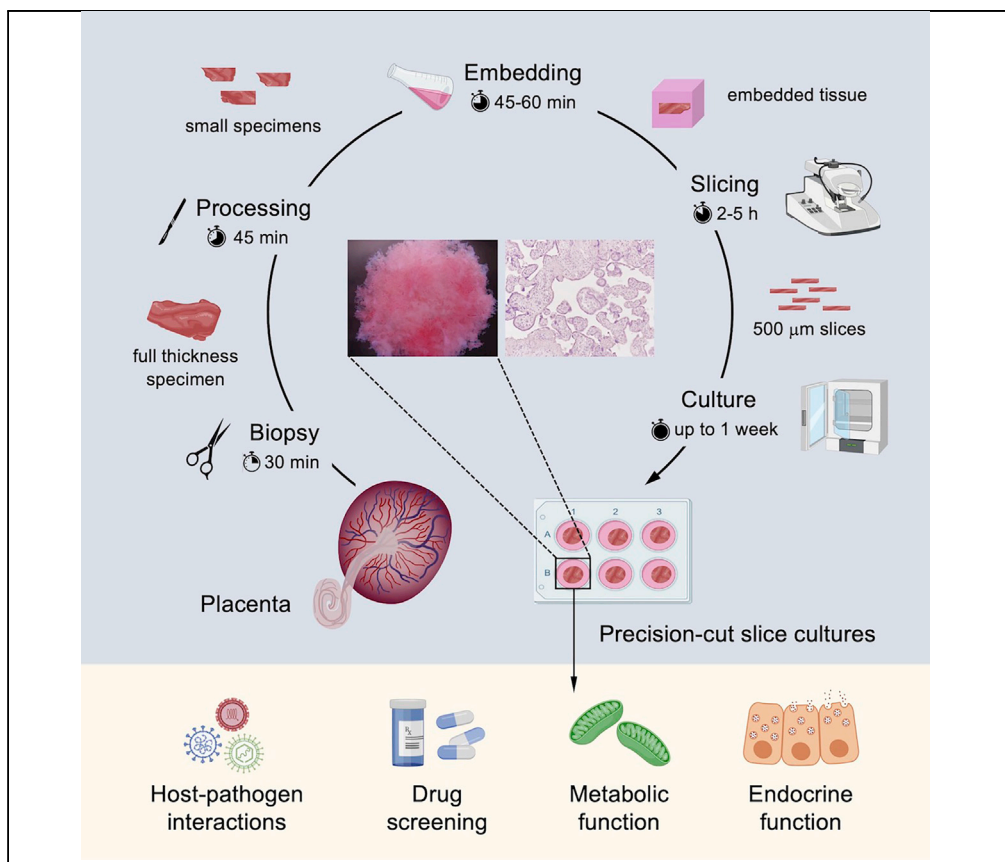


Protocol

Generation of precision-cut slice cultures of human placenta



We present a protocol to generate an advanced *ex vivo* model of human placenta. We use a vibrating tissue slicer to obtain precision-cut slices representative of the entire thickness of human placenta. This approach delivers standardized cultures with a preserved microstructure and cellular composition comparable to the native tissue. We applied this system to study SARS-CoV-2 infection at the maternal-fetal interface. Moreover, this system can be used to investigate the basic functions of the human placenta in health and disease.

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Highlights

Protocol to generate
an advanced model
of the human
maternal-fetal
interface

Standardized cultures
representative of the
full thickness of
human placenta

Cultures preserve the
microstructure and
cellular composition
of the native tissue

Can be applied to
study basic functions
of the human
placenta in health and
disease

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Protocol

Generation of precision-cut slice cultures of human placenta

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SUMMARY

We present a protocol to generate an advanced *ex vivo* model of human placenta. We use a vibrating tissue slicer to obtain precision-cut slices representative of the entire thickness of human placenta. This approach delivers standardized cultures with a preserved microstructure and cellular composition comparable to the native tissue. We applied this system to study SARS-CoV-2 infection at the maternal-fetal interface. Moreover, this system can be used to investigate the basic functions of the human placenta in health and disease. For complete details on the use and execution of this protocol, please refer to Fahmi et al. (2021).

