Video Article

# Isolation and Analysis of Hematopoietic Stem Cells from the Placenta

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## Abstract

Hematopoietic stem cells (HSCs) have the ability to self-renew and generate all cell types of the blood lineages throughout the lifetime of an individual. All HSCs emerge during embryonic development, after which their pool size is maintained by self-renewing cell divisions. Identifying the anatomical origin of HSCs and the critical developmental events regulating the process of HSC development has been complicated as many anatomical sites participate during fetal hematopoiesis. Recently, we identified the placenta as a major hematopoietic organ where HSCs are generated and expanded in unique microenvironmental niches (Gekas, et al 2005, Rhodes, et al 2008). Consequently, the placenta is an important source of HSCs during their emergence and initial expansion.

In this article, we show dissection techniques for the isolation of murine placenta from E10.5 and E12.5 embryos, corresponding to the developmental stages of initiation of HSCs and the peak in the size of the HSC pool in the placenta, respectively. In addition, we present an optimized protocol for enzymatic and mechanical dissociation of placental tissue into single-cell suspension for use in flow cytometry or functional assays. We have found that use of collagenase for single-cell suspension of placenta gives sufficient yields of HSCs. An important factor affecting HSC yield from the placenta is the degree of mechanical dissociation prior to, and duration of, enzymatic treatment.

We also provide a protocol for the preparation of fixed-frozen placental tissue sections for the visualization of developing HSCs by immunohistochemistry in their precise cellular niches. As hematopoietic specific antigens are not preserved during preparation of paraffin embedded sections, we routinely use fixed frozen sections for localizing placental HSCs and progenitors.

## Video Link

The video component of this article can be found at http://www.jove.com/video/742/

## Protocol

### 1. Embryo removal and placental dissection

1. To begin, embryos must first be isolated from pregnant dams.
2. Animals are euthanized according to approved procedures – in our case we are going to use a lethal dose of the anaesthetic isoflurane
3. First, spray the belly with ethanol and make a small incision with a pair of scissors.
4. Tear away the skin with both hands to uncover the abdomen and cut open the peritoneum.
5. With forceps, pick up the uterine horns and collect them using scissors at each distal end. Place the uterine horns in a petri dish filled with PBS and place on ice. Also, remember to wash several times with PBS before isolating the placenta. We can now isolate the placenta.
6. With two forceps, begin carefully peeling away the endometrial tissue surrounding the embryo conceptuses. One should be careful not to puncture or otherwise inflict structural damage to the embryos, as this will complicate subsequent dissection steps.
7. Place the isolated conceptuses in a new petri dish with PBS on ice and continue until all conceptuses have been freed from the endometrium.
8. Transfer one conceptus to a new petri dish with PBS placed under the microscope and with two forceps peel the decidua away from the placenta. Remove as much of the decidua as possible as decidua cells will interfere with single-cell suspension and flow cytometry. A smooth continuous peeling motion is recommended to achieve the best results.
9. Cut the yolk sac away from the placenta at the junction between the two organs and gently pull the yolk sac and vitelline vessels away from the placenta. Care should be taken not to disrupt the chorionic plate of the placenta, especially with early embryos.
10. Carefully holding the chorionic plate of the placenta with one pair of forceps and the umbilical cord with another, pull the umbilical cord and attached embryo away from the placenta.
11. Remove excess giant cell tissue at the edges of the placenta in order to minimize cell clumping during preparation of single-cell suspension.
12. Collect the placenta in a suitable container, such as a 15-ml Falcon tube, with PBS+5%FCS and on ice. Do this for all placentas dissected.
13. At this point, you can either proceed with the preparation of a single-cell suspension for flow cytometry, functional assays, such as in vitro culture or transplantation, or prepare whole placenta for tissue fixation and fixed frozen block embedding for eventual immunohistochemistry.

