}{\partial t} = D\left(\frac{\partial^2 \mathbf{C}}{\partial x^2} + \frac{\partial^2 \mathbf{C}}{\partial y^2} + \frac{\partial^2 \mathbf{C}}{\partial z^2}\right) = D\nabla^2 \mathbf{C} \tag{1}$$

The oxygen concentration at the villous membrane corresponds