BEFORE YOU BEGIN

Ethical and safety aspects

Ensure that all procedures on human specimens are performed with the approval of regulating authorities applicable at your institution. The placenta biopsies used in our study (Fahmi et al., 2021), were obtained from patients undergoing elective C-section or vaginal delivery at term pregnancy. Written informed consent was obtained from all the patients. The study protocol agrees with the Ethics Committee of the Canton of Vaud, Switzerland. Notably, placental tissue may contain infectious agents potentially harmful. Therefore, patient's serology (e.g., HIV, hepatitis, syphilis) should be checked before any investigation. Furthermore, it is recommended whenever possible to work under biosafety level 2 conditions to carry out this protocol.

Placenta biopsies transportation

⌚ Timing: 10–15 min

1. Prepare a bucket full of crushed ice.
2. Prepare sterile surgical scissors.
3. Prepare 50 mL conical bottom tubes with cold sterile phosphate buffer solution (PBS) supplemented with antibiotics (refer to [Materials and equipment](#) for detailed recipe) to store the placental tissue for transportation.



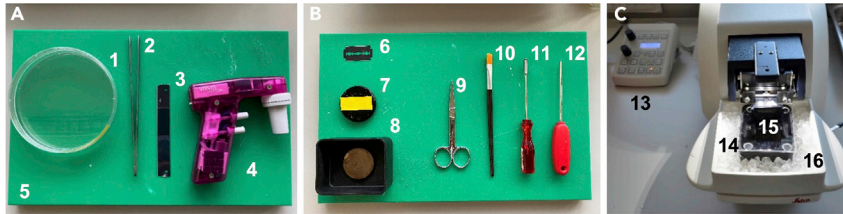


Figure 1. Material and equipment required for the generation of placenta precision-cut slices

(A) Material required for placental tissue processing: petri dish (1), sterile blunt-end forceps (2), microtome blade (3), pipette girl (4), sterile plastic cutting board (5).

(B) Material required for placental tissue slicing: sterile blade (6), specimen plate with protective tape (7), buffer tray (8), sterile surgical scissors (9), sterile flat brush (10), Allen key, size 3.0 (11), Allen key, size 6.0 (12).

(C) Vibrating tissue slicer ready to be used: control panel (13), installed buffer tray filled with media or PBS containing antibiotics (14) and covered with a Plexiglas lid (15), and placed into the ice tray (16).

Preparation for placental tissue processing

⌚ Timing: 20–25 min

4. Prepare sterile material for tissue processing according to [Figure 1A](#).
5. Prepare a bucket full of crushed ice.
6. Cool down a plastic cutting board to 4°C. The board will be used for tissue processing.
7. Prepare culture media: refer to [materials and equipment](#) for the detailed recipe.
8. Prepare cold sterile PBS supplemented with antibiotics. Approximately 100 mL of PBS per placenta biopsy.

Note: Keep culture media and PBS on ice.

Preparation for placental tissue embedding

⌚ Timing: 15–20 min

9. Inside a biological safety cabinet, prepare 1% low-melting point agarose solution in culture media (or PBS) without antibiotics. Per piece of placenta embedded into a mold, approximately 4.5 mL of agarose solution is required.

Note: The percentage of the agarose solution depends on the consistence of the tissue.

Note: Approximately 10–12 precision-cut slices can be obtained per piece of embedded placental tissue.

- a. Fill a sterile glass bottle with 50 mL of culture media without antibiotics.
- b. Weight 500 mg of low-melting point agarose powder and add to the culture media.
- c. Boil the culture media with the agarose powder in a microwave for 2 min at a power of approximately 700 W.

Note: Make sure the lid is loosened while heating the solution.

- d. After boiling, mix thoroughly the agarose solution by a rotary motion of the arm. The agarose powder must be fully dissolved. If required, heat again until agarose solution boils and repeat step (d).

Note: While handling the glass bottle wear heat resistant gloves for your safety.

10. Once all the agarose dissolved, maintain agarose solution at 37°C until further use.

Preparation of vibrating tissue slicer and associated material

⌚ Timing: 20–30 min

11. Prepare equipment needed for tissue slicing according to [Figure 1B](#).
12. Switch on the vibrating tissue slicer ([Figure 1C](#)).

Note: We established the method using the VT1200/S vibrating-blade microtome (Leica Biosystems) but there are other alternatives available on the market that can be used.

13. Insert a new blade into the blade holder according to manufacturer’s instructions.

Note: The blade may blunt during the cutting process. We recommend changing the blade when noticing lower efficiency in cutting and between different experiments.

