RESEARCH

Modeling of human placental capillaries by using a unique 3d reconstruction program

İnsan plasenta kapillerlerinin özgün bir yazılımla 3 boyutlu modellemesi

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Abstract

Purpose: Visualization of the three-dimensional structures is performed by examining two-dimensional sections in histology. It is not always possible to comprehend structure-function integrity. Therefore, it was planned to obtain three-dimensional (3D) models of the capillaries in the placenta terminal villus by using the program developed in the Başkent University's laboratory and used in studies in different disciplines. The program's suitability for histology studies will also be assessed.

Materials and Methods: A term placenta tissue sample was obtained from a 33-year-old healthy woman. Following the plastic embedding procedure, semi-thin serial sections were taken from these blocks. The sections were stained using toluidine blue and examined and photographed by using a light microscope. The 3D modeling procedure was conducted on the light micrographs of serial sections in order to reconstruct villous capillaries.

Results: 3D images were obtained from two-dimensional light micrographs by using the same program. It was determined that the vessels running parallel to each other branched harmoniously, forming a capillary network in the terminal villus. Additionally, challenges encountered during the utilization of this program in histological studies are also presented in this study.

Conclusion: Fetal blood reaches every part of the terminal villus through the branching capillary network. This feature would increase the surface area of vasculo-syncytial membrane and facilitate feto-maternal exchange. This can be clearly visualized by using 3D models. Three-dimensional modeling helps to understand the structure of all organs and tissues. Given that the current program is freely accessible, it will not incur additional costs for researchers and is open to further improvement by the programmer (erhankiziltan@gmail.com).

Keywords:. Capillary, 3D modeling, placenta, villus

Öz

Amaç: Histolojide 3 boyutlu yapıların zihinde canlandırılması iki boyutlu kesitlerin incelenmesi ile sağlanmaktadır. Ancak yapı işlev bütünlüğünü anlamak her zaman mümkün olmamaktadır. Bu nedenle çalışmamızda laboratuvarımızda geliştirilen ve farklı bilim dallarındaki çalışmalarda kullanılan program ile plasenta terminal villusundaki kapillerlerin 3 boyutlu (3D) modellerinin elde edilmesi planlanmıştır. Programın Histoloji çalışmaları için uygunluğu da değerlendirilecektir.

Gereç ve Yöntem: Çalışmamızda 33 yaşında, sağlıklı gebenin term plasenta dokusundan örnek toplanmıştır. Plastik gömme işlemini takiben bloklardan yarı ince seri kesitler alınmıştır. Alınan seri kesitler Toluidin blue ile boyanarak ışık mikroskopta incelenmiş ve fotoğraflanmıştır. Villöz kapillerlerin rekonstrüksiyonu için seri kesitlerin ışık mikrograflarına üç-boyutlu modelleme prosedürü uygulanmıştır.

Bulgular: Mevcut program ile kesitlerden elde edilen iki boyutlu ışık mikrograflardan 3 boyutlu görüntüler elde edilmiştir. Birbirine paralel uzanan damarların uyumlu bir şekilde dallanarak terminal villusta kapiller ağ yapısı oluşturdukları gözlenmiştir. Ek olarak, multidisipliner olarak kullanılan bu programın histolojik çalışmalarda uygulanması sırasında ortaya çıkan zorluklar da sunulmuştur.

Sonuç: Fetal kan, dallanan kapiller ağ sayesinde terminal villusun her yerine ulaşmaktadır. Bu özellik, vaskülo-sinsityal membranın yüzey alanını artırarak feto-maternal değişimi kolaylaştırmaktadır. Bu özellik üç boyutlu modeller kullanılarak açıkça görselleştirilmiştir. Üç boyutlu modellemeler organların ve dokuların yapısını anlamamıza yardımcı olurlar. Mevcut program serbest erişimli olduğu için çalışmalara ek maliyet yaratmayacaktır ve araştırmacıların ihtiyaçları doğrultusunda programcı tarafından geliştirilmeye açıktır (erhankiziltan@gmail.com).