### 2. Preparation of single-cell suspension of placenta tissue

1. We will now show you how to generate a single cell suspension from placenta for FACS analysis.

2. To prepare a single cell suspension from placenta, mechanical and enzymatic dissociation is required. First prepare a 0.1% Collagenase solution in PBS with 10% FCS and 1% Penicillin/Streptomycin solution.
3. Add an appropriate volume of collagenase solution to the placentas, depending on how many you have. A volume that is twice the placenta volume and between 2-5ml is recommended.
4. Use a 16-G needle fitted on a 5-ml syringe to mechanically disrupt the tissue by passaging the collagenase solution and placenta through the needle 3 times. Repeat with an 18-G needle.
5. Place the sample in a 37°C, 5% $CO_2$ incubator for 45 minutes.
6. After the 45 minute incubation in collagenase, passage the cell solution through a 20-G needle, and incubate for an additional 45 minutes, in 37°C.
7. After the 1.5 hr total incubation in collagenase, passage the cell solution through 22-G and 25-G needles 3 times with each needle.
8. Filter the sample through a 50-µm filter attached to a new 15-ml Falcon tube, wash with PBS+5%FCS and centrifuge at 4°C for 5 minutes at 300 × g.
9. At this point, we have a single cell suspension suitable for FACS analysis or functional assays.

**3. Tissue Fixation for Frozen Sections**

1. Immediately after isolating the placenta from each embryo, they are placed in cold 1X PBS. Usually a 96-well plate works best for young placentas (E8.5-11.5), but with older placentas, E12.5 and older, a 24-well plate may be more convenient.
2. Each placenta is transferred into a new well filled with freshly thawed 4% paraformaldahyde or PFA. For the E12.5 placentas, the tissue is moved to a new well by hand, but for the E10.5 you can remove the PBS carefully with a pipette and add the PFA. It is important that the placenta is fully immersed for 2-4 hours at 4°C, depending on the age and size of the tissue.
3. Transfer the tissue into 30% sucrose, or if the tissues are very small and fragile, aspirate the PFA and add the sucrose directly. At this stage, the placentas will be floating in solution. Let the tissues stay overnight at 4°C.
4. After the overnight step in 30% sucrose, the tissues should ultimately sink to the bottom of the well, indicative of proper cryopreservation. Remove half of the sucrose solution and replace the volume with OCT and place the tissue back in 4°C for 1-2 hours. This facilitates OCT penetration.
5. Transfer the tissue to 100% OCT for 1 hour, after which it can be embedded.

**4. Embedding the Fixed Tissue**

1. Label plastic molds with appropriate tissue identification (show example mold).
2. Pull the tissue out of the OCT solution and cut the placenta in half by orienting the disc-shaped placenta with the umbilical cord side facing down and carefully slicing with a clean razor blade making sure to move the blade back and forth before releasing pressure to ensure an even cut. The placenta is cut in half to achieve the best prientation to later visualize the placental HSCs.
3. Place one placenta half at the bottom of the mold with the cut edge in contact with the bottom surface of the mold. Repeat with a new mold for the other half. For E10.5 placentas, both halves may be placed into the same mold using the same technique.
4. When pouring OCT into the mold, it can be difficult to keep the placentas in the proper orientation. Secure the placenta in place by holding the tissue half with forceps. Slowly pour OCT solution into the mold and avoid producing any bubbles. For E10.5 placentas, hold one tissue half by forceps and rest the other tissue half against the outside of the forceps. Slowly pour OCT solution as described for E12.5 placentas.
5. Once the mold is filled with OCT, quickly place the mold onto dry ice, the OCT will turn white flash freezing the tissue
6. Place the mold into a small plastic bag containing Drierite and immediately store in the -80°C freezer.

## Discussion

The experimental procedures described in this protocol will allow for isolation and visualization of placental tissue and hematopoietic stem and progenitor cells. For a comprehensive summary on expected cellular yield and number of HSCs in placenta and other fetal hematopoietic organs throughout fetal development we refer to Gekas, et al. 2005. For localization of developing HSCs and other hematopoietic cells in the placenta we refer to Rhodes, et al. 2008.

## References

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