14. Calibrate the tissue slicer for each new inserted blade according to the manufacturer’s instructions.

Note: Calibration is recommended to prepare high-quality sections.

15. Select the following settings for the different parameters: speed 0.12–0.26 mm/s, amplitude 3 mm.

16. Put some tape on the specimen plate where the embedded tissue will be glued.

Note: We advise not to directly stick the embedded tissue on top of the specimen plate. This allows proper equipment maintenance.

17. Prepare 6-well plates with 4 mL pre-warmed culture media per well for culturing the precision-cut slices.

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Human placenta ^a	Materno-Fetal and Obstetrics Research Unit, Lausanne University Hospital, Switzerland	N/A
Chemicals, peptides, and recombinant proteins		
Phosphate buffer solution (no calcium, no magnesium)	Gibco	Cat# 14200067
Agarose, low melting point	Promega	Cat# V2111
Penicillin-Streptomycin (10,000 U/mL)	Gibco	Cat# 15140-122
DMEM (1×) + GlutaMAX	Gibco	Cat# 32430-027
Fetal bovine serum, qualified	Gibco	Cat# 10270
HEPES (1 M)	Gibco	Cat# 15630-056
MEM non-essential amino acids solution (100×)	Gibco	Cat# 11140-035
Others		
VT1200/S vibrating-blade microtome	Leica Microsystems	Cat# 14 0481 80101
Cyanoacrylate adhesive	Best Klebstoffe, DE	Cat# 4250874203564

(Continued on next page)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Feather double edge blades	Biosystems	Cat# 121-9
Peel-A-Way™ embedding molds	Sigma-Aldrich	Cat# E6032-1CS
Microtome blade	Leica Biosystems	Cat# DB80 HS
1 mL pipette tip	Sarstedt	Cat# 70.3060.255
Serological pipettes, 25 mL	Sarstedt	Cat# 86.1254.001
Pipette Girl	Vitaris	Cat# 6340020
50 mL conical bottom tubes	Greiner Bio-One	Cat# 227 261
37°C Water bath	Hecht	Cat# 8911275
Tape	N/A	N/A
Tissue culture dishes	TPP	Cat# 93150
Cell culture plates, 6-well	TPP	Cat# Z707767
Plastic cutting board	N/A	N/A
Blunt-end forceps	N/A	N/A
Flat brush	N/A	N/A
Small surgical scissor	N/A	N/A
Microwave oven	N/A	N/A

^aWe used term placenta (37–42 weeks of gestation) and the patients included in the study were between 25 and 35 years old.

MATERIALS AND EQUIPMENT

Culture media

Reagent	Final concentration	Amount
DMEM (1×) + GlutaMAX	N/A	435 mL
Fetal bovine serum, qualified	10%	50 mL
HEPES (1 M)	10 mM	5 mL
MEM non-essential amino acids solution (100×)	1×	5 mL
Penicillin-Streptomycin (10,000 U/mL)	100 units/mL, 100 µg/mL	5 mL
Total	N/A	500 mL

Keep sterile and store at 4°C for up to 1 month.

Phosphate buffer solution containing antibiotics

Reagent	Final concentration	Amount
Phosphate buffer solution, no calcium, no magnesium	N/A	495 mL
Penicillin-Streptomycin (10,000 U/mL)	100 units/mL, 100 µg/mL	5 mL
Total	N/A	500 mL

Keep sterile and store at 4°C for up to 1 month.

STEP-BY-STEP METHOD DETAILS

Procurement of tissue

⌚ **Timing: 30 min (+ transportation time)**

This step describes how to obtain representative biopsies of the human placenta.

1. Immediately after birth, cut biopsies of around 5 cm diameter with a sterile scissor halfway between the umbilical cord insertion and the margin of the placenta. Each placenta biopsy should include the full thickness of the placenta with both maternal and fetal sides (Figures 2A and 2B).