Anahtar kelimeler: Kapiller, 3 boyutlu modelleme, plasenta, villus

INTRODUCTION

The placenta is an organ that plays a vital role in normal fetal development and maintenance of pregnancy. It is known that gestational diseases develop due to placental abnormalities. For this reason, the structure of the placenta in detail has always drawn the attention of researchers[1-7]. Feto-maternal exchange takes place in the terminal villi. Terminal villi are also known as "floating villi" because they are found at the end of the villous tree and float freely in the maternal blood. The surface of the floating villi is covered with thin syncytiotrophoblast cloth. Sinusoids, which are enlarged capillaries, occupy a large area in the villus stroma. The endothelium of these enlarged capillaries and syncytiotrophoblast form a vasculo-syncytial membrane that allows or hinders feto-maternal exchange. The presence of numerous vasculo-syncytial membranes at the periphery of terminal villi is noticeable in 2D histological sections. However, it is not clear whether these sinusoidal capillaries reach every point of the villi or whether the capillaries are related to each other. These capillaries play a vital role for the placenta; therefore, creating three-dimensional (3D) models would help understanding the structure-function integrity.

There are studies in the literature suggesting that 3D modeling is useful in many disciplines[8]. For example, it was reported that examining 3D models of tissues taken in pathology allows the observation of complex differentiated structures and helps in tumor staging[9-12]. Radiological studies carried out in recent years emphasized the benefits of 3D reconstruction. In fact, they reported that, since the sections are automatically created in the computer environment in radiology, the margin of error is significantly lower and it also helps in surgical interventions[13-14].

Two-dimensional sections are analyzed to assume the three-dimensional structures of tissues in histology. Researchers occasionally make use of the analysis of serial sections to support their conclusions. However, they still come to subjective conclusions and sometimes the conclusion does not reflect the actual structure. Malandain et al. explained this situation, which they defined as "banana effect" or "z shift", by using the following example. Taking 2D serial sections from a banana and examining the serial sections under a microscope, histologists will see repetitive ellipsoid sections and they may not reconstruct the actual shape of the fruit histologically[15]. Therefore, adding quantitative data to histological studies by making use of technology will increase the reliability of studies. Kartasalo et al. compared free and commercial software to examine 3D reconstruction in histological studies. At the end of their study, they emphasized that 3D reconstruction is very useful for understanding the morphology of cells and tissues in histology, but that programs still need to be improved[16]. This 3D technology, particularly commercial software, frequently increases study costs and puts researchers in a challenging position. For this reason, free-access TT3D-BMMP (TanTuna 3D-Başkent Measuring and Modeling Program) program that was developed in Başkent University's laboratories was used in the present study.

In this study, it was planned to obtain a 3D model of the capillary network in the terminal villus from serial histological sections by using the current program. Thus, the 3D model of the capillaries in the placenta terminal villus will be displayed and, if the program is suitable for histology studies, it can be used free of charge. The hypothesis of this study is that placental capillaries branch and merge, providing access to every point in the villus, and the current 3D program is a suitable method to histologically prove this.

MATERIALS AND METHODS

The ethical approval for this study was obtained from the Başkent University's Institutional Review Board (Project No: DA23/330) and supported by the Başkent University's Research Fund.

Tissue collection

A term placenta was obtained from 33-year-old, healthy woman. A patient without any known chronic disease and/or complications, and followed up in Baskent University's Faculty of Medicine, Department of Obstetrics and Gynecology was involved in the present study. Before tissue collection, the patient was informed about the study and signed the "Informed Consent Form for Scientific Research".

