DEVELOPMENT

Watching embryos develop

Embryonic development is typically hidden from view, but a window preparation technique now sheds light on this phase in the life of a mouse.

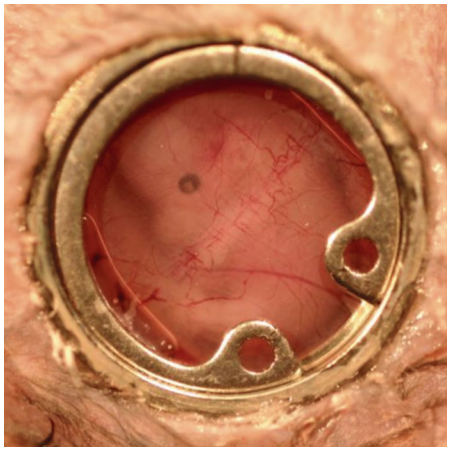
Intravital imaging provides a live view of organs such as the brain, the spinal cord, the colon or the small intestine. Long-term optical access in the mouse can be achieved through the implantation of lenses or windows. However, embryonic development within the uterus had only been visualized via ultrasound or magnetic resonance imaging, which provide limited spatial resolution.

Xiling Shen from Duke University and his colleagues address this limitation. Shen had previously used window preparations to image the gut. "Everyone loves images, but we asked ourselves the hard question: what can intravital imaging do that other techniques absolutely cannot? The answer is that it can see internal dynamic changes at cellular resolution in vivo," says Shen.

Shen says that the idea for intravital imaging of embryos arose serendipitously. Joint first author Qiang Huang, who is now a postdoc in the lab, was a practicing pediatric gastrointestinal surgeon and originally came to Shen's lab to learn some techniques. While helping another student with a surgery, he came up with the idea for imaging live embryos. Shen remembers saying, "it can't be that simple. If it can be done, someone would have tried it already."

It turns out that the window preparation was less difficult than anticipated. "The reality is there's no secrets and anyone who can handle animals can easily learn it. Embryos are a lot more resilient than we assumed," says Shen. The main differences from other window preparations in the abdomen are that the uterus needs to be surgically attached to abdominal muscles to stabilize it and that the decidua, a mucous layer that lines the uterus in early pregnancy, needs to be removed for imaging between embryonic days 9.5 and 12. The technique is simple enough that Shen's team reaches up to 85% embryonic survival rates. Embryos developing underneath the window exhibit a slightly reduced weight but otherwise show no deficits and grow up without abnormalities.

Shen and his team used the window technique to image the neuroepithelium and development of the neural crest cell lineage in a mouse strain expressing tdTomato in these cells. Among other things, they observed that neural crest cells differentiated into perivascular cells in the brain. Furthermore, the team imaged stem cells in the developing intestine, as well as the process of autophagocytosis in the embryonic retina. The window preparation also provides access to the developing brain and allows imaging of neuronal activity.



Window preparation showing a mouse embryo. Credit: Qiang Huang and Xiling Shen, Duke University

The window preparation can be combined with genetic manipulation of embryos, either via in utero electroporation or via transduction with adeno-associated viruses (AAVs) as gene vehicles. Shen and his team found that they could inject AAV8 and AAV9 into the tail vein of pregnant mice and observe reporter gene expression in embryos three days after injection. "Selected AAV vectors can easily penetrate the placenta and deliver payload to the embryos efficiently (which was quite a surprise)," he concludes. Finally, the window preparation can be harnessed to monitor cell fate in chimeric embryos.

Shen plans to use the window technique to "understand how neural crest cells migrate, differentiate, and innervate internal organs all over the body to form the peripheral nervous system" and to "study neural progenitor cells' dynamics." In addition, his lab is happy to share their expertise with others who would like to learn the technique.

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