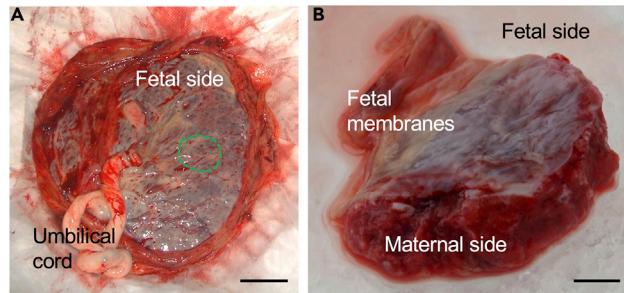


Figure 2. Placenta biopsy

(A) Representative picture of a human placenta after delivery. The biopsy is taken halfway between the umbilical cord insertion and the margin of the placenta (green dotted circle). Scale bar, 5 cm.
(B) Picture showing a representative placenta biopsy. Scale bar, 1 cm.

2. Store placenta biopsies in sterile PBS containing antibiotics at 4°C until processing to preserve the viability of the tissue.

Note: We recommend using fresh tissue for the protocol. However, others demonstrated the use of tissue preserved 16 h at 4°C (Cirelli et al., 2000).

Processing of placental tissue

⌚ Timing: 45 min

This step describes how to process the placental tissue before embedding and slicing. This step should be performed inside a biological safety cabinet.

3. Rinse the placenta biopsies until the surrounding blood is cleared off (Figure 3A).

Note: To facilitate the rinsing of the well-vascularized placental tissue, the use of cold sterile PBS containing antibiotics is recommended.

⚠ **CRITICAL:** Handle tissue with care and be careful not to disrupt/damage the tissue. Use blunt-end forceps to move the placental tissue by using the borders of the fetal membranes (Figure 3B).

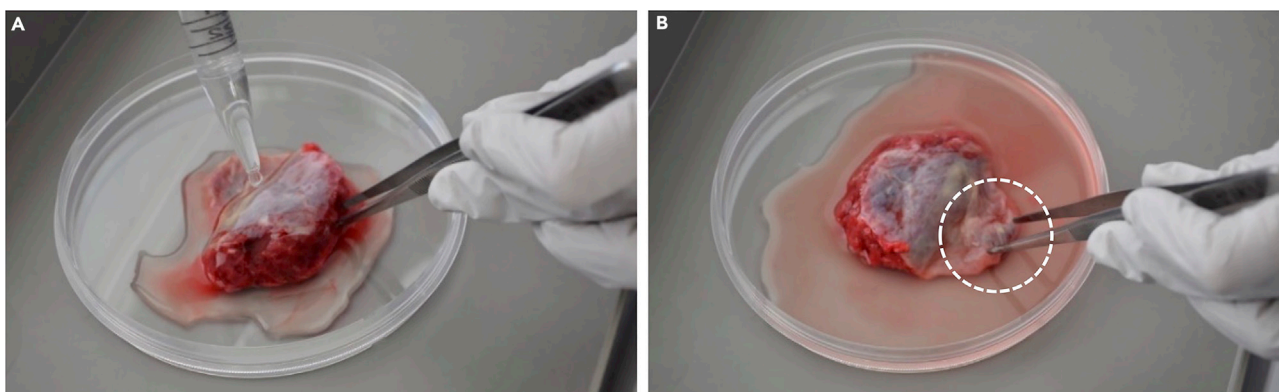


Figure 3. Handling and rinsing of human placental tissue

(A) Gentle rinse of placental tissue with cold sterile PBS supplemented with antibiotics until the tissue is cleared from the surrounding blood.
(B) Manipulation of placental tissue only at the borders of the fetal membranes with sterile blunt-end forceps to not harm the tissue (white dotted circle).