Processing of semi-thin serial sections

Placenta tissue samples were fixed in a 2% glutaraldehyde solution after the birth. Following the primary fixation, the samples were washed using

Sorenson phosphate buffer and then fixed in 1% osmium tetroxide for about an hour. Tissue samples were then dehydrated by using graded ethanol series, substituted with propylene oxide, and then embedded in epoxy resin. Serial semi-thin sections with a thickness of 1000nm were taken from tissues embedded in epoxy resin[17]. Subsequently, the semi-thin sections were stained by using toluidine blue. They were then examined under the light microscope (Leica DM3000, Germany), and digital images were taken with the attached digital camera (Zeiss Axiocam 506 color, Germany) at 40X magnification. Sixty light micrographs obtained from serial sections were aligned by using the Adobe Photoshop program. During the alignment process, the villus in the first light micrograph was used as the reference, and the other light micrographs were aligned with the reference photograph by reducing their opacity and applying the "edit >free transform" option for rotating and replacing the new image overlapping the reference image.

TT3D-BMMP software

Before starting the analysis, pixel values were calibrated by using the bar in the light micrograph. The boundaries of the capillaries were determined (segmentation) in 60 aligned light micrographs and the 3D model was reconstructed by using TT3D-BMMP software. Then, the model was visualized by using the GMSH© software[18], and real-like surface views (a 3D image) were obtained. GMSH is a free 3D finite element mesh generator with built-in post-processing facilities and can be used under the General Public License (GPL).

RESULTS

The boundaries of the capillaries in the aligned light micrographs were determined by using the TT3D-BMMP program (Figure 1).
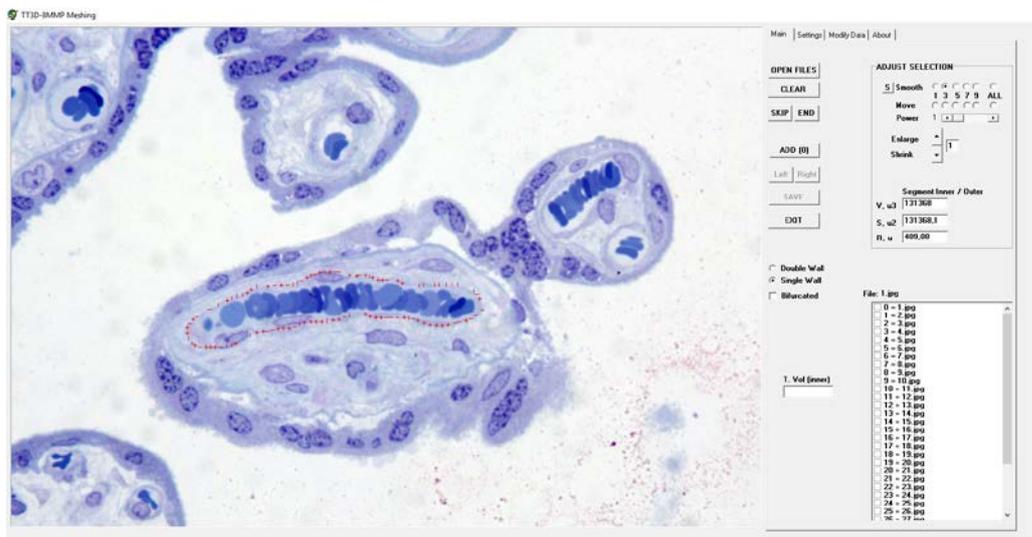

Figure 1. Drawing of the borders of capillary selected in the villus in the TT3D-BMMP program.

Even though the TT3D-BMMP program has automatic and semi-automatic segmentation options, the manual segmentation option was preferred in this study because of the irregular capillary borders and similar dye tones of the objects to be segmented. Then, the necessary corrections were made on the segmented points. The scroll wheel of the mouse was used to zoom the region of the targeted capillaries, and shift+left click buttons were used to draw the

capillary borders. As it is a common feature in many graphic design, 3D modeling, or data visualization software, this program was designed to help users manually edit and refine the segmented points. The inappropriate points were moved to their appropriate places by using the "adjust selection" option of the program when necessary. In the "adjust selection" option, one can smooth or move on the inappropriate points as many points as they choose

(1-3-5-7-9 or all points). Although the program automatically smoothens in the "smooth" option, one can move the nodes point-to-point manually in the "move" option. The options in the "adjust selection" are presented in Table 1.