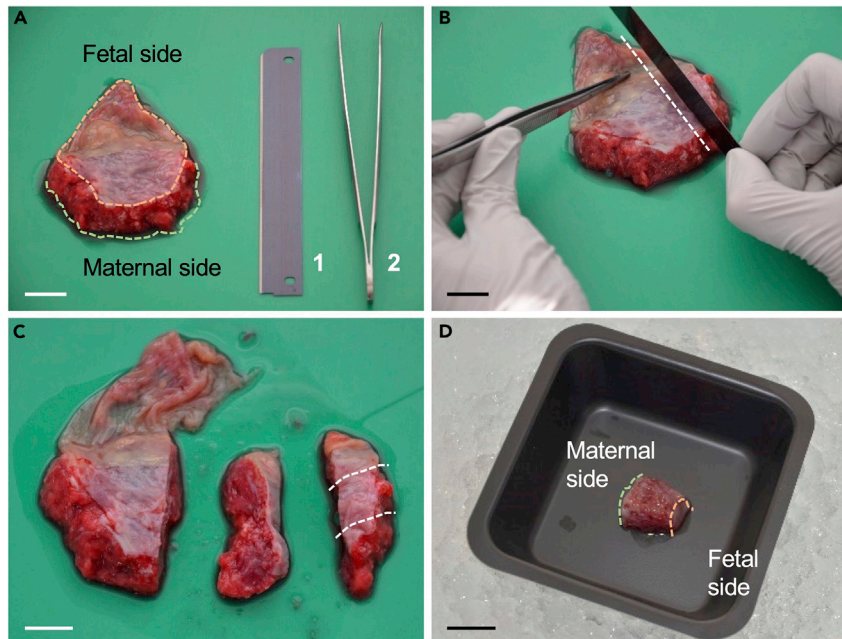


Figure 4. Dissection process of human placenta biopsies with preservation of fetal-maternal orientation

(A) Picture showing a representative placenta biopsy and the material required to cut and handle the tissue: sterile microtome blade (1) and sterile blunt end-forceps (2). Scale bar, 2 cm.

(B and C) The placenta biopsy is cut into smaller pieces while maintaining the fetal-maternal orientation. Scale bar, 2 cm.

(D) The specimens are stored on ice until further processing. Scale bar, 1 cm.

4. Put placenta biopsies on the cooled cutting board (Figure 4A).
5. Manually cut placental tissue into smaller pieces using a sterile blade (Figures 4B and 4C).

Note: Cut the tissue by preserving the orientation of the maternal-fetal interface.

Note: The pieces weigh about 1–1.8 g and should be 0.8–1 × 0.8–1 cm, and 2 cm thick representing the full thickness of the human placenta.

Note: Prepare several pieces, depending on the number of slices required (expect to obtain approximately 10–12 slices per embedded piece of placenta). Ensure you prepare enough material since not every tissue piece gives the desired number of slices.

6. Keep the placenta pieces at 4°C until embedding (Figure 4D).

⚠ **CRITICAL:** Do not store the small placenta specimens in PBS or culture media before embedding, since this leads to loosening of the placental tissue, which can impair the cutting process.

Embedding of placental tissue

⌚ **Timing:** 45–60 min

This step describes how to embed placenta pieces into agarose solution. This step should be performed inside a biological safety cabinet.

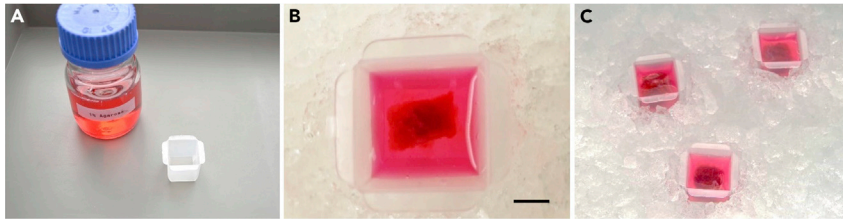


Figure 5. Embedding step for small placenta specimens

- (A) Placenta pieces are embedded into agarose solution in square-formed molds and kept on ice.
 (B) Placenta pieces are placed horizontally in the middle of the mold and are surrounded completely by agarose solution. Scale bar, 0.5 cm.
 (C) Embedded tissue is kept on ice until further slicing.

△ **CRITICAL:** Make sure the prepared agarose solution maintains a temperature of 37°C. Agarose solution solidifies at room temperature (20°C) and too hot temperature may harm the tissue.

7. Pour 1.5 mL 1% agarose solution (prepared in step 9 in [before you begin](#) section) into a Peel-A-Way embedding mold to cover the bottom ([Figure 5A](#)).
8. Place the mold on ice.
9. Wait approximately 60 ± 5 s for the agarose solution to start solidifying.

△ **CRITICAL:** Don't let the agarose solution fully solidify but just enough so that the embedded placenta piece does not sink to the bottom. This ensures full coverage of the tissue with agarose.

10. Place the placenta piece horizontally in the middle of the mold already containing a layer of agarose solution.
11. Cover the tissue with 1% agarose solution (approximately 3 mL) ([Figure 5B](#)).
12. Keep the mold on ice to let the agarose solution fully solidify ([Figure 5C](#)).

△ **CRITICAL:** While embedding, the work surface should be even to ensure a homogenized distribution of the agarose solution around the tissue and to avoid displacement of the embedded tissue.

Generation of precision-cut slices

⌚ **Timing:** 2–5 h depending on the number of slices required

This step describes how to generate precision-cut slices from agarose-embedded tissue using a vibrating tissue slicer. The procedure is carried out on a laboratory workbench.

13. Fill the buffer tray of the tissue slicer with cold sterile culture media (prepared in step 7 in [before you begin](#) section) and place it into the ice tray.

Note: Cold sterile PBS containing antibiotics can be used as well.

14. Cover the filled buffer tray with the Plexiglas lid to avoid any ice in the culture media.
15. Fill the ice tray with crushed ice.

Note: Fill up the ice tray when required to keep the culture media in the buffer tray at 4°C during all the procedures.



Figure 6. Sectioning process of embedded placenta pieces into precision-cut slices

- (A) Molds are cut using a sterile surgical scissor.
 (B) Agarose block is released from the mold without breaking it.
 (C and D) Agarose block is glued on the specimen plate.
 (E) Agarose block on the specimen plate is transferred into the buffer tray containing media or PBS supplemented with antibiotics.
 (F) The section being sliced.
 (G) During slicing, the section is supported by a flat brush.
 (H) Surgical scissors can be used to fully separate the section from the agarose block.

16. Cut at the edges of the mold and down the corners (Figure 6A). Open the mold and carefully detach the agarose block (Figure 6B).
17. Glue the agarose block using the cyanoacrylate adhesive onto the specimen plate (Figures 6C and 6D).

△ **CRITICAL:** Keep the cyanoacrylate adhesive on ice to maintain its viscosity.

18. Place the specimen plate into the buffer tray using the provided screw and adjust it to the desired position (Figure 6E).

△ **CRITICAL:** Orient the agarose block so that the blade starts cutting at the edge of the block. This reduces the pressure of the blade onto the agarose block, which reduces the risk to detach from the specimen plate.

△ **CRITICAL:** Make sure the whole agarose block is covered with culture media. This helps the blade to better slice once in contact with the tissue.

19. Adjust the clearance angle of the blade holder following the manufacturer's instructions.

Note: A clearance angle of 18° is recommended.

20. Define the cutting window edges to have a defined start and endpoint for cutting following the manufacturer's instructions.
21. Wet the blade with culture media from the tray using a sterile brush.

22. Trim the agarose block until the blade is in the proximity of the embedded tissue.

Note: To accelerate the process of trimming, choose an appropriate thickness and higher speed.

Optional: Manual trimming of the agarose using a blade or scalpel can be performed before gluing the agarose block onto the specimen plate.

23. Enter the thickness of interest between 500-700 μm .

24. Start the sectioning and discard the first 1–2 sections since they have not yet the defined thickness (Figure 6F).

Note: During the sectioning process, if required, support slices gently with a sterile brush to avoid that the tissue is pushed down and to keep its contact with the blade (Figure 6G).

△ CRITICAL: Be careful not to damage the slices by pushing and avoid pressing down the piece of tissue to not impact the thickness of the section.

Note: In case the slice did not fully detach from the specimen block, use a sterile surgical scissor, and carefully cut to separate the slice from the specimen block (Figure 6H).

25. Collect the floating slices using a flat sterile brush and transfer into the 6-well plate (prepared in step 17 in [before you begin](#) section) as you go along with cutting.

Punching of precision-cut slices

⌚ **Timing:** 15–30 min depending on the number of slices required

This step describes how to punch the precision-cut slices to obtain comparable sizes. This step should be performed inside a biological safety cabinet.

26. To punch the precision-cut slices (Figure 7A), use the top of a sterile 1 mL pipette tip (8 mm diameter). Position the top of the tip in the middle of the slice, push it down, and then gently turn the tip while pushing it down (Figure 7B).

△ CRITICAL: Maintain the maternal-fetal interface in the placental precision-cut slices by punching in the middle of the sections.

△ CRITICAL: Be careful not to damage the slices while punching. Use a small sterile scissor and forceps if the punched slice does not detach completely.

Note: From one raw placenta precision-cut slice, we obtain 1 punched slice.

Optional: A disposable, sterile biopsy puncher can be used instead of a 1 mL pipette tip.