Table 1. The options of the adjust selection section

Button/option	Function
Scroll wheel of the mouse	Expands the relevant area
Shift + Left click	Draw manually
Alt + Smooth + Number of point	Automatically smooth selected points
Alt + Move + Number of point	Moves selected points manually
Enlarge	Automatically expands the segmentation points
Shrink	Automatically shrinks the segmentation points

Once the segmentation was fixed with the region of interest, the "add" option was clicked, and the segmentation was saved as points in the program. The "bifurcation" option was selected, and the two branching capillaries were drawn separately as "left" and "right" in the sections, where the capillaries branched. After defining the segmented points in all slices (Figure 2a), the 3D surface mesh is constructed by clicking "save" button (Figure 2b) and the complete data is saved on the computer permanently for further evaluation. Finally, the rendered 3D surface mesh of the capillary is visualized by using the GMSH software (Figure 2c).
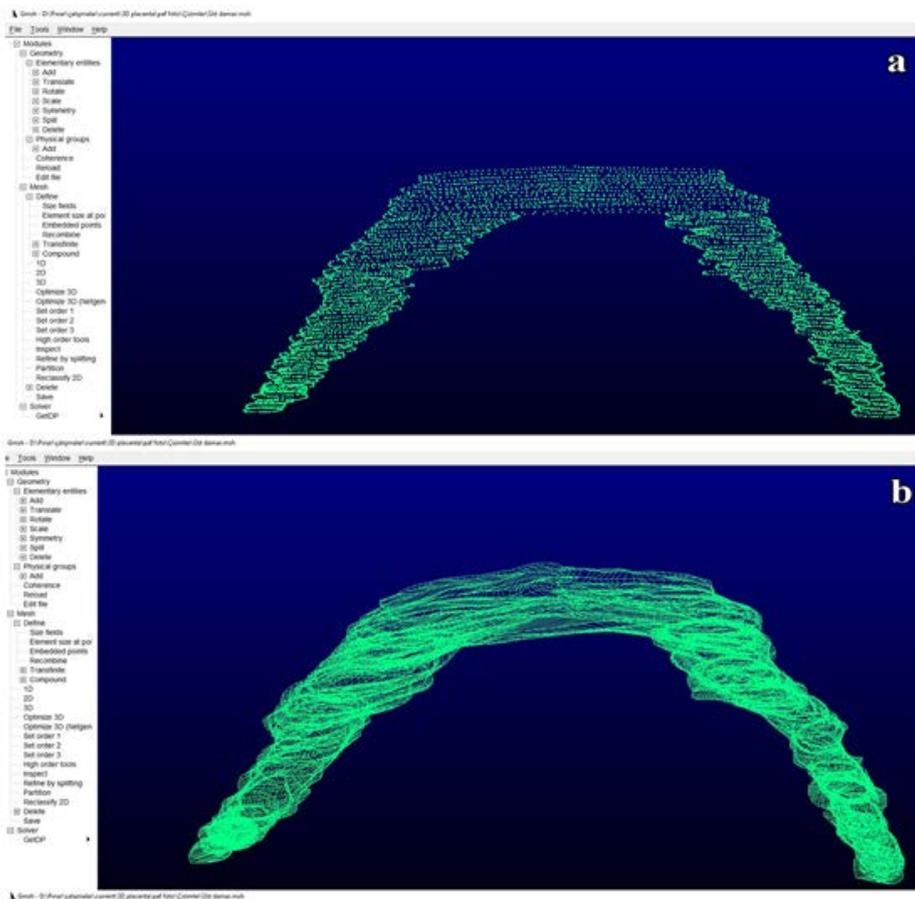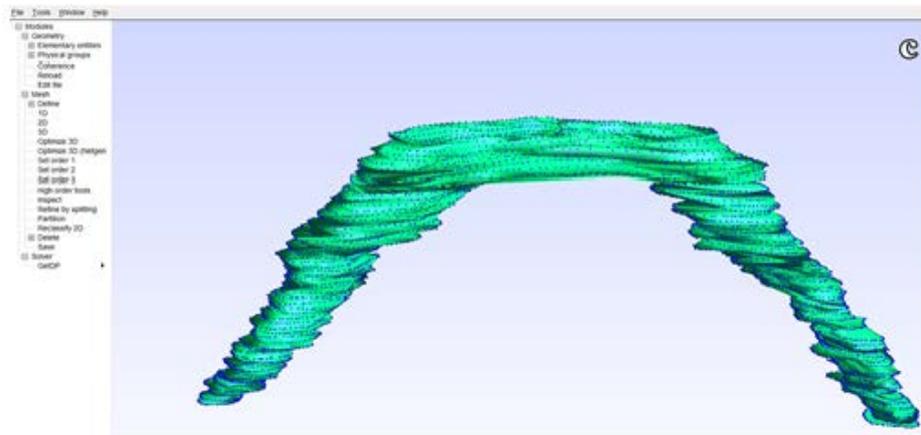
Figure 2: a) Segmentation image of the capillary located higher in the two-dimensional villus section in the GMSH program b) Following the segmentation, the points in each segment were connected with lines c) Surface mesh and also a 3D image of the capillary was created.

The same procedures were repeated for the other capillaries in the terminal villus serial sections. Three-dimensional models of both capillaries were created and merged in the GMSH program. Thus, both the individual courses of the capillaries and their relationships with each other were revealed. It was observed in the 2D terminal villus section that the upper capillaries were divided into two branches, and the branches continued throughout the serial sections. It was determined that the capillaries located lower in the villus section started off small and solitary, but they grew and branched in the following sections. They even extended deep into the villus from the space between branches. It was noted that these capillaries in the villus branched in harmony with each other, forming a capillary network within the villus. So, these parallel capillaries formed a network reaching every point of the villus (Figure 3).
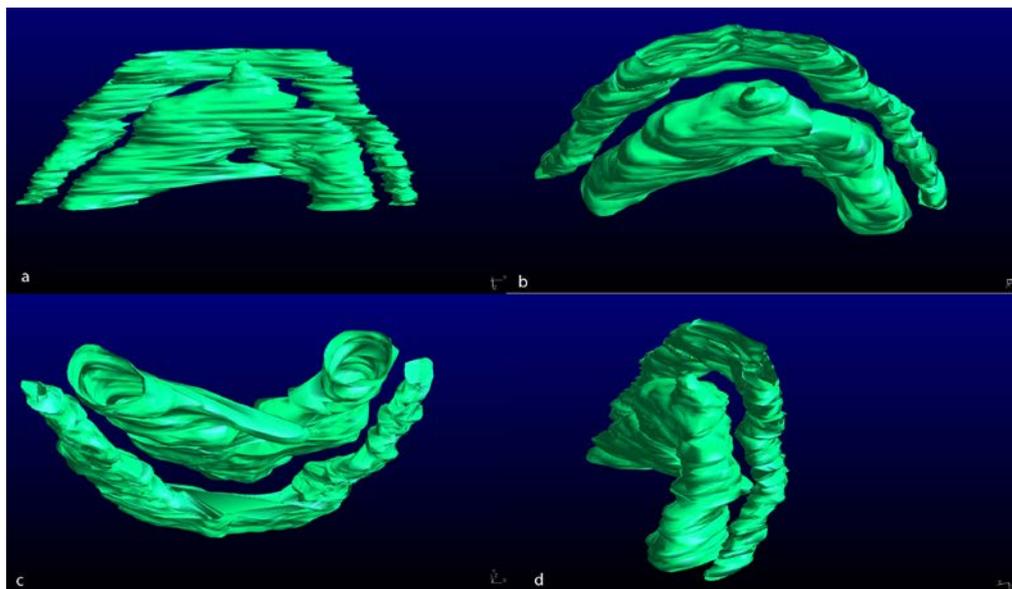

Figure 3: a-b-c-d) 3D models of the villous capillaries at different angles.