27. Discard the surrounding tissue (Figure 7C) and place the slices in 4 mL of culture media at a rate of one slice per well in a 6-well plate at 37°C, 5% CO₂ (Figure 7D).

28. To limit the effect of injury caused by tissue slicing/punching, maintain the cultures two days prior to use at 37°C, 5% CO₂ and change the media every 24 h (Fahmi et al., 2021; Gilligan et al., 2012).

EXPECTED OUTCOMES

Precision-cut slices preserve the microstructure of the native placental tissue and can be cultivated up to one week, and potentially longer, following specimen procurement (Figures 8A–8C). As an

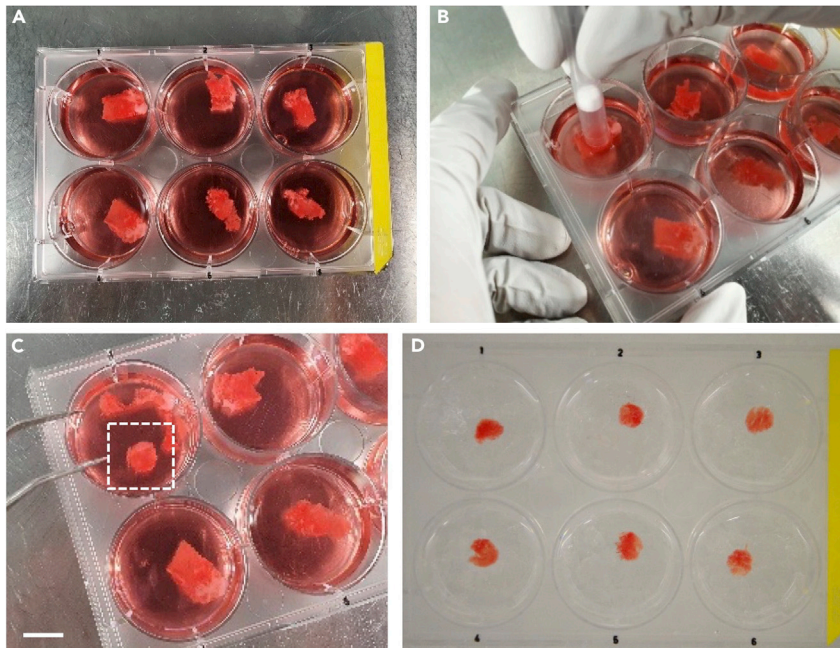


Figure 7. Punching and culturing placenta precision-cut slices

(A) Six-well plate containing the raw sliced sections.

(B and C) Punching of sections with a 1 mL pipette tip to obtain precision-cut slices (shown in white square following punching) of comparable sizes.

(D) Scale bar, 1 cm (D) Placental precision-cut slices ready for culture (here placed in PBS for illustration purpose).

advanced *ex vivo* model of the human placenta, it represents a relevant approach for the study of host-pathogen interactions at the maternal-fetal interface, as we reported previously for SARS-CoV-2. We used precision-cut slices to evaluate the susceptibility of the human placenta to SARS-CoV-2 replication and to further investigate the cellular tropism of SARS-CoV-2 (Fahmi et al., 2021). This system could be applied to other pathogens potentially targeting the placenta. Moreover, placenta precision-cut slices could be used as a translational platform for the screening of antivirals or other pharmacological compounds. Considering the high degree of interspecies placenta diversity, this system could also be of help to study placenta-related diseases and more generally, to study basic endocrine and metabolic functions of the human placenta.

LIMITATIONS

Placenta precision-cut slices do not constitute an appropriate system for the assessment of the placental barrier and long-term outcomes influenced by immune components. Also,

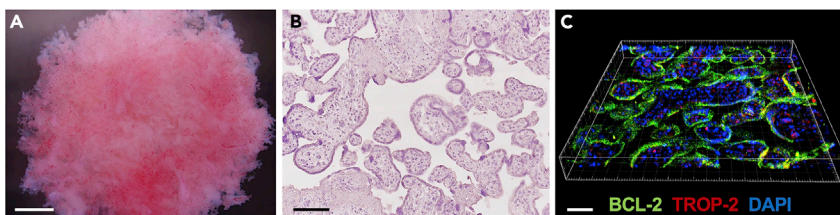


Figure 8. Villous and microstructure are well preserved in placenta precision-cut slices

(A) Representative stereomicroscopic image showing a preserved villous microstructure of placental precision-cut slices after 3–4 days in culture. Scale bar, 500 μm .