DISCUSSION AND CONCLUSION

In terminal villi, capillaries must get closer to the syncytiotrophoblast to increase feto-maternal exchange. Examining 3D models, it was determined that these parallel capillaries formed a network, increasing the vasculo-syncytial membrane surface area and facilitating feto-maternal exchange in line with the literature[19-23]. Moreover, it was also observed that the blood supply to the villus via capillary branching and side extensions will be sufficient. As can be seen in this study, 3D modeling allows us to better understand the structure of organs and tissues and can serve as a guide in creating scaffolds in artificial organ studies. Using 2D images to visualize the 3D structure of tissues and organs is really challenging for histologists. In addition, examinations are susceptible to subjectivity when only 2D examinations are conducted, affecting results depending on the histological experience of the researcher. To minimize the subjective effect, researchers also use 3D reconstruction in histological studies[8,16,24,25]. It is important to make use of technology so that the results of the studies are measurable, comparable, and user-independent. The present study suggests that 3D modeling programs, which are generally used in fields such as radiology, surgical interventions, and medical education, can also be used in histological sections and will be very useful[26-28]. Using the TT3D-BMMP program in radiological imaging methods with more regular borders and black-white tones will be quite easy since it is semi-automatic. This program employs the automatic object recognition feature by using "radial map" algorithm in structures with smooth surfaces and distinct tonal differences while performing a segmentation. Since the borders of the capillaries are irregular and there is not much difference in the tone of histological dye, manual drawing was preferred as the first step in the present study. However, even though the manual segmentation was employed, the program was found to be easily applicable with its user-friendly interface. There are several adjust selection options to correct the segmentation as described above. These options make the program easier, despite the omitted semiautomatic section. Taking the serial sections and aligning these serial sections in Adobe Photoshop program are the stages that challenge researchers. Taking serial sections with a thickness of 1000nm without any tissue loss is a procedure that requires mastery. In addition, alignment is a challenging process that requires both great attention and patience. Although manual drawing is a more labor-intensive process when compared to automatic selection, it becomes easier thanks to the "adjust selection" option of the program. Since paid programs will increase the cost of studies, researchers cannot easily access such programs. Therefore, the program used in this study is offered to researchers cost-free for academic purposes. Limitations of using this software in histological studies include the difficulty of automatic selection due to the closeness of histological dye tones and the irregularity of the boundaries of the structures, the difficulty of serial sectioning without loss of tissue, and the alignment of light micrographs. However, these limitations can be overcome as described above.

Three-dimensional reconstruction will enable us to understand not only the histology but also the physiopathology of tissues and will also contribute to the construction of scaffolds in artificial organ studies. Therefore, this software will be useful for different organs and larger areas. It is also open to improvements by programmers in line with the needs of researchers (For program supply and updates according to your needs, please contact with the programmer erhankiziltan@gmail.com).

In conclusion, given the results achieved in the present study, the potential advantages of using free-access software for histological studies:

1. Cost-Effective: Researchers can use the software without the need for expensive licenses or subscriptions, making it accessible to a wider range of individuals and institutions.

2. Community Collaboration: Free-access software often fosters collaboration and a sense of community among researchers, who can share their findings and improvement solutions on the software.

3. Accessibility: It promotes inclusivity by ensuring that anyone with an internet connection can use the tool, regardless of their location or available resources.

4. Customization: Users may have the flexibility to customize or modify the software in parallel with their specific research requirements and necessities.

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