(B) Five μm cross-sections of placenta precision-cut slices counterstained with hematoxylin. Scale bar, 200 μm .

(C) Representative 3D-rendering micrograph of the syncytiotrophoblast (BCL-2, green) and trophoblast (TROP-2, red) layers in placenta precision-cut slice cultures. DAPI, blue. Scale bar, 50 μm .

conclusions regarding early pregnancy cannot be drawn using precision-cut slices generated from term placenta. However, the method could potentially be adapted to preterm placenta specimens.

TROUBLESHOOTING

Problem 1

Disruption of agarose block while detaching it from the mold or while cutting (steps 16 and 24 in section [generation of precision-cut slices](#)).

Potential solution

Make sure the agarose solution has completely solidified in step 12 in [embedding of placental tissue](#) section. Otherwise, keep it longer at 4°C. Carefully detach the agarose block from the mold. Avoid tearing the mold too hard and cut well down the edges using surgical scissors. The first layer of agarose solution solidified too much before placing the placenta piece in step 9 in [embedding of placental tissue](#) section. During the 60 s, check the stiffness from time to time using blunt-end forceps. The agarose should be viscous but not solid yet. Incomplete embedding of the placenta piece. Make sure the tissue is surrounded completely with agarose solution. This stabilizes the tissue for a proper cutting process. Place the placental piece centrally into the mold. To avoid that the entire block falls off while cutting, we recommend being generous with the cyanoacrylate adhesive.

Problem 2

The placenta piece disassembles from the agarose block (step 24 in section [generation of precision-cut slices](#)).

Potential solution

Adjust the speed and the amplitude. We recommend using 0.12–0.26 mm/s and 3 mm, respectively. Inappropriate embedding. See [problem 1](#) in the [troubleshooting](#) section.

Problem 3

Poor quality of precision-cut slices (step 25 in section [generation of precision-cut slices](#)).

Potential solution

Work with fresh tissue, handle tissue with care, and do not store placenta pieces in PBS or culture media before embedding to ensure good quality of tissue before cutting. Connective tissue within the placenta piece may lead to disruption of the slice. The blade may be blunt. Insert a new blade into the blade holder (step 13) and repeat step 14 in [before you begin](#) section. Adjust the speed and the amplitude. We recommend using 0.12–0.26 mm/s and 3 mm, respectively. Rule of thumb: the softer the tissue, the lower the speed and the higher the amplitude. We recommend choosing a thickness of the slice of 500 μm. Depending on the tissue quality, increasing the thickness from 500 to 700 μm may help.

Problem 4

Compromised viability of the cultures (step 27 in section [punching of precision-cut slices](#)).

Potential solution

Keep tissue at 4°C during transportation and all the procedure. Handle tissue with care during all procedures to not disrupt/damage the tissue. E.g., by using blunt-end forceps and by using the borders of the fetal membranes for manipulation of the tissue. Use sharp blades for the cutting procedures. For the embedding of placental tissue, agarose solution at a temperature above 37°C should not be used. Immediately after sectioning, carefully transfer the slices to a cell culture plate with culture media and keep them at 37°C, 5% CO₂ afterward. Change culture media every 24 h.

Problem 5

Contamination of the cultures (step 27 in section [punching of precision-cut slices](#)).

Potential solution

Whenever possible, carry out the whole procedure inside a biological safety cabinet. All items placed inside the biological safety cabinet must be sprayed with 70% ethanol. All the workbench surfaces must be sprayed with 70% ethanol. Instruments such as scissors, forceps, blades, and brushes must be soaked in 70% ethanol for 20 min followed by air-drying prior use. If not too heavy or too big, the tissue slicer can be placed inside a biological safety cabinet.

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Dr. Marco P. Alves (marco.alves@vetsuisse.unibe.ch).

Materials availability

The study did not generate new unique reagents.

Data and code availability

No data or code was generated or analyzed in this protocol.

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AUTHOR CONTRIBUTIONS

Conception and design, M.P.A. and D.B.; data acquisition, A.F., M.B., B.Z., and B.I.O.E.; data analysis and interpretation, M.P.A., D.B., A.F., and M.B.; drafting the manuscript, A.F., M.B., D.B., and M.P.A.; final approval of the manuscript, all authors.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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